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Pilot Study: Absorption and Efficacy of Multiple Hormones Delivered in a Single Cream Applied to the Mucous Membranes of the Labia and Vagina

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Key Words
Gonadal steroid hormones · Absorption · Vaginal creams · Estradiol · Estriol · Testosterone · Progesterone

Abstract
Background/Aims: There is a lack of evidence in the literature supporting vaginal application of a combination hormone-containing cream for local and systemic symptom relief. This pilot study examined the extent of absorption of a single cream containing estriol, estradiol, progesterone, DHEA, and testosterone. ***Methods:*** A combination cream was administered to 12 postmenopausal women in two differing doses over two independent time periods. Following 28 days (arm 1) and an additional 14 days (arm 2), measurement of hormones in saliva and blood and measurements of symptom relief, patient tolerability, and health-related quality of life (HRQoL) were obtained. ***Results:*** The dosage and time of evaluation for study arm 1 was not ideal for providing documented increases in hormone levels. HRQoL measurements supported measured improvement in this arm. The second arm did document absorption of the various hormones when given vaginally. ***Conclusion:*** This study is the first documenting systemic absorption of multiple hormones by both saliva and blood as well as improvement of HRQoL. This therapy was generally well-tolerated with only 2 patients experiencing minor irritation, not necessitating discontinuation. Additional studies in larger numbers of patients will provide better knowledge for clinicians wanting to provide similar therapy at the lowest effective dose.

Introduction

Vaginal delivery of hormones, including estriol, estradiol, estrone, progesterone and testosterone, has been well established in the literature. It has been shown in multiple studies that hormones administered vaginally are absorbed systemically, bypass hepatic metabolism and are biologically active [1–12]. It has also been shown that hormones applied to the mucous membranes are more readily absorbed than hormones applied to the skin [13–16]. Testosterone, applied to the mucous membranes of the labia, has been shown to be absorbed and have systemic effects [15, 29]. Hormones applied vaginally achieve higher plasma levels than if taken orally and the vaginal route appears to be more adequate than the oral one for hormone replacement therapy [16].

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Table 1. Patient characteristics (n = 12)

	Average	Range
Age, years	58.3	48–70
Time since last menstrual period, years	12.1	1–26

Symptomatic relief of genital urinary symptoms as well as systemic climacteric symptoms with vaginally administered hormones has been described and is dose dependent [11, 17–19].

The long-term safety of vaginal estrogen therapy has been established in the literature. Vaginal estrogens do not increase the risk of breast cancer [30–37, 57]. Vaginal estriol use in breast cancer patients does not increase the risk of recurrence (RR 0.57) or death [33]. Vaginal estriol does not increase the risk of endometrial hyperplasia or uterine cancer [30, 38–40]. Unlike oral estriol, vaginal estriol has been shown to increase bone density [38, 41]. There is no accumulation of hormones or metabolites with vaginal estrogen or progesterone therapy [10, 16, 39, 42–44].

Vaginal progesterone has preferential distribution to the uterus and protects the uterine lining [20–22]. Unlike the oral synthetic progestins, vaginal progesterone does not negate the beneficial effects of estrogen on the heart and enhances the effect of estrogen on exercise-induced myocardial ischemia [12]. Progesterone has not been associated with an increase in breast cancer, unlike the synthetic progestins [34, 35]. Progesterone, applied vaginally, has a high local effect on the endometrium without systemic side effects (bloating, sedation, persistent hot flushes) due to high plasma progesterone levels and metabolites [21, 22, 43–46]. Vaginal administration of progesterone is preferred in patients with cardiovascular disease, liver disease or hepatic overload [47].

The safety of nonoral, nonsynthetic testosterone has been established. Testosterone has been used to treat breast pain, breast cancer, endometriosis, fibroids and other uterine pathology. Testosterone's action is antiproliferative and pro-apototic and is mediated through the androgen receptor [48–50]. Testosterone has been shown to prevent breast proliferation, decrease estrogen receptor alpha and prevent the stimulation of breast tissue from estrogen/progestin therapy [51, 52, 55]. Testosterone has also been shown to lower the risk of breast cancer when given with estrogen/progestin therapy [54] and has been used to treat breast cancer patients. It is highly unlikely that vaginal testosterone would have any long-term negative effect on breast tissue unlike *oral* synthetic methyl-testosterone [56, 57]. The safety of vaginal dehydroepiandrosterone (DHEA) has been described [9].

This is the first published study to use a combination of the hormones estriol, estradiol, progesterone, testosterone, and DHEA delivered in a single cream to treat both local and systemic symptoms. It has previously been demonstrated that the addition of progesterone to vaginal estradiol cream does not affect the absorption of the estradiol [8]. In 1981, after documenting the superiority of intravaginal application of progesterone, it was hypothesized that 'full hormone replacement could be accomplished in the deficient states by cyclic vaginal application of both steroids', i.e. estrogen and progesterone [28].

Goals for this pilot study were to examine the extent of absorption of a combination cream containing estriol, estradiol, progesterone, DHEA, and testosterone. In addition to objective measurements of steroid hormones in saliva and blood, qualitative measurements of symptom relief, patient tolerability, and health-related quality of life (HRQoL) were obtained to determine efficacy of the combination therapy.

Materials and Methods

Patients

Twelve postmenopausal females aged 49–74 years were recruited to participate in the study. Baseline salivary hormone levels [estrone, estradiol, estriol, progesterone, testosterone, dehydroepiandrosterone sulfate (DHEA-S), and cortisol] were obtained. Baseline serum levels (estrone, estradiol, free estradiol, progesterone, testosterone, free testosterone and DHEA-S) were obtained. Patients were not taking additional prescription or nonprescription hormonal or natural products which might interfere with measurement of levels during the timeframe of the present study. Patients enrolled and completed the study on a volunteer basis; a formal IRB consent was not requested or obtained. Patient characteristics are shown in table 1.

Preparation and Application of Hormones

In the first arm of the study a compounded hormone cream was prepared by a compounding pharmacy using Versabase™, micronized progesterone USP (Professional Compounding Centers of America, Houston, Tex., USA), micronized estriol USP, micronized estradiol USP, micronized DHEA, micronized testosterone propionate USP (Hawkins Pharmaceutical Group, Minneapolis, Minn., USA) in a final concentration of: estriol 2 mg, estradiol 0.5 mg, progesterone 100 mg, DHEA 5 mg, and testosterone 1 mg per ml of cream. This was dispensed in prefilled 1-ml syringes.

Patients were instructed to apply 0.25 ml of cream to the mucous membranes of the labia and vagina each morning using their index finger, supplying a daily dose of: estriol 0.5 mg, estradiol

0.125 mg, progesterone 25 mg, DHEA 1.25 mg, and testosterone 0.25 mg.

On day 28, serum was collected at 6 h and saliva was collected at 24 h following the application of hormone. Saliva was collected at 24 h, which is typically the collection time recommended for topical (skin) application (24–48 h following last dose).

After initial evaluation of hormone levels, it was felt that the delayed collection of saliva at 24 h following the application of hormones might have underestimated the absorption of hormones. It was also felt that the dose of some of the hormones might have been inadequate. Therefore, in the second arm of the pilot study, seven of the post menopausal study participants with low baseline levels of DHEA-S and testosterone were selected to use a second compounded cream with higher concentrations of hormones. A combined cream was prepared similarly in Versabase in the following final concentrations: estriol 1 mg, estradiol 1 mg, progesterone 100 mg, DHEA 100 mg, and testosterone 1 mg per ml of cream.

Patients were instructed to apply 0.5 ml of cream to the mucous membranes of the labia and vagina each morning using their index finger, supplying a daily dose of: estriol 0.5 mg, estradiol 0.5 mg, progesterone 50 mg, DHEA 50 mg and testosterone 0.5 mg.

After 14 days of therapy, saliva was collected at 6 h (vs. 24 h in the first arm) following the last dose and hormone levels were again measured. A summary of the differing study arms is shown in table 2.

Saliva Collection

Saliva (minimal 5 ml) was collected in polypropylene tubes in the morning before breakfast (7–9 a.m.) at baseline and in the first arm of the study and at 6 h after application of hormone cream in the second arm of the study. Food and beverages (except water) were avoided 2 h prior to saliva collection. Saliva samples were shipped within 24 h for laboratory analysis.

Saliva Processing

Saliva was processed by adding 50 μl of 0.14 mg/ml dithiothreitol (DTT) per ml of saliva to break up mucins that interfere with saliva extraction. Steroids were then extracted from 1.5 ml of saliva by C-18 column chromatography. Samples were gently pulled through the columns by vacuum. Control and calibrator samples were prepared from Biorad Lyphocheck diluted 1/100 in phosphate-buffered saline (PBS) buffer containing DTT. Following absorption to C-18 columns, the samples, controls, and calibrators were washed with PBS buffer and the steroids eluted with alcohol solvent. The eluted solvent containing the steroids was dried under nitrogen and then reconstituted in PBS buffer containing 0.1% T904 detergent and 0.05% Proclin antimicrobial (assay buffer).

Steroid Testing

Steroids in the extracted/reconstituted saliva were quantified by enzyme immunoassay (EIA) with commercial kits from DRG, Germany. Standards were prepared in assay buffer from a concentrated stock of each hormone with serial dilution. Inter- and intra-assay coefficients of variation for low and high controls for all steroids tested were 10% or less. ZRT Laboratory has performed weekly approximately 1,500 samples of each of the steroids (estradiol, progesterone, testosterone, DHEA, and cortisol). Ranges were based on gender, age, menstrual status (e.g. follicular vs. luteal phase of the menstrual cycle), and hormone therapy.

Table 2. Dosing and evaluation of hormones

	Study arm 1	Study arm 2
Daily dose of hormones		
Estriol, mg	0.5	0.5
Estradiol, mg	0.125	0.5
Progesterone, mg	25	50
DHEA, mg	1.25	50
Testosterone, mg	0.25	0.5
Length of therapy, days	28	14
Salivary collection time from last dose, h	24	6

Serum Testing

A description of the testing methodologies for serum testing is summarized in table 3.

Quality of Life Measurement

General health-related quality of life was measured using the short-form 12 version 2 (SF-12v2, Quality Metric, Inc., Lincoln, R.I., USA). Patients self-administered the written surveys on days 0 and 28. Scoring was completed using SF Outcomes Scoring Software™ (Quality Metric).

Results

Measurement of Serum and Salivary Hormones during Study Arms 1 and 2

During study arm 1, baseline (day 0) and day 28 comparative serum and salivary hormone levels were available for 12 and 9 patients, respectively (tables 4, 5). Salivary specimens for 1 patient were lost in transit and two samples were contaminated. Estriol in serum was not sensitive as it was measured in ng/ml vs. pg/ml. Other studies have show elevation of serum levels with 0.5 mg of estriol delivered vaginally when measured in pg/ml or nmol/l and by suppression of LH and FSH [2–5, 10, 18, 19].

Estradiol and free estradiol were elevated in serum and saliva. Estrone levels did not significantly change with vaginal estradiol as has been previously demonstrated in the literature [3].

Progesterone levels also increased in serum and saliva. Statistical significance was not demonstrated at day 42 compared to day 0, although 6 of 7 patients had at least two-fold increases in salivary levels when compared to baseline day 0 (data not shown). Systemic absorption of progesterone has also been previously demonstrated in the literature with vaginal administration of a progesterone gel [20–22].

Table 3. Description of serum hormone testing methodologies

Hormone	Testing methodology	Comments
Estriol	ICMA	
Estradiol	ADVIA Centaur (competitive immunoassay direct chemiluminescent)	The ADVIA Centaur estradiol-6 III assay measures estradiol concentrations up to 1,000 pg/ml with a minimum detectable concentration (sensitivity) of 7.0 pg/ml. Sensitivity is defined as the concentration of estradiol that corresponds to the RLUs that are 2 SDs less than the mean RLUs of 20 replicate determinations of the estradiol-6 III zero standard.
Estradiol free	ADVIA Centaur (competitive immunoassay direct chemiluminescent)	
Estrone	RIA	
Testosterone total	ICMA	
Testosterone free	Direct analog/RIA	This assay used a labeled testosterone analogue that has a low binding affinity for both SHBG and albumin but is bound by anti-testosterone antibody used in the assay. Since the analogue is unbound in the plasma, it competes with free testosterone for binding sites on the anti-testosterone antibody that is immobilized on the surface of the polyproplyene tube.
DHEAS	ICMA	
Progesterone	ICMA	

ADVIA™ Bayer HealthCare immunoassay. ICMA = Immunochemiluminometric assay; RIA = radioimmunoassay.

Table 4. Study arm 1: serum hormone levels at days 0 and 28

Hormone	Day 0 (n = 12) level ± unbiased SD	Day 28 (n = 12) level ± unbiased SD	p value
Estradiol, pg/ml	14.75 ± 6.33	30.83 ± 17.43	0.01
Free Estradiol, pg/ml	0.253 ± 0.112	0.483 ± 0.287	0.29
Estriol, ng/ml	<3	<3	
Estrone, pg/ml	68.53 ± 27.94	67.17 ± 33.87	0.91
Progesterone, ng/ml	0.467 ± 0.107	2.925 ± 1.157	<0.01
Testosterone, ng/dl	48.25 ± 7.94	38.75 ± 10.172	0.03
Free testosterone, pg/ml	0.775 ± 0.54	1.767 ± 1.04	<0.01
DHEA-S, μg/dl	93.58 ± 50.98	90.67 ± 55.73	0.98

Table 5. Study arm 1: salivary hormone levels at days 0 and 28 collected 24 h post-dose

Hormone	Day 0 (n = 9) level ± unbiased SD	Day 28 (n = 9) level ± unbiased SD	p value
Estradiol, pg/ml	1.178 ± 0.315	1.7 ± 0.622	0.04
Estriol, pg/ml	3.64 ± 0.932	4.31 ± 2.242	0.42
Estrone, pg/ml	1.344 ± 0.959	1.7 ± 1.038	0.46
Progesterone, pg/ml	16.125 ± 2.8	59.5 ± 53.722	0.06
Testosterone, pg/ml	20.222 ± 8.151	23 ± 13.458	0.50
DHEA-S, ng/ml	5.978 ± 2.742	4.854 ± 2.305	0.36

In the first arm of the study, total serum testosterone declined. Serum and salivary DHEA were not altered. Serum free testosterone was elevated at 6 h and correlated with relief of symptoms related to low testosterone levels.

The dose of DHEA in the first arm of the study was felt to be inadequate, not measurable in serum or saliva, and was increased in the second arm of the study. It was felt that the delayed collection of saliva (24 h) could have missed the rise in testosterone. Therefore, the second arm of the study examined higher doses and earlier collection of saliva. Absorption of *all hormones* was documented at 6 h following use of the higher strength cream applied to the mucous membranes of the labia and the vagina. This is consistent with previous studies which have shown absorption of vaginally administered hormones (estriol, es-

Table 6. Study arm 2: salivary hormone levels at days 0 and 42 (after administration of the higher strength cream for 14 days) collected 6 h postdose

Hormone	Day 0 (n = 7) level ± unbiased SD	Day 42 (after 14 days' higher dosage) (n = 7) level ± unbiased SD	p value
Estradiol, pg/ml	1.286 ± 0.212	6.143 ± 3.375	0.01
Estriol, pg/ml	4.386 ± 1.369	28.814 ± 28.287	0.06
Estrone, pg/ml	1.343 ± 0.68	2.529 ± 1.329	0.07
Progesterone, pg/ml	19.857 ± 12.851	443.143 ± 760.833	0.18
Testosterone, pg/ml	15.714 ± 5.619	78 ± 45.92	0.01
DHEA-S, ng/ml	5.157 ± 2.72	17.314 ± 9.703	0.02

Table 7. Summary scores from SF-12v2

Health construct	Day 0 mean ± SD	Day 28 mean ± SD	p value	Change
Physical function	42.51 ± 10.81	44.66 ± 12.1	0.600	2.15
Role physical	42.2 ± 10.84	48.25 ± 7.23	0.073	6.05
Bodily pain	40.25 ± 12.74	47.25 ± 12.34	0.125	7.00
General health	44.74 ± 10.56	49.73 ± 8.9	0.159	4.99
Vitality	44.6 ± 12.03	49.64 ± 10.53	0.218	5.04
Social function	47.1 ± 8.62	51.52 ± 7.37	0.130	4.42
Role emotional	38.61 ± 10.18	45.59 ± 8.87	0.047	6.98
Mental health	44.35 ± 11.93	48.54 ± 9.14	0.274	4.19
Physical component score	42.98 ± 12.78	47.32 ± 11.14	0.313	4.34
Mental component score	43.98 ± 12.69	49.42 ± 8.38	0.162	5.44

tradiol and progesterone) with serum levels peaking at 1–8 h [1, 2, 10, 11,19, 20, 28, 39, 42–43]. Estriol levels approached statistical significance comparing baseline to day 42 and were increased 2- to 6-fold in all but 1 patient (table 6). Estrone was not significantly elevated (table 6).

Measurement of HRQoL

Systemic symptoms were relieved with therapy as documented on the SF-12v2 quality of life survey done on days 0 and 28 (table 7).

The eight health constructs and two summary component scores (PCS and MCS) have been widely used and validated in the medical literature [23–27]. Norm-based scoring of the SF-12 tool assigns a score of 50 as the norm for any given construct, with a scaled standard deviation of 10 [23]. So, (a) the scores for most of the measured constructs were within 1 SD below the US population norm other than emotional health (slightly greater than 1 SD below US population norm at time point 0), and (b) while some of the individual patients within the study had a worsening of a single construct, when examined as a group, all of the measured health constructs (and summary scores) improved over the 28-day timeframe. These effects were between a mean change of 2.15 for physical functioning (least effect) to a mean change of 6.98 and 7.00 for role emotional and bodily pain, respectively (greatest effect). Measurement of role emotional was the only construct demonstrating statistical significance when comparing all of the summary scores and health constructs.

Tolerability and Side Effects

In the second arm of the study, one patient had complaints consistent with androgen excess. The 50-mg dose of DHEA was felt to be excessive. The vaginal dose for DHEA has not been established. The 1.25-mg vaginal dose of DHEA was not measurable in serum or saliva.

Patient satisfaction with this method of delivery was high with all 12 patients choosing to continue with hormone therapy. Two of 12 patients had minor irritation with the cream base. Patients found the once daily, single cream, mucous membrane/vaginal method convenient and easy to use. Vaginal delivery of hormones provided relief of systemic symptoms along with relief of vaginal and urinary symptoms; 87% of patients in this study had genital urinary symptoms before therapy and all patients had relief of genital urinary symptoms with therapy at day 28.

Discussion and Conclusions

This study, like others [1–16] that have examined absorption of the individual hormones, confirmed adequate absorption when a combination cream was applied vaginally in a sufficient dosage. Prior studies used serum to measure absorption of vaginally administered hormones. In the initial arm of this study both serum and saliva were measured to confirm absorption. In the second arm of this study, salivary levels alone were used to measure absorption. In accordance with previous studies (serum), measurable hormone levels were demonstrated at 6 h in saliva as compared to 24 h. This correlated with historical studies, which showed that vaginal hormones are absorbed and peak between 1 and 8 h, returning to baseline (serum) at 24 h [1, 2, 8, 10, 16, 18, 19, 21, 22, 39, 42–44].

This study included the generalized HRQoL measure in an effort to determine whether the included patients obtained benefit from the therapy. The 2005 National Institutes of Health Consensus Statement [58] indicated the need for researchers and clinicians to closely examine therapies that translate into improved HRQoL for women with menopausal symptoms. As previously mentioned, all 12 patients chose to continue therapy following the conclusion of the study, which may indicate a patient preference for treatment effects versus choosing no treatment.

Again, this pilot study did not include a large sample size and was not powered to determine statistical significance between differing time-points when examining HRQoL average measures. Additionally, caution must be used when examining HRQoL small group mean values and some [59] suggest examining repeat measures with an individual patient as a more robust method of analysis, which was also examined in this study. Longer monitoring, or use of a disease-specific measurement tool, such as the Menopause Rating Scale [60–61] or the combination of a general HRQoL and disease-specific scale may be important for future studies.

Limitations of this study include the small sample size and relatively short study period. It is likely that the small sample size and variability of measurements may have led to an under-powered study when examining some of the measurements and comparing day 0 and day 42 measurements. The authors were inclined to accept this in an effort to determine dose-response and individual patient response. Additional information gained regarding inter- and intra-patient variability will allow for future studies examining a sufficient number of patients to reduce type 1 and type 2 statistical errors.

As in most therapies, not every patient is expected to respond to an initial dosage regimen and dosages may need to be adjusted upwards or downwards with extended therapy. In practice, many patients receiving topical hormone therapy, including intravaginal therapy, are given some freedom to make minor adjustments while monitoring for effect and reporting this back to the prescribing physician. Additionally, there was no attempt to blind patients to therapy which may have biased HRQoL results. This limitation would not have an effect on objective serum/salivary measurements.

Additional studies would be useful to determine individual patient characteristics that would suggest tolerability and efficacy at a given dosage. Finally, longer monitoring of patients receiving vaginal combination hormone therapy will help determine the persistency of effect and the safety and tolerability profile.

This pilot study documented the systemic absorption of multiple hormones by both saliva and blood testing as well as relief of systemic symptoms by a standardized HRQoL questionnaire. The combination cream utilized in this pilot study is balanced (with a progesterone to estradiol ratio of at least 100:1) and in practice, patients may increase or decrease the amount of cream used depending on symptoms. The cream may be applied intravaginally, to the external mucous membranes, or both. Based upon results of this pilot, absorption is adequate with either application site and patients generally tolerated this method of administration with a high level of satisfaction. Vaginal hormone therapy has been successfully used in practice by the author for over five years in approximately 2,000 patients with excellent clinical results. Current dosing, which relieves local and systemic symptoms without noted side effects, is estriol 0.5 mg, estradiol 0.1 mg, progesterone 25 mg and testosterone 0.5 mg in a 0.25-ml volume of nonirritating cream base 3–6 days per week.

Additional benefits of this method of administration include the avoidance of first-pass hepatic metabolism. The hormones did not appear to accumulate or form metabolites during the timeframe of this study. Finally, vaginal delivery provided relief of climacteric symptoms with a strong safety profile.

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