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Efficacy of intravaginal dehydroepiandrosterone (DHEA) on moderate to severe dyspareunia and vaginal dryness, symptoms of vulvovaginal atrophy, and of the genitourinary syndrome of menopause

Fernand Labrie, MD, PhD,[1] David F. Archer, MD,[2] William Koltun, MD,[3] Andrée Vachon, MD,[4] Douglas Young, MD,[5] Louise Frenette, MD,[6] David Portman, MD,[7] Marlene Montesino, MD,[1] Isabelle Côté, BSc,[1] Julie Parent, PhD,[1] Lyne Lavoie, MSc,[1] Adam Beauregard, BSc, MBA,[1] Céline Martel, PhD,[1] Mario Vaillancourt, BSc, MBA,[1] John Balser, PhD,[8] Érick Moyneur, BSc, MA,[9] and the members of the VVA Prasterone Research Group

Abstract

Objective: The aim of this study is to confirm the local beneficial effects of intravaginal dehydroepiandrosterone (DHEA, Prasterone) on moderate to severe dyspareunia or pain at sexual activity, the most frequent symptom of vulvovaginal atrophy due to menopause or genitourinary syndrome of menopause (GSM).

Methods: In a prospective, randomized, double-blind, and placebo-controlled phase III clinical trial, the effect of daily intravaginal 0.50% DHEA (6.5 mg) (Prasterone, EndoCeutics) was examined on four coprimary objectives, namely percentage of parabasal cells, percentage or superficial cells, vaginal pH, and moderate to severe pain at sexual activity (dyspareunia) identified by the women as their most bothersome vulvovaginal atrophy symptom. The intent-to-treat population included 157 and 325 women in the placebo and DHEA-treated groups, respectively.

Results: After daily intravaginal administration of 0.50% DHEA for 12 weeks, when compared to baseline by the analysis of covariance test, the percentage of parabasal cells decreased by 27.7% over placebo ($P < 0.0001$), whereas the percentage of superficial cells increased by 8.44% over placebo ($P < 0.0001$), vaginal pH decreased by 0.66 pH unit over placebo ($P < 0.0001$), and pain at sexual activity decreased by 1.42 severity score unit from baseline or 0.36 unit over placebo ($P = 0.0002$). On the other hand, moderate to severe vaginal dryness present in 84.0% of women improved at 12 weeks by 1.44 severity score unit compared to baseline, or 0.27 unit over placebo ($P = 0.004$). At gynecological evaluation, vaginal secretions, epithelial integrity, epithelial surface thickness, and color all improved by 86% to 121% over the placebo effect ($P < 0.0001$ for all comparisons with placebo). Serum steroid levels remained well within the normal postmenopausal values according to the involved mechanisms of intracrinology. The only side effect reasonably related to treatment is vaginal discharge due to melting of the vehicle at body temperature and this was reported in about 6% of the participants.

Conclusions: The daily intravaginal administration of 0.50% (6.5 mg) DHEA (Prasterone) has shown clinically and highly statistically significant effects on the four coprimary parameters suggested by the US Food and Drug Administration. The strictly local action of Prasterone is in line with the absence of significant drug-related adverse events, thus showing the high benefit-to-risk ratio of this treatment based upon the novel understanding of the physiology of sex steroids in women.

Key Words: Dehydroepiandrosterone – Dryness – Dyspareunia – Genitourinary syndrome of menopause – Intracrinology – Prasterone – Vulvovaginal atrophy.

Atrophy of the epithelial surface of the vaginal mucosa is accompanied at menopause by reduced fluid secretion, reduced levels of lactobacilli, and increased vaginal pH.[1] These epithelial changes are responsible for the best known symptoms of vulvovaginal atrophy (VVA) or genitourinary syndrome of menopause (GSM),[2] namely vaginal dryness, pain at sexual activity, and irritation/itching.[3-5] Bleeding associated with sexual

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From the [1]EndoCeutics Inc, Quebec City, Quebec, Canada; [2]CONRAD Clinical Research Center, Norfolk, VA; [3]Medical Center for Clinical Research, San Diego, CA; [4]Clinique Médicale St-Louis (recherche) Inc, Quebec City, Quebec, Canada; [5]Northern California Research, Sacramento, CA; [6]Q&T Recherche Sherbrooke, Sherbrooke, Quebec, Canada; [7]Columbus Center for Women's Health Research, Columbus, OH; [8]Veristat, Holliston, MA; and [9]StatLog Consulting Inc, Ottawa, Ontario, Canada.

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Address correspondence to: Fernand Labrie, MD, PhD, EndoCeutics, 2975, Laurier Boulevard, Suite 500, Quebec City, QC G1V 4M7, Canada. E-mail: Fernand.Labrie@endoceutics.com

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activity, pain and burning during urination (dysuria), and urinary tract infections with vaginal discharge commonly accompany VVA. In addition to the local negative effects in the vagina, VVA is frequently associated with emotional distress and reduced quality of life,[6-8] with extension to the sexual partner.[9] The VVA symptoms are reported in up to approximately 60% of postmenopausal women.[7,10,11]

Despite their benefits on vasomotor symptoms, 40% of women receiving systemic estrogen therapy have persistent vaginal symptoms.[12] Consequently, local estrogen therapy is recommended over systemic administration, except if other symptoms of menopause, especially hot flushes, are present. All intravaginal estrogens apparently have comparable efficacy for the treatment of VVA.[13] As per the data of the Women's Health Initiative Study,[14] the recommendation has been to use the lowest dose of intravaginal estrogen possible for the shortest duration of treatment possible.[13,15,16]

Significant changes recently took place in our understanding of sex steroid physiology in women. Whereas it was well known that the ovary stops making estrogens at menopause, thus resulting in very low and biologically inactive serum estradiol (E_2) levels,[17] it was important to recognize that the adrenal and, to a small extent, the ovary[18] secrete in the blood a compound inactive by itself, namely dehydroepiandrosterone (DHEA), which is the exclusive precursor of all sex steroids after menopause.[18] Thus, acting through the mechanisms of intracrinology, DHEA provides estrogens and/or androgens only to the cells/tissues which possess the required enzymes to transform DHEA.[19]

The secretion of DHEA markedly decreases with age, with an average 60% loss between the age of 30 years and the menopause at about 50 years.[20-23] This marked reduction in the secretion of DHEA[23] results in a parallel fall in the formation of androgens and estrogens in peripheral target tissues—a situation most likely responsible for the hormone deficiency-related symptoms and signs of menopause. In addition to markedly decreasing with age, the serum levels of DHEA are highly variable, with some women having barely detectable serum concentrations of DHEA, whereas others have values of up to 9 to 10 ng/mL in the normal premenopausal range.[18,20]

Our recent placebo-controlled, randomized, double-blind, and prospective clinical trials have shown clinical and statistically significant benefits of daily intravaginal administration of DHEA on the maturation index (MI) of the vaginal cells, vaginal pH, as well as on moderate to severe dyspareunia, considered as the most bothersome symptom (MBS), as well as on moderate to severe vaginal dryness.[5,24-27] The objective of the present clinical trial, performed in 558 postmenopausal women enrolled with moderate to severe dyspareunia as their most bothersome VVA symptom, is to confirm the previous data obtained with daily intravaginal administration of DHEA for 12 weeks while reaching the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)-required number of 1,500 women exposed to the intravaginal DHEA treatment and the US Food and Drug Administration (FDA) guidelines.[28]

METHODS

This was a phase III, placebo-controlled, double-blind, prospective, and randomized study (ERC-238; NCT02013544, ClinicalTrials.gov) to confirm the efficacy of daily intravaginal administration of 0.50% DHEA ovules (suppositories) for 12 weeks on moderate to severe pain at sexual activity as the MBS of VVA. Women were randomized in a 2:1 ratio between the 0.50% DHEA and placebo ovules.

Independent ethics committee or Institutional Review Board

The protocol was approved by the Institutional Review Board (IRB) Services (Central IRB) for all investigational sites, except for the Eastern Virginia Medical School, Department of Obstetrics and Gynecology, and Clinical Research Center, where a local IRB gave approval before the start of the study.

Informed consent

A written informed consent was obtained from all participants before the performance of any study-related procedure.

Inclusion criteria

The inclusion criteria were as follows:

(1) Postmenopausal women (non-hysterectomized or hysterectomized) must satisfy either of the following:
   (a) No menses for at least 1 year for non-hysterectomized women, or
   (b) Follicle-stimulating hormone (FSH) levels above 40 IU/L or above the postmenopausal value of the laboratory where the FSH assay is performed (a woman with previously measured elevated serum FSH meets the inclusion criteria) in women with no menses for more than 6 months, but less than 12 months, or in hysterectomized women who were premenopausal at the time of hysterectomy, or
   (c) Six months or more of day 1 visit after bilateral oophorectomy with or without hysterectomy.
(2) Women between 40 and 80 years of age.
(3) Women who have self-identified at screening and baseline (day 1) pain at sexual activity as moderate to severe and as the most bothersome VVA symptom.
(4) Women having 5% or less of superficial cells on vaginal smear at screening and baseline (day 1).
(5) Women having a vaginal pH above 5 at screening and baseline (day 1).
(6) Women who currently have intercourse or other sexual activity (masturbation, etc) at least once a month (with or without a partner), or who had intercourse or other sexual activity at least once a month in the past, but later decreased sexual activity due to excessive pain or vaginal dryness.
(7) Normal mammogram (American College of Radiology Breast Imaging-Reporting and Data System [BI-RADS] category 1 or 2) within 9 months of study initiation (day 1).

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(8) Normal breast examination.
(9) A normal Pap smear (which includes inflammatory changes) within the past 12 months (of day 1) for both non-hysterectomized and hysterectomized women after specimen collection (see "exclusion criteria no. 14").
(10) Willing to participate in the study and sign an informed consent.
(11) No former or present narcotic addiction or alcoholism.
(12) For non-hysterectomized women, willing to have an endometrial biopsy during the screening period to exclude endometrial pathology.

Exclusion criteria

The exclusion criteria were as follows:

(1) Previous enrollment in EndoCeutics studies performed with intravaginal DHEA (ERC-210, ERC-213, ERC-230, ERC-231, or ERC-234).
(2) Previous diagnosis of cancer, except skin cancer (non-melanoma).
(3) Active or history of thromboembolic disease (thromboembolic event after an accident, a surgery, or immobilization is not an exclusion criterion).
(4) Clinically significant metabolic or endocrine disease (including diabetes mellitus) not controlled by medication.
(5) Use of estrogen alone injectable drug therapy or progestin implant within 6 months before study entry (screening visit).
(6) Use of estrogen pellet or progestin injectable drug within 6 months before study entry (screening visit).
(7) Oral estrogen, progestin, or DHEA exposure or intrauterine progestin therapy in the 8 weeks before baseline assessments (screening visit).
(8) Vaginal hormonal products (rings, creams, gels, or tablets) or transdermal estrogen alone or estrogen/progestin products in the 8 weeks before baseline assessments (screening visit).
(9) Previous treatment with androgens or anabolic steroids within 6 months before screening visit.
(10) Natural oral estrogenic products in the 4 weeks before baseline assessments (screening visit), whether intended or not, for the relief of symptoms of VVA and/or hot flushes.

Regarding exclusion criteria 5 to 10, participants can washout as indicated below, but the questionnaire on VVA, as well as the evaluation of cell maturation and pH, must be answered or evaluated after the required washout period.

- At least an 8-week washout period for prior oral estrogen, DHEA, and/or progestin therapy.
- At least an 8-week washout period for prior transdermal hormone therapy (HT).
- At least an 8-week washout period for locally delivered hormone replacement therapy for vaginal dryness (rings, creams, gels, or tablets).
- Eight weeks or longer for prior intrauterine progestin therapy.
- At least 6 months for prior estrogen pellet therapy or progestin injectable drug therapy.
- Six months or longer for prior progestin implants and estrogen alone injectable drug therapy.
- Six months or longer for previous treatment with androgens or anabolic steroids.
- Four weeks or longer for prior natural oral "estrogenic" products.

(11) Confirmed clinically significant depression (not controlled by standard therapy) or confirmed history of severe psychiatric disturbance.
(12) The administration of any investigational drug within 30 days of screening visit.
(13) Clinically significant abnormal serum biochemistry, urinalysis, or hematology.
(14) Baseline cervical cytology showing atypia of squamous cells of undetermined significance (ASC-US) or worse. Since ASC-US can be due to atrophy, a woman with ASC-US without history of abnormal Pap within the past 2 years and a negative human papillomavirus (HPV) test can be enrolled.
(15) Palpable fibroids, or grade 2 uterine prolapse (when the cervix reaches labia minora) by gynecologic examination.
(16) Endometrial hyperplasia (simple or complex hyperplasia with or without atypia), cancer, or endometrial histology showing proliferative, secretory, or menstrual-type characteristics at histologic evaluation of endometrial biopsy performed at screening.
(17) Women who suffer from vulvar lichen sclerosis.
(18) Endometrial polyps.
(19) Women who had endometrial ablation.

Randomization

Randomization was done centrally by Veristat Inc. A randomization with permuted blocks of three women at a 2:1 ratio of DHEA (group B) and DHEA-placebo (group A), respectively, was performed to ensure a balanced allocation to the two treatments by investigational site. A list of randomization provided the association between each randomization number and a specific medication container. Each container contained 0.50% DHEA or DHEA placebo ovules.

Dosing

Participants randomized into groups A and B were instructed to insert one vaginal ovule (suppository) daily before bedtime (usually in the evening) for 12 weeks. Before first administration of the study treatment, proper instructions were given to the participants on how to apply the vaginal ovule using the single-use applicator provided.

Compliance and drug accountability

Study drug containers were collected and verified at all visits by designated site personnel. Participants were questioned regarding study drug application technique, and use of any additional topical or systemic products. The time of drug application was to be recorded on a daily basis in the diary card provided by the Sponsor.

The person from the clinical trials team who was responsible for treatment dispense and return had to keep an accurate count of the number of investigational units received,

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dispensed to the participants, returned to the Investigator by the women, and the number of units returned to EndoCeutics during and at the completion of the study. Any discrepancy in the study drug accountability was to be reconciliated and documented.

Vaginal cell maturation

Vaginal smears were collected at screening, baseline (day 1), week 6 and week 12 (or at discontinuation visit, if applicable). Vaginal smears were obtained by gently scraping the middle (second third) of the side wall of the vagina using a wooden or plastic spatula gently applied to a glass slide and immediately fixed with Cytospray or its equivalent. Vaginal smears were not collected if the participant had sexual intercourse within the preceding 24 hours and/or has used vaginal products (lubricant, cream, vaginal douching, etc) within 48 hours before sampling. Vaginal smears were sent to the central laboratory (Centre Hospitalier Universitaire de Québec) for determination of the MI.

All samples were examined by an experienced cytopathologist blinded to the treatment regimens. A 100-cell count was performed to classify cells as parabasal (P) (including the basal), intermediate (I), and superficial (S) squamous cell types (a number greater than 100 cells is usually counted, and the numbers obtained for each of the three cell populations are divided to be reported as a total of 100).

Vaginal pH

The vaginal pH was determined at screening, baseline (day 1), week 6 and week 12 (or at discontinuation visit, if applicable). A pH strip fixed on an Ayre spatula (or equivalent) was applied directly to the lateral wall of the vagina opposite to the side scraped to obtain the vaginal smear.

Vaginal Atrophy Symptoms Questionnaire

Self-assessment of symptoms of vulvovaginal atrophy associated with menopause were evaluated at screening, baseline (day 1), week 6, and week 12 (or at discontinuation visit, if applicable) by a questionnaire.[29] The self-reported symptom score takes the following values: none, mild, moderate, or severe, and was analyzed using values of 0, 1, 2, or 3, respectively.

Observations at vaginal examination

The aspect of the mucosa and tolerance to treatment were verified by gynecological examinations performed at screening, day 1, and weeks 6 and 12 (or at discontinuation visit, if applicable)[29] as follows:

(1) Vaginal secretions:
   (a) No atrophy: normal clear secretions noted on vaginal walls.
   (b) Mild: superficial coating of secretions, difficulty with speculum insertion.
   (c) Moderate: scant and covering entire vaginal vault, may need lubrication with speculum insertion to prevent pain.
   (d) Severe: none, inflamed, ulceration noted, need lubrication with speculum insertion to prevent pain.
(2) Vaginal epithelial integrity:
   (a) No atrophy: normal.
   (b) Mild: vaginal surface bleeds with scraping.
   (c) Moderate: vaginal surface bleeds with light contact.
   (d) Severe: vaginal surface has petechiae before contact and bleeds with light contact.
(3) Vaginal epithelial surface thickness:
   (a) No atrophy: rugation and elasticity of vault.
   (b) Mild: poor rugation with some elasticity noted of vaginal vault.
   (c) Moderate: smooth, some elasticity of vaginal vault.
   (d) Severe: smooth, no elasticity, constricts in upper 1/3 of vagina or loss of vaginal tone (cystocele and rectocele).
(4) Vaginal color:
   (a) No atrophy: pink.
   (b) Mild: lighter in color.
   (c) Moderate: pale in color.
   (d) Severe: transparent—either no color or inflamed.

The above-indicated parameters (secondary endpoints), evaluated as no atrophy, mild, moderate, and severe, were analyzed using values of 1, 2, 3, and 4, respectively.

Safety

Physical examination, gynecological examination (for inspection of vaginal mucosa at day 1, wk 6, and wk 12) and clinical laboratory tests were performed. Adverse events (AEs) were recorded for safety evaluation and then coded into system organ class and preferred terms using the Medical Dictionary for Regulatory Authorities (MedDRA version 16.1). The potential influence of DHEA on participant's male partner was investigated as secondary objective. Clinical laboratory measurements were done at a central laboratory (URMC Laboratories, Rochester, NY). Serum steroids (namely DHEA, DHEA sulfate [DHEA-S], testosterone [Testo], dihydrotestosterone [DHT], androstenedione [4-dione], androst-5-ene-3β,17β-diol [5-diol], estrone [E_1], 17β-estradiol [E_2], estrone sulfate [E_1-S], androsterone glucuronide [ADT-G], and androstane-3α,17β-diol-17 glucuronide [3α-diol-17G]) were measured at the Central Laboratory of EndoCeutics Bioanalytical Unit by validated liquid chromatography tandem mass spectrometry (LC-MS/MS) methodology.[30-33]

Statistical analysis

Statistical analyses were performed at the two-sided significance level of 0.05. The primary analyses in this study were based on the comparison of DHEA to placebo, and statistical significance of this comparison permits to demonstrate the superiority of DHEA to placebo in the treatment of VVA.

The coprimary endpoints for the analysis consist of the following:

(1) Pain at sexual activity (dyspareunia)

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(2) Percentage of parabasal cells
(3) Percentage of superficial cells
(4) Vaginal pH

No *P* value adjustment was used for the analysis of these four coprimary endpoints for the primary statistical comparisons of DHEA to placebo, since statistical significance of all four is required to reach a conclusion of superiority of the DHEA over placebo. The four endpoints were analyzed as continuous measurements.

The symptom score parameters of the other two symptoms of vaginal atrophy, namely vaginal dryness and vulvovaginal irritation/itching, were tested statistically as second-order and third-order endpoints. Therefore, the primary symptom score parameter of dyspareunia must be statistically significant before the test for vaginal dryness, which in turn must be significant before the test for significance of vulvovaginal irritation/itching. This procedure controls the type 1 error rate for these secondary and tertiary comparisons.

The analysis of primary and secondary endpoints was performed using analysis of covariance (ANCOVA), with the treatment group as the main factor and the baseline value as the covariate. Two separate ANCOVAs were used for the 6 and 12-week data. The *P* value for the baseline adjusted least square mean (LSM) difference between groups was presented (specifically, *P* values for 0.50% DHEA vs placebo).

Analysis populations

Efficacy

The intent-to-treat (ITT) population consists of all women who have received at least one dose of the study drug (based on diary card) with a baseline (day 1) evaluation meeting the study entry criteria. This analysis population was considered as the primary analysis population for efficacy parameters. Women in this population who had missing observations had the last value carried forward for efficacy analyses. In particular, women must have met all the following inclusion criteria both at screening and at day 1: 5% or less of superficial cells on vaginal smear, a vaginal pH above 5, and who have self-identified moderate to severe vaginal pain at sexual activity (dyspareunia) as their MBS; the observations at day 1 before treatment were used for the change from baseline analysis. If some data of efficacy parameters were missing at day 1, the screening value was used for baseline.

Safety

The safety population consists of all women who received an administration of any amount of test article (DHEA or placebo) (based on diary card and/or drug accountability), and who had any post-baseline safety information available. This analysis population was considered the primary analysis population for safety parameters.

Data processing

Electronic forms (including diaries and questionnaires) were filled out by clinical site personnel. For parameters described herein, verification of the data entry with the source was performed 100% by clinical research associates for all case report form pages completed by the clinical site personnel. The verified-source data were captured in the electronic data capture (EDC) system.

The web-based BioClinica Express EDC system provided and hosted by BioClinica was used for this study. The MI results were captured by EndoCeutics' personnel in the EDC system and were 100% verified by a second person, whereas the Clinical laboratory results provided by the University of Rochester Medical Center (URMC) central laboratory were uploaded in the EDC system using the software import functionalities. Data from participant source records were captured in the EDC system by designated clinical site personnel.

A database quality assurance audit was performed at the end of the study. To generate study data after locking of the database, a parallel programming process was used to minimize potential errors related to SAS programming and/or interpretation of the statistical analysis plan. In this process, two programmers independently executed all planned analyses of the statistical analysis plan, starting from the xml export of the clinical database and SAS datasets (for steroid data) approved in SOLABS (Montreal, Canada). Sets of tables and listings obtained from both programmers were matched; after all discrepancies had been resolved, the complete set of tables/listings was assigned a "QC passed" or "final" mark.

Disposition of participants

Please refer to Figure 1 for disposition of participants. For 23 women in the randomized placebo group and 49 women in the DHEA group, all the study entry criteria (including VVA criteria) were met at screening, but one or more required study entry criteria (including VVA criteria) was not present at baseline (day 1), thus automatically excluding these participants from the ITT population. These women were kept on study treatment (placebo or DHEA as randomized) to obtain additional exposure and safety data, but they were not part of the efficacy analysis due to their lack of compliance with the study entry criteria on day 1.

RESULTS

Demographics and baseline characteristics

A summary of the demographics and baseline characteristics of the safety population is presented in Table 1. The demographics and baseline characteristics of the women enrolled in the two treatment groups were similar, considering the 2:1 ratio of the DHEA-to-placebo distribution. Overall, the average age of women enrolled in the study was 59.5 years (median 59.0 y; range 40-80 y). Similar age distributions were observed in both treatment groups.

Moreover, women assigned to the two treatment groups had similar reproductive histories: an average of 13.9 years (from 13.4 y [placebo] to 14.1 y [0.50% DHEA]) had elapsed

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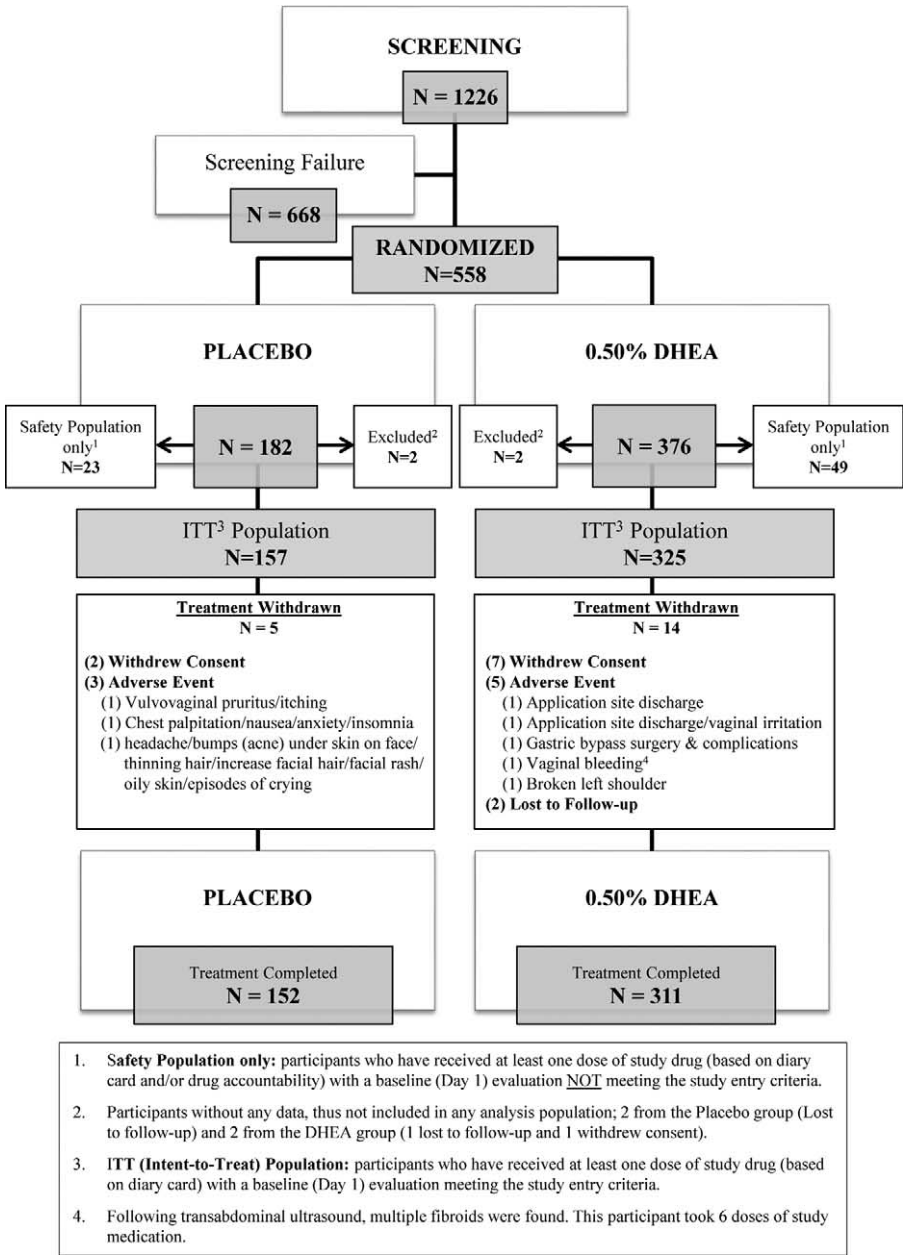

FIG. 1. Participant disposition. DHEA, dehydroepiandrosterone.

between their last menses and their participation in this study. Menstrual cycles ceased naturally in 64% of the population (from 63% [0.50% DHEA] to 67% [placebo]). The average age at which the women had their last menstrual period ranged from 48.6 years (0.50% DHEA) to 48.9 years (placebo) in women who had a natural menopause, and from 39.8 years (0.50% DHEA) to 40.6 years (placebo) in women who had surgical menopause.

As illustrated in Figure 2, the percentage (%) of parabasal cells in the placebo group decreases from $51.7 \pm 3.00\%$ at baseline to $39.7 \pm 2.68\%$ at 12 weeks for a decrease of $12.0 \pm 2.36\%$ (-23.2%) versus baseline. In contrast, the % of parabasal cells decreases from $54.3 \pm 2.14\%$ at baseline to $12.7 \pm 1.02\%$ at 12 weeks in the 0.50% DHEA group (-76.6% vs baseline). In the ANCOVA model, with the treatment group as the main factor and the baseline value as the covariate, the least square mean difference shows a value for the DHEA group of -27.7% at 12 weeks over placebo ($P < 0.0001$).

In the same groups, the % of superficial cells increases in the placebo group from $1.04 \pm 0.11\%$ at baseline to $2.78 \pm 0.27\%$ at 12 weeks ($+167\%$), whereas in the DHEA group, the % of superficial cells increases from $1.02 \pm 0.08\%$ at baseline to $11.2 \pm 0.56\%$ at 12 weeks ($+1000\%$). In the ANCOVA test, the 5.8-fold greater effect of DHEA over placebo is measured at a P value less than 0.0001 (Fig. 3).

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TABLE 1. *Overview of demographics and baseline characteristics: safety population*

Parameters	Placebo	0.50% DHEA	Total
Number of participants (%)	180 (100)	374 (100)	554 (100)
Race (number of participants, %)			
White	163 (91)	338 (90)	501 (90)
Black or African American	13 (7)	28 (7)	41 (7)
Asian	2 (1)	4 (1)	6 (1)
American Indian or Alaska Native	0	1 (0)	1 (0)
Native Hawaiian or Other Pacific Islander	0	1 (0)	1 (0)
White, Black or African American	2 (1)	2 (1)	4 (1)
Ethnicity (number of participants, %)			
Not Hispanic or Latino	166 (92)	330 (88)	496 (90)
Hispanic or Latino	14 (8)	44 (12)	58 (10)
Age, y			
Mean	59.6	59.5	59.5
Median	59.0	59.0	59.0
Range (min-max)	47-75	40-80	40-80
Anthropometric measurements (mean)			
Height, cm	161.7	161.0	161.2
Weight, kg	67.8	69.2	68.7
Body mass index, kg/m^2	26.0	26.7	26.4
Reproductive history			
Years since last menses (mean)	13.4	14.1	13.9
Cause of last menses, %			
Natural	67	63	64
Surgical	33	37	36
Age at last menses, y (mean)			
All women	46.2	45.4	45.6
Natural menopause	48.9	48.6	48.7
Surgical menopause	40.6	39.8	40.0
Hysterectomy, %	36	39	38
Ovariectomy, %			
Any ovariectomy	24	27	26
Bilateral ovariectomy	17	19	19
Previous hormone therapy, %	42	42	42
Baseline medical history, %			
Any medical history abnormality	100	100	100
Any physical examination abnormality	14	14	14
Any vital sign abnormality	4	6	6

DHEA, dehydroepiandrosterone.

When vaginal pH is examined (Fig. 4), a small decrease is observed in the placebo group from 6.32 ± 0.05 pH units at baseline to 6.05 ± 0.07 pH units at 12 weeks (−4.3%), whereas the pH decreases from 6.34 ± 0.04 at baseline to 5.39 ± 0.05 pH units at 12 weeks in the 0.50% DHEA group (−15.0%). When comparing to placebo in the ANCOVA test, there is a 3.4-fold greater effect of DHEA ($P < 0.0001$ vs placebo).

When the change of the moderate to severe pain at sexual activity (dyspareunia) is examined, the symptom evaluated by all women as being their MBS of VVA, it can be seen in Figure 5 that the severity score decreased from 2.56 ± 0.04 units at baseline in the placebo group to 1.50 ± 0.08 unit at 12 weeks (−1.06 unit). In the DHEA group, on the contrary, the severity score decreased from 2.54 ± 0.03 units at baseline to 1.13 ± 0.05 units at 12 weeks (−1.42 units). In the ANCOVA test, there is a 0.36-unit greater decrease of the intensity score in the DHEA group compared with the placebo group (+34%) ($P = 0.0002$ vs placebo).

It is of interest to see in Table 2 that of the 482 women who had moderate (n = 217, 45%) or severe (n = 265, 55%) pain at sexual activity (dyspareunia) as their most bothersome VVA symptom at baseline, 283 (59%) women had moderate and 122 (25%) had severe vaginal dryness.

Since the moderate to severe symptoms of VVA, at least for dyspareunia, vaginal dryness, and irritation/itching, respond similarly if the moderate to severe symptom is considered most bothersome or not by the women,[27] it is of interest to see in Figure 6 that the severity score of moderate to severe vaginal dryness decreases from a severity score of 2.30 ± 0.04 units at baseline to 1.13 ± 0.08 unit at 12 weeks (−1.17 units) in the placebo group, whereas, in the DHEA-treated group, the severity score decreased from 2.30 ± 0.03 units to 0.86 ± 0.05 units (−1.44 units). In the ANCOVA test, the difference due to treatment groups shows a 0.27 severity score unit superiority of DHEA compared with placebo (+23.1%; $P = 0.004$ vs placebo).

To further assess the effect of treatment on the vaginal mucosa, visual vaginal examination with a speculum was performed at baseline and after 6 and 12 weeks of randomized treatment. The index of vaginal secretion decreased from 2.63 ± 0.05 to 2.24 ± 0.06 units in the placebo group, compared with a decrease from 2.70 ± 0.04 to 1.97 ± 0.04 units at 12 weeks in the DHEA-treated group, for a difference of −0.34 (+87%) in favor of DHEA ($P < 0.0001$ over placebo) (Fig. 7).

Vaginal epithelial integrity, on the other hand, decreased in the placebo group from 2.43 ± 0.06 at baseline to 2.06 ± 0.06

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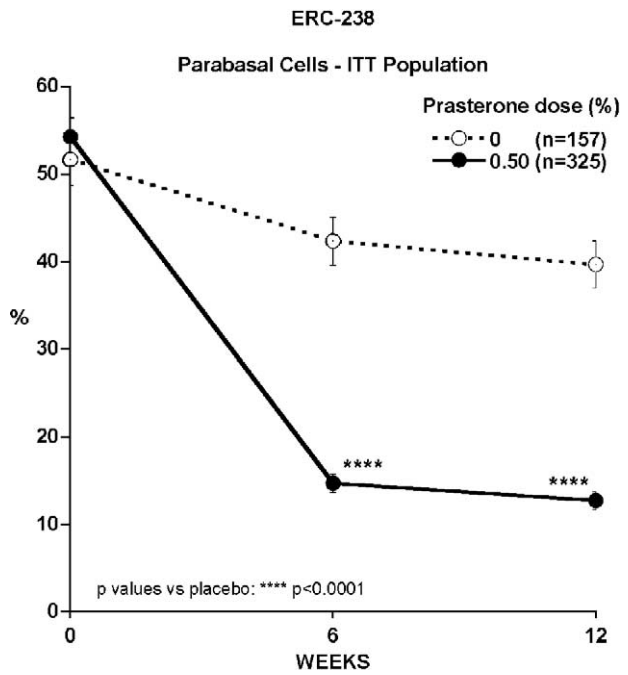

FIG. 2. Effect of Prasterone on parabasal cells (ITT). Effect of daily intravaginal application of 0.0% (n = 157) and 0.50% (n = 325) dehydroepiandrosterone (DHEA; Prasterone) for 6 and 12 weeks on the percentage (%) of vaginal parabasal cells in postmenopausal women. Data are expressed as means ± SEM; the *P* value for the Prasterone dose is compared with placebo at all time intervals. ITT, intention to treat.

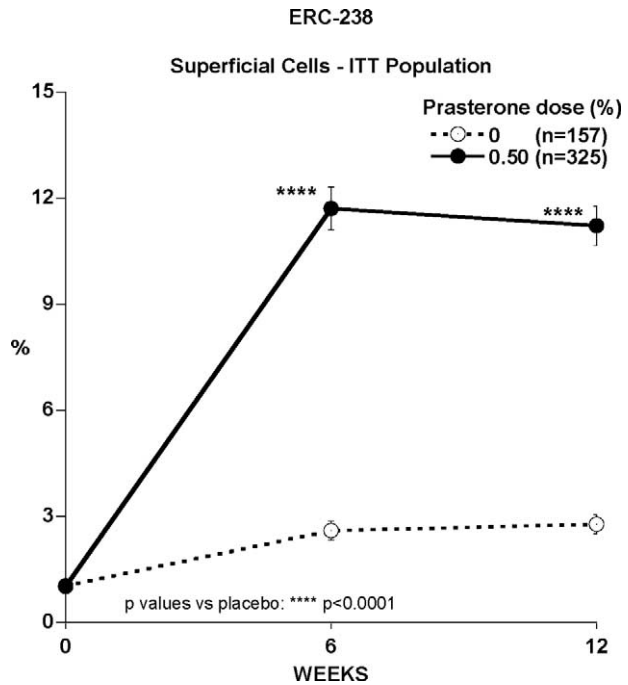

FIG. 3. Effect of Prasterone on superficial cells (ITT). Effect of daily intravaginal application of 0.0% (n = 157) and 0.50% (n = 325) dehydroepiandrosterone (DHEA; Prasterone) for 6 and 12 weeks on the percentage (%) of vaginal superficial cells in postmenopausal women. Data are expressed as means ± SEM; the *P* value for the Prasterone dose is comparison with placebo at all time intervals. ITT, intention to treat.

units at 12 weeks, whereas, in the DHEA-treated women, the decrease was from 2.45 ± 0.05 to 1.75 ± 0.04 units for a DHEA superiority of -0.32 ($+86\%$) severity score unit ($P < 0.0001$ vs placebo) (Fig. 8). Evaluation of the epithelial surface thickness has shown a decrease from 2.76 ± 0.05 units at baseline to 2.41 ± 0.05 units at 12 weeks, whereas the decrease was in the DHEA group from 2.83 ± 0.03 units at baseline to 2.09 ± 0.04 at 12 weeks, for a superiority of -0.38 units ($+106\%$) in favor of DHEA ($P > 0.0001$ vs placebo) (Fig. 9). Similarly, vaginal color decreased from 2.67 ± 0.05 at baseline to 2.34 ± 0.05 units at 12 weeks in the placebo group, whereas, in the DHEA-treated group, the score went from 2.75 ± 0.03 units at baseline to 2.03 ± 0.04 units at 12 weeks for a difference of -0.40 units ($+121\%$) in favor of DHEA compared with placebo ($P < 0.0001$) (Fig. 10).

Safety

Table 3 indicates the most frequently reported treatment-emergent AEs (TEAE) ($\geq$3% in one or both the treatment groups) presented by primary system organ class (SOC). Almost half (42.8% in the placebo group and 47.9% in the 0.50% DHEA group) experienced at least one TEAE of any nature.

The most frequently affected SOC (Table 3) was "infections and infestations," with 10.6% and 14.2% in the placebo and DHEA groups, respectively. No relevant difference in frequencies of the MedDRA terms was observed between the two groups. On the basis of the known metabolism and local action of DHEA, the application site discharge is the only AE which can reasonably be drug-related. This is due to melting of the vehicle in the vagina at body temperature with the possible addition of increased vaginal secretions due to treatment. This was reported in 5.6% of women in the placebo group and 6.1% in the DHEA group. Two women discontinued treatment due to this AE.

No clinically significant abnormality was observed in the hematology, blood chemistry, or urinalysis. Serum DHEA and its main metabolites (namely DHEA-S, Testo, DHT, 4-dione, 5-diol, E_1, E_2, E_1-S, ADT-G, and 3α-diol-17G) measured at baseline and week 12 (or at discontinuation visit, if applicable) by validated liquid chromatography tandem mass spectrometry[28,30-33] remained well within the normal postmenopausal values.

Compliance

In the ITT population, 152/157 (97%) of those receiving placebo have completed the study, whereas 96% (311/325) have completed the study in the DHEA group.

DISCUSSION

The present data confirm the beneficial effects of intravaginal DHEA on the coprimary parameters recommended by the US FDA[28] for evaluation of therapeutic efficacy on the symptoms and signs of VVA. The present study is in close agreement with the data obtained in our previous clinical trials,[5,24,26,34] and completes the requirements of the ICH

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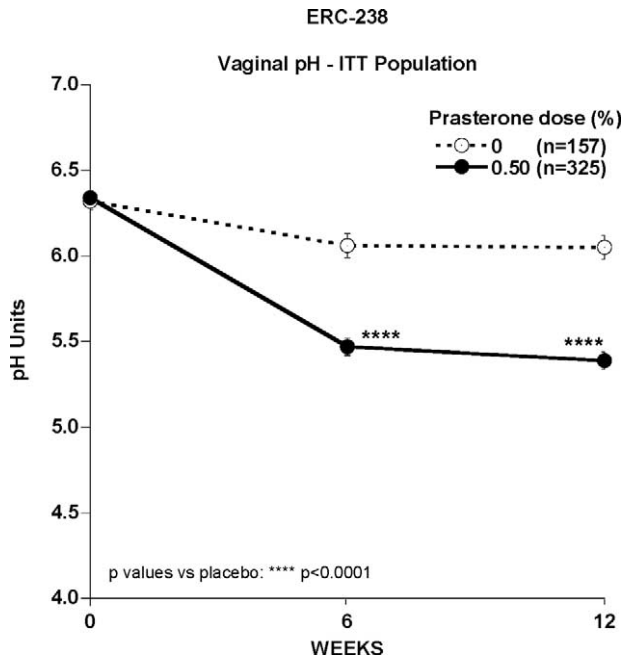

FIG. 4. Effect of Prasterone on vaginal pH (ITT). Effect of daily intravaginal application of 0.0% (n = 157) and 0.50% (n = 325) dehydroepiandrosterone (DHEA; Prasterone) for 6 and 12 weeks on vaginal pH in postmenopausal women. Data are expressed as means ± SEM; the *P* value for the Prasterone dose is comparison with placebo at all time intervals. ITT, intention to treat.

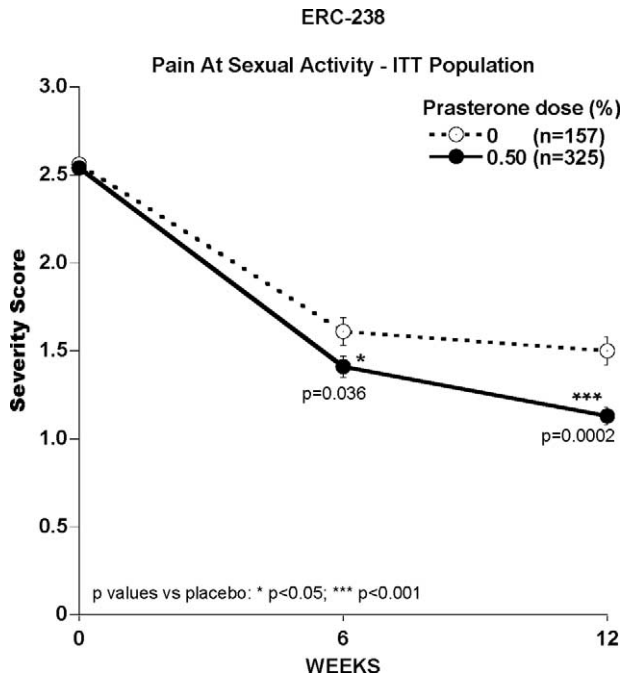

FIG. 5. Effect of Prasterone on pain at sexual activity (ITT). Effect of daily intravaginal application of 0.0% (n = 157) and 0.50% (n = 325) dehydroepiandrosterone (DHEA; Prasterone) for 6 and 12 weeks on pain at sexual activity in postmenopausal women. Data are expressed as means ± SEM; the *P* value for the Prasterone dose is comparison with placebo at all time intervals. ITT, intention to treat.

guideline for 1,500 participants exposed to a new chemical entity.

The arrest of estrogen secretion by the ovaries at menopause,[18] combined with the decreased secretion of the sex steroid precursor DHEA by the adrenals starting at the age of 30 years and continuing after menopause,[23,35] is accompanied by a long series of symptoms/signs associated with the aging process in the vagina, genitourinary system, vasomotor system, skin, skeleton, brain, cardiovascular system, and probably at various degrees in all other body systems.[36,37] VVA has been reported in more than 60% of women after the fourth year of menopause.[38-40]

The intracellular transformation of DHEA into estrogens is apparently responsible for the maturation of the parabasal cells which are transformed into intermediate and then into superficial cells.[5] DHEA has also been shown in preclinical studies to induce mucification of the epithelium or superficial layer of the vaginal mucosa while increasing the density of the collagen fibers in the second layer (lamina propria) and stimulating the muscular third layer.[41]

TABLE 2. *List of number of women reporting vaginal atrophy symptoms of various degrees of severity at baseline (ITT population)*

Treatment	Severity	Pain at sexual activity n (%)	Vaginal dryness n (%)	Irritation/itching n (%)
Placebo	None = 0	NA	5 (3)	55 (35)
	Mild = 1	NA	20 (13)	38 (24)
	Moderate = 2	69 (44)	92 (59)	51 (32)
	Severe = 3	88 (56)	40 (25)	13 (8)
	Total	157	157	157
0.50% DHEA	None = 0	NA	15 (5)	95 (29)
	Mild = 1	NA	37 (11)	104 (32)
	Moderate = 2	148 (46)	191 (59)	84 (26)
	Severe = 3	177 (54)	82 (25)	42 (13)
	Total	325	325	325
Overall (placebo + DHEA)	None = 0	NA	20 (4)	150 (31)
	Mild = 1	NA	57 (12)	142 (29)
	Moderate = 2	217 (45)	283 (59)	135 (28)
	Severe = 3	265 (55)	122 (25)	55 (11)
	Total	482	482	482

DHEA, dehydroepiandrosterone; ITT, intention to treat.

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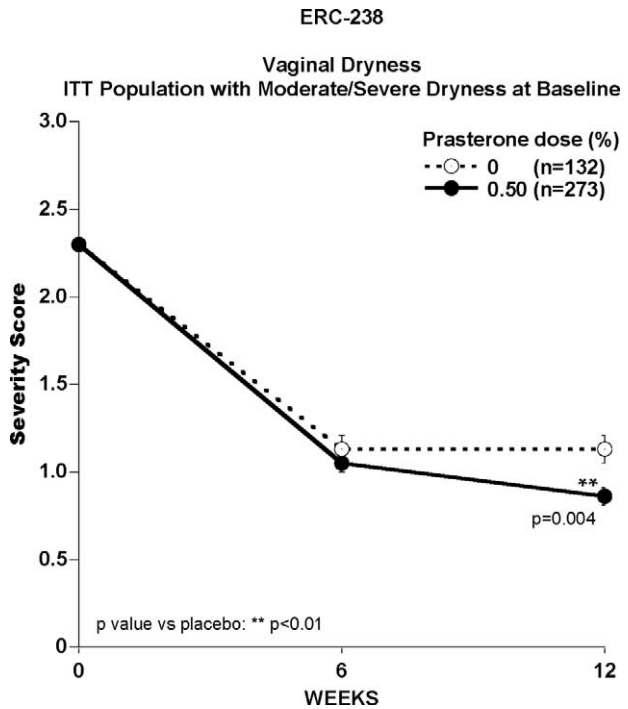

FIG. 6. Effect of Prasterone on vaginal dryness (ITT with moderate to severe dryness at baseline). Effect of daily intravaginal application of 0.0% (n = 132) and 0.50% (n = 273) dehydroepiandrosterone (DHEA; Prasterone) for 6 and 12 weeks on vaginal dryness in postmenopausal women. Data are expressed as means ± SEM; the *P* value for the Prasterone dose is comparison with placebo at all time intervals. ITT, intention to treat.

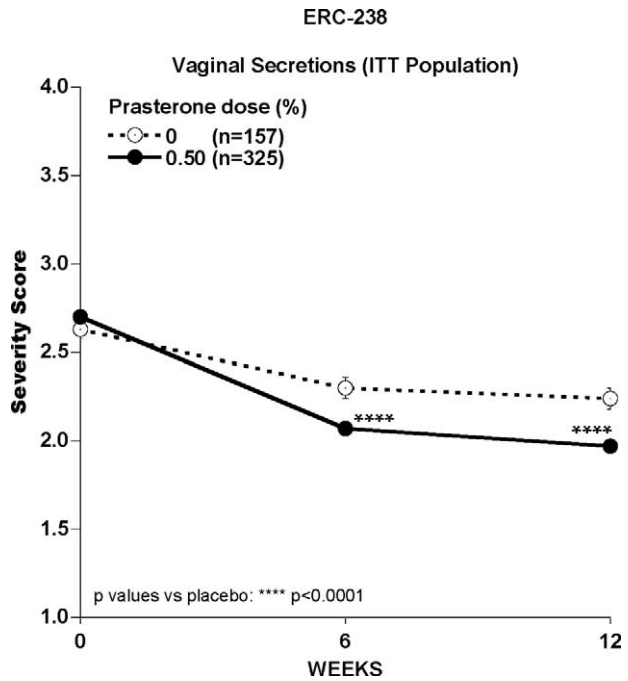

FIG. 7. Effect of Prasterone on vaginal secretions (ITT). Effect of daily intravaginal application of 0.0% (n = 157) and 0.50% (n = 325) dehydroepiandrosterone (DHEA; Prasterone) for 6 and 12 weeks on vaginal secretions in postmenopausal women. Data are expressed as means ± SEM; the *P* value for the Prasterone dose is comparison with placebo at all time intervals. ITT, intention to treat.

Systemic HT does not permit control of the urogenital atrophy in up to 45% of the women.[38] It is estimated that only about 7% of postmenopausal women suffering from VVA receive treatment (Kingsberg S, personal communication). The REVIVE (REal Women's VIews of Treatment Options for Menopausal Vaginal ChangEs)[42] has indicated that women have a lack of symptom relief with the over-the-counter products and have concerns about the safety of estrogen-based therapies. As far as nonhormonal moisturizers/lubricants are concerned, there is no evidence that they have an effect other than placebo[38] and certainly do not correct the problems responsible for the VVA symptoms.

The VVA symptomatology can affect daily activities, including walking, sitting, and exercising.[43] The urogenital symptoms not only represent a much more common problem than is recognized not only by the medical community but also by women themselves, who are often unwilling or too embarrassed to complain about these symptoms while believing that it is an unavoidable part of getting older. The effects on the individual can be serious when they markedly impact their sexuality, redefining their feminine role and ultimately negatively impacting their physical and mental well being.[40]

So far, VVA treatment with estrogens has essentially been limited to the most superficial or epithelial layer, which is largely responsible for pain at sexual activity or dyspareunia. Contraction of the introitus, and shortening and narrowing of the vaginal canal are not related to the superficial layer, but rather depend upon loss of collagen and muscular components in the lamina propria and muscularis layers, respectively.[41] In animal models, androgens have been shown to maintain structural integrity of vaginal tissues and to enhance vaginal hemodynamics independently from estrogens.[44]

The loss of rugosity and elasticity of the vagina related to menopause is due to the breakdown of collagen supporting the vaginal epithelium.[45,46] Our preclinical studies[41] have clearly demonstrated a stimulatory effect of the androgens derived from DHEA on collagen formation in the second layer of the vagina, namely the lamina propria. This effect is in agreement with the recently described stimulatory effect of DHEA on collagen formation in the human skin dermis, the equivalent of the lamina propria in the vagina.[47]

The decrease of glycogen in VVA is accompanied by increased pH, which modifies the vaginal microflora with the loss of protective microorganisms and the increased risk of overgrowth of pathogenic species.[40,48] Recurrent urinary tract infections are thus more common in postmenopausal women, with a higher incidence of infection complications including pyelonephritis.[49]

The International Menopause Society Writing Group, due to the progressive severity of VVA, has recommended to start treatment early upon onset of menopause before irreversible atrophic changes occured.[1,50] If VVA is left untreated, these is increased probability of the epithelium becoming friable, thus causing ulcerations, petechiae, and tears, with bleeding at

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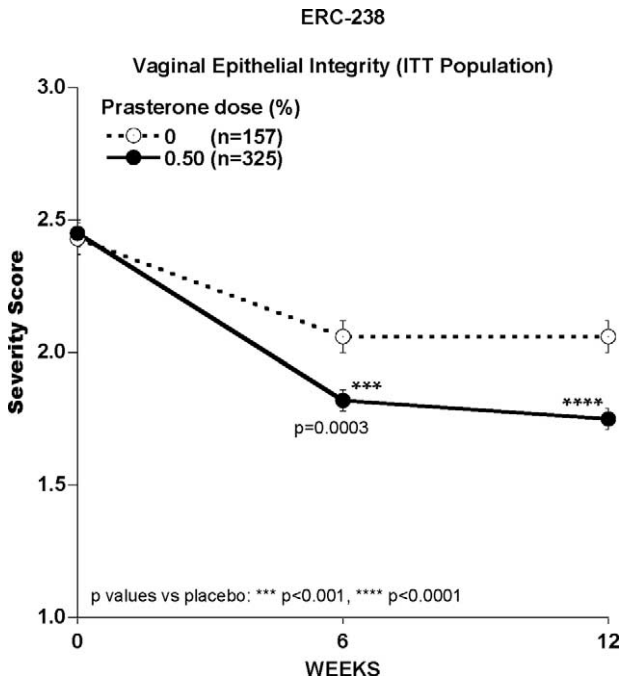

FIG. 8. Effect of Prasterone on vaginal epithelial integrity (ITT). Effect of daily intravaginal application of 0.0% (n = 157) and 0.50% (n = 325) dehydroepiandrosterone (DHEA; Prasterone) for 6 and 12 weeks on vaginal epithelial integrity in postmenopausal women. Data are expressed as means ± SEM; the *P* value for the Prasterone dose is comparison with placebo at all time intervals. ITT, intention to treat.

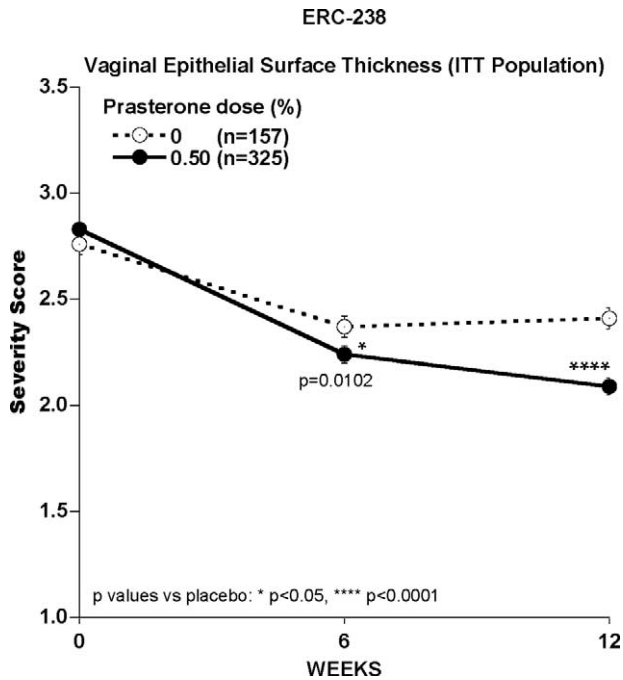

FIG. 9. Effect of Prasterone on vaginal epithelial surface thickness (ITT). Effect of daily intravaginal application of 0.0% (n = 157) and 0.50% (n = 325) dehydroepiandrosterone (DHEA; Prasterone) for 6 and 12 weeks on vaginal epithelial surface thickness in postmenopausal women. Data are expressed as means ± SEM; the *P* value for the Prasterone dose is comparison with placebo at all time intervals. ITT, intention to treat.

minimal trauma (intercourse, insertion of a speculum, etc).[51,52] Since vaginal atrophy is a chronic condition, which, unlike hot flushes, does not tend to diminish over time, long-term treatment is frequently required as symptoms may recur upon cessation of therapy.[53] In fact, VVA symptoms persist and increase in severity with time in the absence of treatment.[8]

Safety is thus particularly important for VVA therapy since treatment needs to be continued to maintain the benefits.[1] Nonestrogenic treatments for VVA are required to avoid the serious concerns regarding the potential stimulatory effects of estrogens on the endometrium and breast.[54-57] A sign of systemic estrogenic effect of ospemifene—a mixed estrogenic/antiestrogenic compound—is the 49% increase in serum sex hormone binding globulin (SHBG), an estrogen-sensitive parameter, from 54.48 ± 25.19 at baseline to 81.31 ± 3.90 nmol/L at 52 weeks.[58] No change was observed in the placebo group (59.33 ± 25.43 nmol/L at baseline vs 54.37 ± 26.44 nmol/L at 52 wks). At the same 60-mg daily dose of ospemifene, there was a 52% increase in endometrial thickness. Stimulatory effects with ospemifene have also been observed on the endometrium.

Major progress in the field of intracrinology has been made by the elucidation of the structure of most of the tissue-specific genes that encode the steroidogenic enzymes

TABLE 3. *Participants with treatment-emergent adverse events by primary system organ class, preferred term (MedDRA version 16.1), and subgroup*

Primary system organ class preferred term (MedDRA version 16.1)	Placebo (n = 180)	0.50% DHEA (n = 374)	Total (N = 554)
Participants with at least one adverse event	77 (42.8)	179 (47.9)	256 (46.2)
General disorders and administration site conditions	14 (7.8)	29 (7.8)	43 (7.8)
Application site discharge[a]	10 (5.6)	23 (6.1)	33 (6.0)
Infections and infestations	19 (10.6)	53 (14.2)	72 (13.0)
Urinary tract infection	5 (2.8)	17 (4.5)	22 (4.0)
Investigations	10 (5.6)	35 (9.4)	45 (8.1)
Weight decreased	6 (3.3)	11 (2.9)	17 (3.1)
Reproductive system and breast disorders	25 (13.9)	47 (12.6)	72 (13.0)
Hot flush	7 (3.9)	6 (1.6)	13 (2.3)

Safety population (preferred terms with incidence ≥3% in any treatment group). Data are presented as n (%). DHEA, dehydroepiandrosterone; MedDRA, Medical Dictionary for Regulatory Activities.
[a]Application site discharge is the only adverse event considered to be reasonably drug-related (by Sponsor's assessment).

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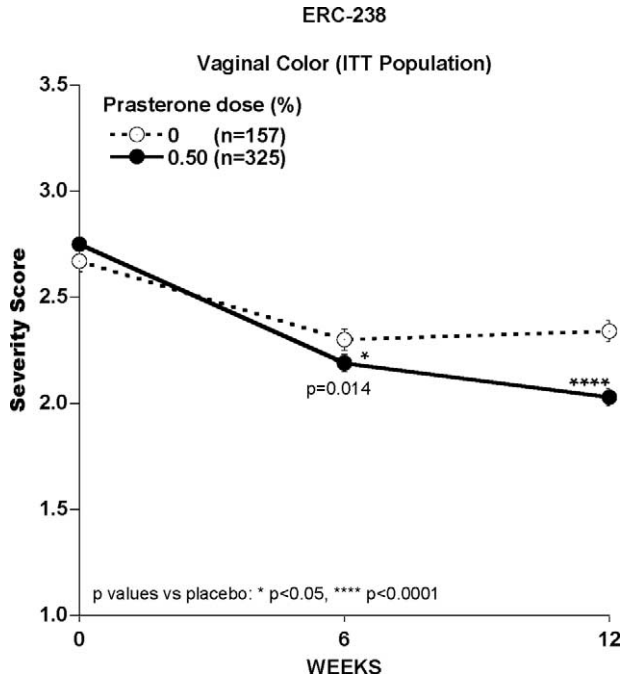

FIG. 10. Effect of Prasterone on vaginal color (ITT). Effect of daily intravaginal application of 0.0% (n = 157) and 0.50% (n = 325) dehydroepiandrosterone (DHEA; Prasterone) for 6 and 12 weeks on vaginal color in postmenopausal women. Data are expressed as means ± SEM; the *P* value for the Prasterone dose is comparison with placebo at all time intervals. ITT, intention to treat.

responsible for the transformation of DHEA into androgens and/or estrogens locally in peripheral tissues.[17,59-64] This cell-specific transformation of DHEA is, however, exerted at different levels, and ratios of estrogens and androgens in different cell types. Most importantly, to protect the uterus and other tissues after menopause, the active hormones made in peripheral tissues are inactivated at their site of synthesis before being released outside the cells as inactive metabolites, thus explaining why there is no or no biologically significant leakage of the active sex steroids into the circulation after their formation from DHEA.[17,35]

A most important characteristic of DHEA is the absence of possible stimulatory effect on the endometrium.[65] In fact, even if women continue to be exposed to relatively high circulating levels of DHEA after menopause, it is well recognized that endometrial atrophy is observed in all normal women as a characteristic of menopause.[20,21] This finding is in agreement with the absence of enzymes, especially aromatase, able to transform DHEA into estrogens in the human endometrium, despite its exposure to DHEA from the circulation.[66-68] Protection of the endometrium is the most obvious reason why evolution over 500 million years has succeeded in engineering an endocrine system unique to the human species and able to protect women from systemic exposure to estrogens after menopause.

Most importantly, no biologically significant leakage into the circulation of the active sex steroids made intracellularly takes place with intravaginal DHEA, thus explaining the highly beneficial effects observed in the vagina in the absence of significant changes in circulating estrogens or androgens.[5,25,26,69-73] In fact, as mentioned above, the active steroids are inactivated locally in the vagina before being released as inactive metabolites into the general circulation from which they are eliminated by the kidneys and the liver.

In agreement with this well recognized observation of nature of an absence of effect of DHEA on the endometrium, the endometrial atrophy observed in all women at the start of treatment remained unaffected at 12 months of percutaneous DHEA administration, even if serum DHEA was increased by 5.5-fold.[74] Moreover, no change in endometrial thickness was found after 6 months of daily oral dosing with 50 mg DHEA in postmenopausal women.[75]

In addition to the observation that serum DHEA remains within normal postmenopausal values after intravaginal administration of 0.50% DHEA,[18,69,70,72] the absence of aromatase in the human endometrium[67] explains the absence of estrogen formation from DHEA in the endometrium of normal women and the endometrial atrophy observed in all normal postmenopausal women. To further support the above explanation, we have obtained endometrial histology in 422 women who had endometrial biopsy at baseline and at exit of study after intravaginal administration of 0.50% DHEA for 52 weeks.[65] Other studies with intravaginal DHEA doses of 3.25 mg (n = 126), 6.5 mg (n = 129), and 13 mg (n = 30) were performed for 12 weeks in women who similarly had baseline and end-of-study biopsies.[65] Endometrial tissue for histology was available in a total of 668 women at both baseline and end-of-study exposure. Endometrial atrophy or inactive endometrium was found in all women treated with intravaginal DHEA.[65]

All estrogen-based vaginal formulations, on the contrary, increase serum estrogens, even at low dose.[76] In fact, the vagina seems to be able to transport E_2 towards the circulation with little or no metabolism,[77,78] thus resulting in higher serum E_2 concentrations.[79]

It seems of interest to mention that despite the complete arrest of estrogen secretion by the ovaries at the time of menopause, while serum E_2 levels are kept at biologically inactive concentrations in all women, not all postmenopausal women suffer from menopausal symptoms despite a common absence of biologically active circulating estrogens. Consequently, the hormonal difference between postmenopausal women suffering from vaginal atrophy symptoms (approximately 75% of women) and those without symptoms (approximately 25%) is not related to the estrogens, which remain at biologically inactive serum levels in all women. In fact, the possible hormonal difference between the two groups of women may be the difference in the availability of DHEA—the exclusive source of sex steroids in women after menopause.[18]

CONCLUSIONS

The current study describes the efficacy of DHEA for the management of VVA. Intravaginal DHEA improved vaginal pH, superficial and basal epithelial cell counts, and relieved

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dyspareunia, the MBS found in the majority of postmenopausal women. Moderate to severe vaginal dryness accompanied dyspareunia in 84% of women and was similarly improved by DHEA (Prasterone) treatment. Physical assessment of the vagina with a speculum demonstrated improvement in vaginal secretions, epithelial integrity, epithelium thickness, and also vaginal color in the DHEA arm, compared with placebo. Overall, DHEA acting by strictly local mechanisms in the vagina shows clinical and highly statistically significant improvement of all recognized subjective and objective elements of VVA and dyspareunia compared with placebo.

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REFERENCES

1. Sturdee DW, Panay N. Recommendations for the management of postmenopausal vaginal atrophy. *Climacteric* 2010;13:509-522.
2. Portman DJ, Gass ML; Vulvovaginal Atrophy Terminology Consensus Conference Panel. 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;21:1063-1068.
3. Bachmann GA, Nevadunsky NS. Diagnosis and treatment of atrophic vaginitis. *Am Fam Physician* 2000;61:3090-3096.
4. Castelo-Branco C, Cancelo MJ, Villero J, Nohales F, Julia MD. Management of post-menopausal vaginal atrophy and atrophic vaginitis. *Maturitas* 2005;52(suppl 1):S46-S52.
5. Labrie F, Archer DF, Bouchard C, et al. Intravaginal dehydroepiandrosterone (Prasterone) a physiological and highly efficient treatment of vaginal atrophy. *Menopause* 2009;16:907-922.
6. Goldstein I. Recognizing and treating urogenital atrophy in postmenopausal women. *J Womens Health (Larchmt)* 2010;19:425-432.
7. Santoro N, Komi J. Prevalence and impact of vaginal symptoms among postmenopausal women. *J Sex Med* 2009;6:2133-2142.
8. Calleja-Agius J, Brincat MP. Urogenital atrophy. *Climacteric* 2009; 12: 279-285.
9. Nappi RE, Polatti F. The use of estrogen therapy in women's sexual functioning (CME). *J Sex Med* 2009;6:603-616; quiz 18-19.
10. Bachmann GA, Komi JO; Ospemifene Study Group. Ospemifene effectively treats vulvovaginal atrophy in postmenopausal women: results from a pivotal phase 3 study. *Menopause* 2010;17:480-486.
11. Pinkerton JV. Vaginal impact of menopause-related estrogen deficiency. *OBG Management* 2010;22:S2-S7.
12. Johnston SL, Farrell SA, Bouchard C, et al. The detection and management of vaginal atrophy. *J Obstet Gynaecol Can* 2004;26:503-515.
13. Suckling J, Lethaby A, Kennedy R. Local oestrogen for vaginal atrophy in postmenopausal women [Review]. *Cochrane Database System Rev* 2006;18:CD001500.
14. Women's Health Initiative. Risks and benefits of estrogen plus progestin in healthy postmenopausal women. *JAMA* 2002;288:321-333.
15. Tan O, Bradshaw K, Carr BR. Management of vulvovaginal atrophy-related sexual dysfunction in postmenopausal women: an up-to-date review. *Menopause* 2012;19:109-117.
16. The North American Menopause Society. The 2012 hormone therapy position statement of: The North American Menopause Society. *Menopause* 2012;19:257-271.
17. Labrie F. All sex steroids are made intracellularly in peripheral tissues by the mechanism of intracrinology after menopause. *J Steroid Biochem Mol Biol* 2015;145:133-138.
18. 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;18:30-43.
19. Labrie F. Intracrinology. *Mol Cell Endocrinol* 1991;78:C113-C118.
20. Labrie F, Bélanger A, Bélanger P, et al. Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women. *J Steroid Biochem Mol Biol* 2006;99:182-188.
21. Labrie F, Luu-The V, Bélanger A, Lin SX, Simard J, Labrie C. Is DHEA a hormone? Starling review. *J Endocrinol* 2005;187:169-196.
22. Labrie F, Luu-The V, Labrie C, et al. Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. *Endocr Rev* 2003;24:152-182.
23. Labrie F, Bélanger A, Cusan L, Gomez JL, Candas B. Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. *J Clin Endocrinol Metab* 1997;82:2396-2402.
24. Labrie F, Archer DF, Bouchard C, et al. Intravaginal dehydroepiandrosterone (prasterone), a highly efficient treatment of dyspareunia. *Climacteric* 2011;14:282-288.
25. Bouchard C, Labrie F, Archer DF, et al; VVA Prasterone Group. Decreased efficacy of twice-weekly intravaginal dehydroepiandrosterone on vulvovaginal atrophy. *Climacteric* 2015;18:590-607.
26. Archer DF, Labrie F, Bouchard C, et al; VVA Prasterone Group. Treatment of pain at sexual activity (dyspareunia) with intravaginal dehydroepaindrosterone (prasterone). *Menopause* 2015;22:950-963.
27. Labrie F, Archer DF, Bouchard C, et al; VVA Prasterone Group. Prasterone has parallel beneficial effects on the main symptoms of vulvovaginal atrophy: 52-week open-label study. *Maturitas* 2015;81: 46-56.
28. US Food and Drug Administration. Center for Drug Evaluation and Research (CDER). *Guidance for Industry: Estrogen and Estrogen/Progestin Drug Products to Treat Vasomotor Symptoms and Vulvar and Vaginal Atrophy Symptoms: Recommendations for Clinical Evaluation.* Washington, DC: US Government Printing Office; 2003. Available at: http://www.fda.gov/downloads/Drugs/DrugSafety/InformationbyDrug Class/UCM135338.pdf.
29. Davila GW, Singh A, Karapanagiotou I, et al. Are women with urogenital atrophy symptomatic? *Am J Obstet Gynecol* 2003;188:382-388.
30. Ke Y, Bertin J, Gonthier R, Simard JN, Labrie F. A sensitive, simple and robust LC-MS/MS method for the simultaneous quantification of seven androgen- and estrogen-related steroids in postmenopausal serum. *J Steroid Biochem Mol Biol* 2014;144:523-534.
31. Dury AY, Ke Y, Gonthier R, Isabelle M, Simard JN, Labrie F. Validated LC-MS/MS simultaneous assay of five sex steroid/neurosteroid-related sulfates in human serum. *J Ster Biochem Mol Biol* 2015;14:1-10.
32. Ke Y, Gonthier R, Isabelle M, et al. A rapid and sensitive UPLC-MS/MS method for the simultaneous quantification of serum androsterone glucuronide, etiocholanolone glucuronide, and androstan-3alpha, 17beta diol 17-glucuronide in postmenopausal women. *JSBMB* 2015;149:146-152.
33. Labrie F, Ke Y, Gonthier R, Belanger A. Why both LC-MS/MS and FDA-compliant validation are essential for accurate estrogen assays? *JSBMB* 2015;149C:89-91.
34. Labrie F, Archer DF, Bouchard C, et al. Lack of influence of dyspareunia on the beneficial effect of intravaginal prasterone

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(dehydroepiandrosterone, DHEA) on sexual dysfunction in postmenopausal women. *J Sex Med* 2014;11:1766-1785.
35. Labrie F, Labrie C. DHEA and intracrinology at menopause, a positive choice for evolution of the human species. *Climacteric* 2013;16:205-213.
36. The Practice Committee of American Society of Reproductive Medecine. Estrogen and progestogen therapy in postmenopausal women. *Fertil Steril* 2004;82(suppl 1):S70-S80.
37. Chakraborty TR, Gore AC. Aging-related changes in ovarian hormones, their receptors, and neuroendocrine function. *Exp Biol Med (Maywood)* 2004;229:977-987.
38. Al-Baghdadi O, Ewies AA. Topical estrogen therapy in the management of postmenopausal vaginal atrophy: an up-to-date overview. *Climacteric* 2009;12:91-105.
39. Lobo RA, Belisle S, Creasman WT, et al. Should symptomatic menopausal women be offered hormone therapy? *MedGenMed* 2006;8:40.
40. Santos I, Clissold S. Urogenital disorders associated with oestrogen deficiency: the role of promestriene as topical oestrogen therapy. *Gynecol Endocrinol* 2010;26:644-651.
41. Berger L, El-Alfy M, Martel C, Labrie F. Effects of dehydroepiandrosterone, Premarin and Acolbifene on histomorphology and sex steroid receptors in the rat vagina. *J Steroid Biochem Mol Biol* 2005;96:201-215.
42. Kingsberg SA, 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;10:1790-1799.
43. Krychman M. Impact of vaginal atrophy on quality of life and sexuality. *OBG Management* 2010;22:S14-S19.
44. Traish AM, Kim SW, Stankovic M, Goldstein I, Kim NN. Testosterone increases blood flow and expression of androgen and estrogen receptors in the rat vagina. *J Sex Med* 2007;4:609-619.
45. Tinelli A, Malvasi A, Rahimi S, et al. Age-related pelvic floor modifications and prolapse risk factors in postmenopausal women. *Menopause* 2010;17:204-212.
46. Moalli PA, Talarico LC, Sung VW, et al. Impact of menopause on collagen subtypes in the arcus tendineous fasciae pelvis. *Am J Obstet Gynecol* 2004;190:620-627.
47. Calvo E, Luu-The V, Morissette J, et al. Pangenomic changes induced by DHEA in the skin of postmenopausal women. *J Steroid Biochem Mol Biol* 2008;112:186-193.
48. Graziottin A, Rovei V. Sexual pain disorders. In: Tepper MS, Owens AF, eds. *Sexual Health*. Westport, CT: Greenwood Publishing Group; 2006: 287-312.
49. Barlow DH, Cardozo LD, Francis RM, et al. Urogenital ageing and its effect on sexual health in older British women. *Br J Obstet Gynaecol* 1997;104:87-91.
50. Archer DF. Efficacy and tolerability of local estrogen therapy for urogenital atrophy. *Menopause* 2010;17:194-203.
51. Nilsson K, Risberg B, Heimer G. The vaginal epithelium in the postmenopause: cytology, histology and pH as methods of assessment. *Maturitas* 1995;21:51-56.
52. Simon J, Nachtigall L, Gut R, Lang E, Archer DF, Utian W. Effective treatment of vaginal atrophy with an ultra-low-dose estradiol vaginal tablet. *Obstet Gynecol* 2008;112:1053-1060.
53. Skouby SO, Al-Azzawi F, Barlow D, et al. Climacteric medicine: European Menopause and Andropause Society (EMAS) 2004/2005 position statements on peri- and postmenopausal hormone replacement therapy. *Maturitas* 2005;51:8-14.
54. Hammond CB, Jelovsek FR, Lee KL, Creasman WT, Parker RT. Effects of long-term estrogen replacement therapy. II. Neoplasia. *Am J Obstet Gynecol* 1979;133:537-547.
55. Lethaby A, Suckling J, Barlow D, Farquhar CM, Jepson RG, Roberts H. Hormone replacement therapy in postmenopausal women: endometrial hyperplasia and irregular bleeding. *Cochrane Database Syst Rev* 2004; CD000402.
56. Smith DC, Prentice R, Thompson DJ, Herrmann WL. Association of exogenous estrogen and endometrial carcinoma. *N Engl J Med* 1975; 293:1164-1167.
57. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* 2002;288:321-333.
58. Simon JA, Lin VH, Radovich C, Bachmann GA. One-year long-term safety extension study of ospemifene for the treatment of vulvar and vaginal atrophy in postmenopausal women with a uterus. *Menopause* 2013;20:418-427.
59. Labrie F, Simard J, Luu-The V, Bélanger A, Pelletier G. Structure, function and tissue-specific gene expression of 3β-hydroxysteroid dehydrogenase/5-ene-4-ene isomerase enzymes in classical and peripheral intracrine steroidogenic tissues. *J Steroid Biochem Mol Biol* 1992;43:805-826.
60. Labrie F, Sugimoto Y, Luu-The V, et al. Structure of human type II 5 alpha-reductase gene. *Endocrinology* 1992;131:1571-1573.
61. Labrie Y, Durocher F, Lachance Y, et al. The human type II 17β-hydroxysteroid dehydrogenase gene encodes two alternatively-spliced messenger RNA species. *DNA Cell Biol* 1995;14:849-861.
62. Luu-The V, Zhang Y, Poirier D, Labrie F. Characteristics of human types 1, 2 and 3 17β-hydroxysteroid dehydrogenase activities: oxidation-reduction and inhibition. *J Steroid Biochem Mol Biol* 1995;55:581-587.
63. Labrie F, Simard J, Luu-The V, et al. The 3β-hydroxysteroid dehydrogenase/isomerase gene family: lessons from type II 3β-HSD congenital deficiency. In: *Signal Transduction in Testicular Cells, Ernst Schering Research Foundation Workshop, Supplement 2*. Berlin: Springer-Verlag; 1996:185-218.
64. Labrie F, Luu-The V, Lin SX, et al. The key role of 17β-HSDs in sex steroid biology. *Steroids* 1997;62:148-158.
65. Portman DJ, Labrie F, Archer DF, et al; VVA Prasterone Group. Lack of effect of intravaginal dehydroepiandrosterone (DHEA, prasterone) on the endometrium in postmenopausal women. *Menopause* 2015;22:1289-1295.
66. Baxendale PM, Reed MJ, James VH. Inability of human endometrium or myometrium to aromatize androstenedione. *J Steroid Biochem* 1981;14:305-306.
67. Bulun SE, Lin Z, Imir G, et al. Regulation of aromatase expression in estrogen-responsive breast and uterine disease: from bench to treatment. *Pharmacol Rev* 2005;57:359-383.
68. Toda K, Simpson ER, Mendelson CR, Shizuta Y, Kilgore MW. Expression of the gene encoding aromatase cytochrome P450 (CYP19) in fetal tissues. *Mol Endocrinol* 1994;8:210-217.
69. Labrie F, Cusan L, Gomez JL, et al. Effect of intravaginal DHEA on serum DHEA and eleven of its metabolites in postmenopausal women. *J Steroid Biochem Mol Biol* 2008;111:178-194.
70. Labrie F, Cusan L, Gomez JL, et al. Corrigendum to: Effect of intravaginal DHEA on serum DHEA and eleven of its metabolites in postmenopausal women. *J Steroid Biochem Mol Biol* 2008;112:169.
71. Labrie F, Archer DF, Bouchard C, et al. Effect on intravaginal dehydroepiandrosterone (prasterone) on libido and sexual dysfunction in postmenopausal women. *Menopause* 2009;16:923-931.
72. Labrie F, Archer DF, Bouchard C, et al. Serum steroid levels during 12-week intravaginal dehydroepiandrosterone administration. *Menopause* 2009;16:897-906.
73. 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.
74. Labrie F, Diamond P, Cusan L, Gomez JL, Bélanger A, Candas B. Effect of 12-month dehydroepiandrosterone replacement therapy on bone, vagina, and endometrium in postmenopausal women. *J Clin Endocrinol Metab* 1997;82:3498-3505.
75. Stomati M, Monteleone P, Casarosa E, et al. Six-month oral dehydroepiandrosterone supplementation in early and late postmenopause. *Gynecol Endocrinol* 2000;14:342-363.
76. Eugster-Hausmann M, Waitzinger J, Lehnick D. Minimized estradiol absorption with ultra-low-dose 10 microg 17beta-estradiol vaginal tablets. *Climacteric* 2010;13:219-227.
77. Schiff I, Tulchinsky D, Ryan KJ. Vaginal absorption of estrone and 17beta-estradiol. *Fertil Steril* 1977;28:1063-1066.
78. Martin PL, Greaney MO, Burnier AM, Brooks PM, Yen SS, Quigley ME. Estradiol estrone and gonadotropin levels after use of vaginal estradiol. *Obstet Gynecol* 1984;63:441-444.
79. Tourgeman DE, Gentzchein E, Stanczyk FZ, Paulson RJ. Serum and tissue hormone levels of vaginally and orally administered estradiol. *Am J Obstet Gynecol* 1999;180(6 pt 1):1480-1483.

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