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Pharmacokinetics and Tolerability of a Novel 17 β -Estradiol and Progesterone Intravaginal Ring in Sheep

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ABSTRACT

This study reports the preparation, *in vitro* release, pharmacokinetics, and local tolerability of novel ethylene-vinyl acetate intravaginal rings (IVRs) delivering 17 β -estradiol (E₂) and progesterone (P), in drug-naïve ovariectomized female Dorset crossbred sheep. After preparation and assessment of *in vitro* release of E₂ and P, animals were randomized to treatment groups 1 or 2 (comparator rings releasing 50 or 100 μ g/d E₂, respectively), groups 3 or 4 (ethylene-vinyl acetate IVRs, 160 μ g/d E₂ with 4 [160/4 IVR] or 8 mg/d P [160/8 IVR], respectively), or group 5 (160 μ g E₂ and 10 mg P administered intravenously). IVRs were placed on day 1 and remained in place through day 29. Animals underwent daily examinations to confirm ring placement, and vaginal irritation was scored from 0 (none) to 4 (severe). Blood samples were taken at scheduled times for pharmacokinetic analysis. Postmortem examinations performed on groups 1–4 were macroscopic and microscopic evaluations, including irritation scoring and histopathology. IVRs were retained over 28 days in all but 1 animal (group 4). In all animal groups, clinical observations showed no significant abnormal findings. Pharmacokinetic analysis in the animals showed sustained release of E₂ and P over a 28-day period. Irritation scores and microscopic assessments were consistent with foreign object placement. A novel 2-drug IVR delivery system was well tolerated in a sheep model and pharmacokinetic release was as expected over a 28-day release period. These results will guide future human clinical studies.

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Introduction

During menopause, there is a decline in ovarian function, with the precipitous fall of circulating levels of estrogen leading to physiological changes and the associated symptoms of menopause. While the causal relationship between low estradiol E₂ levels and genitourinary symptoms is well understood,¹ the mechanism behind vasomotor symptoms (VMS) is less well elucidated, but it is clear that E₂, although not solely responsible, is a major endocrine influencer.²

The recently published position statement of the North American Menopause Society states that hormonal therapy (HT) is the most

effective treatment for VMS and genitourinary symptoms, with both local and systemic treatments widely used. HT with low-dose vaginal estrogen therapy is recommended for isolated genitourinary symptoms associated with vulvovaginal atrophy (VVA). Treatment with E₂ alone or in combination with a progestogen is recommended as the first-line therapy for VMS in women without contraindications, where the addition of a progestogen is needed to provide endometrial protection from unopposed systemic estrogen.^{3–5} The use of progesterone (P) is preferred by some over progestins in patients at risk of cardiovascular disease because of favorable effects on lipids, atherosclerosis, and vascular reactivity.^{6,7}

There are currently 2 E₂-only silicone-based intravaginal rings (IVRs) approved in the United States for the treatment of menopausal women: a 17 β -estradiol acetate vaginal ring (Femring; equivalent to release of 50 μ g/d and 100 μ g/d) approved to treat

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VMS and local VVA symptoms and an E₂ vaginal ring (Estring: 7.5 µg/d) approved only for local vaginal symptoms. A combination product that delivers E₂ and P via an IVR over a 28-day period, which treats women for VMS and VVA, a known urogenital symptom of menopause, while providing endometrial protection, would be novel and desirable. However, there are currently no approved nonoral combination products in the United States that contain both E₂ for treatment of VMS and VVA and progesterone, necessary for endometrial protection.

A novel IVR technology that allows for E₂ and P to be integrated into a single ethylene-vinyl acetate (EVA) ring delivery system is currently under development. This IVR is being designed to treat VMS in women with an intact uterus, while also aiming to reduce the symptoms of VVA. This IVR is designed to either deliver 80 or 160 µg/d E₂ with an adequate rate of P for endometrial protection over a 28-day period. These target release rates were based on previous reports using IVRs comprising an inner core of silicone elastomer matrix containing E₂ and P, and a silicone elastomer overcoat, have demonstrated the effectiveness of delivering 160 µg/d E₂ with either 10 or 20 mg/d P in 20 postmenopausal women.⁸ Results showed a significant reduction in the incidence of hot flashes and night sweats from 2 weeks through 16 weeks, with ultrasound monitoring of the endometrium suggesting effective protection against endometrial hyperplasia. In another related study,⁹ IVRs releasing E₂ at 150 µg/d and P at 5 or 9 mg/d resulted in significant reduction in the incidence of hot flashes and night sweats in a group of 55 women.

This study was conducted *in vivo* in sheep, a relevant nonclinical model, in preparation for a clinical development program, to determine the pharmacokinetics (PK) and local tolerability of E₂ and P combination IVRs compared with a marketed 17β-estradiol acetate IVR Femring® (Warner Chilcott, Rockaway, NJ), that releases the equivalent of E₂ at either 50 µg/d or 100 µg/d. A sheep model was selected for use in this study because the vagina and cervix in sheep are reported to be of a size similar to those in women,¹⁰ thereby allowing for the testing of IVRs without modification of ring dimensions. Sheep are routinely used in the nonclinical assessment of intravaginal compounds and IVRs^{10,11} as the vagina is composed of a stratified squamous epithelium that is more comparable to humans than other commonly used models of intravaginal administration (e.g., rabbits). Finally, the response of the sheep vagina to endogenous and exogenous estrogenic and progestogenic stimulation is well described.^{12,13}

Methods

Intravaginal Rings

Intravaginal rings capable of releasing E₂ and P were prepared in a manner similar to that described previously.¹⁴ Manufacturing of IVRs was performed by QPharma, Malmö, Sweden.

IVRs capable of releasing E₂ (EP; Aspen Oss B.V., Oss, the Netherlands) at the desired rates are prepared by using fibers of varying length and drug loading. The 2 IVRs evaluated release E₂ at a rate of 160 µg/d and P released at 4 mg/d (160/4 IVR) or 8 mg/d (160/8 IVR). All IVRs are 57 mm in overall diameter and a cross-sectional diameter of 5 mm. The EVA fiber containing E₂ was prepared at a concentration of 10 w/w in EVA (28% vinyl acetate content; VitalDose®, Celanese Corporation, Boucherville, Canada). The E₂ fiber length in the finished IVR is 15 mm in both the 160/4 and 160/8 IVRs. IVRs releasing 4 and 8 mg/d P were prepared using EVA (28% vinyl acetate content; VitalDose®) with a final drug loading of 27% w/w. To create the 160/4 IVR, the P-containing segment length was 74.5 mm with a placebo segment length of 74 mm. To create the 160/8 IVR, 27% loaded EVA fiber was 148.5 mm in length.

There is no placebo segment in the 160/8 IVR. The theoretical loading of E₂ in both 160/4 and 160/8 IVRs is 28.9 mg; theoretical loading of P in the 160/4 IVR is 387.1 mg while that of the 160/8 IVR is 771.6 mg.

In Vitro Release of E₂ and P From IVRs

The release rates of E₂ and P from the 160/4 and 160/8 IVR formulations were measured *in vitro* to determine whether the target release rates had been attained. Release rates were tested using 200 mL 0.5% sodium dodecyl sulfate as a release medium, in shakers operating at 130 ± 2 rpm at 37°C. These conditions were based on solubility assessments of both E₂ and P using several surfactants (sodium dodecyl sulfate, Tween 80, hexadecyl trimethyl ammonium bromide) under varying degrees of agitation. Sampling (2 mL) was conducted at 6 h, days 1–4, 7–11, 14, 15, 18, 21, 22, 25, and 28. Concentrations of E₂ and P were determined using a validated reverse-phase liquid chromatography method. The column used was a Phenomenex Luna C8(2), 150 mm × 3.0 mm, 5 µm, and the guard column was Phenomenex C8 (4 mm × 3 mm). The mobile phase was acetonitrile 45% in purified water (55%), v/v. The injection volume was 10 µL. Detection of E₂ was based on fluorescence (279 nm excitation with 306 nm emission); P was detected by UV at 245 nm. The standard curve range for E₂ was 0.25–3.5 µg/mL; the range for P was 0.00625–0.25 mg/mL. Both curves were linear (correlation coefficient >0.997). Six IVRs were tested at each dissolution time point.

Animal Study Design

The purpose of this study was to evaluate the *in vivo* PK and local tolerability of 160/4 and 160/8 IVRs in drug-naïve ovariectomized female Dorset crossbred sheep and to establish the comparative bioavailability with 50 or 100 µg/d E₂ comparator IVR products (Femring) over a 28-day exposure period, and with a single IV administration of a 160 µg E₂ and 10 mg of P.

The study was conducted at MPI Research, A Charles River Co., which is an American Association for Accreditation of Laboratory Animal Care–accredited contract research organization. It was conducted in compliance with the US Food and Drug Administration Good Laboratory Practices Regulations and the US Department of Agriculture Animal Welfare Act.

A total of 27 experimentally naïve, female, uniparous, Dorset crossbred sheep, approximately 15.5 to 19 months of age at receipt, were received from Ehrhardt Farm, Eaton Rapids, Michigan and from Lauwers Lamb, Capac, Michigan. Animals were identified by implanted microchips and by individual ear tags.

During acclimation, the animals were observed daily with respect to general health and any signs of disease. All animals were given a detailed examination, and body weights were recorded within 3 days of receipt and again before the operating procedures. All animals were negative for *Cryptosporidium* and *Giardia* species. *Strongyloides* and *Coccidia* were detected in stool samples from almost all animals. Animals were treated with a single administration of fenbendazole (10 mg/kg orally). Animals weighed 57.5 to 77.0 kg at randomization.

Between 25 and 54 days before the scheduled dosing, all animals underwent a surgical procedure to remove the ovaries, in accordance with the research facility's standard operating procedures.

Animals were allowed to recover for 25 to 54 days before dosing. During this recovery period, body weight measurements and other observations were performed weekly. Ovariectomy surgery was performed successfully in all animals as determined by undetectable levels of endogenous hormones.

During the course of the study, all animals were observed twice daily for morbidity, mortality, injury, and the availability of food and water. Detailed examination of each animal was performed weekly during the study. These observations included evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior.

Animals were randomly allocated to 1 of 5 treatment groups: group 1 ($n = 5$) comparator IVR Femring (50 μg E_2 /day); group 2 ($n = 5$) comparator IVR Femring (100 μg E_2 /day); group 3 ($n = 5$) 160/4 IVR; group 4 ($n = 5$) 160/8 IVR; and group 5 ($n = 3$) bolus injection of 160 μg E_2 and 10 mg P.

The 160/4 and 160/8 IVRs were stored at 2°C to 8°C until use and were allowed to warm to room temperature for 30 to 120 min before use; comparator IVRs were stored at room temperature in accordance with labeling. All IVRs were inserted on day 1 and were to remain in place until removal at day 29. Vaginal ring insertion was performed as a clean procedure. The IVR was photographed before being digitally inserted into the cranial vagina using a gloved finger. During the treatment period, animals were digitally examined daily to confirm that the IVR was still in place.

After completion of treatment on day 29, the IVRs were removed from each animal and were photographed and stored at 2°C to 8°C before being returned for analysis of residual E_2 and P content. The theoretical mass balance was calculated by adding the amount of E_2 and P present in the device after use with the amount released by the IVR (taken from the initial release testing of the IVRs *in vitro*) and dividing by the theoretical drug content. This was calculated as a gross check on IVR performance, and results were not intended to be correlated with PK findings.

E_2 and P were administered to group 5 on day 1 via 2 separate intravenous injections into the jugular vein, at a dose volume of 1 mL/dose. P was administered first, followed within 1 to 3 min by E_2 . The control articles were used as received from the supplier (Sigma Aldrich, Milwaukee, WI), formulated to achieve nominal concentrations of 0.16 mg/mL E_2 or 10 mg/mL P in ethanol/propylene glycol/sterile water (3:3:4, v/v/v), and filtered through a 0.22 μm polyvinylidene fluoride syringe filter before administration.

Pharmacokinetics

Blood samples taken periodically from 2 to 672 h were placed in tubes containing K_2 -EDTA and were centrifuged under refrigerated conditions within 60 min of sample collection. The resulting plasma was stored frozen at -60°C to -90°C within 120 min of sample collection. Plasma samples were shipped on dry ice for analysis (Pyxant Labs, Inc., Colorado Springs, CO). Plasma samples were analyzed using liquid chromatography–mass spectrometry/mass spectrometry methods validated according to bioanalytical method guidelines. The standard curve range for E_2 was 5–500 pg/mL; the range for P was 0.1–20 ng/mL. Based on quality control samples, accuracy ranged from 96.7%–101% for E_2 and 96.5%–98.0% for P. Precision (%CV) was less than 4.2% for E_2 and less than 7.5% for P. The lower limit of quantitation for E_2 was 5.0 pg/mL and 0.1 ng/mL for P; the upper limit of quantitation for E_2 was 100 pg/mL and 20 ng/mL for P. Concentrations below the lower limit of quantitation were set to zero for PK analyses.

Standard noncompartmental PK analysis methods were used. Pharmacokinetic parameters were determined for E_2 and P (as applicable). The values of area under the concentration–time curve (AUC) were estimated by the trapezoidal rule, and intravenous clearance was estimated as $\text{dose}/\text{AUC}_{\text{INF}}$. The C_{avg} after IVR administration was calculated as $\text{AUC}_{0-672 \text{ h}}/672 \text{ h}$.

Tolerability: Description of Assessments, Grading Scale

For animals in groups 1 through 4, examination of the external vagina (the vulva and the externally visible portion of the vestibule) was conducted before insertion of the IVRs, and daily examinations were conducted on days 2 through 29, before the daily ring checks. The external vagina was observed for gross signs of irritation (i.e., erythema and edema) and any other signs of local or systemic effect. Irritation was scored based on the Draize scale¹⁵; erythema and edema formation were rated on a scale of 0 (none) to 4 (severe). The same scales were used at necropsy on day 29 to score irritation of the internal vagina (the portion not visible during in-life assessments); any other signs of local or systemic effects were also recorded.

Necropsy

On day 29, after external vaginal irritation scoring and ring removal, animals in all groups were euthanized. At necropsy, a macroscopic examination of the reproductive organs and surrounding tissues was performed and the uterus, cervix, and vagina were collected and fixed in 10% neutral buffered formalin. Microscopic examination of reproductive tissues (processed hematoxylin and eosin-stained slides) was conducted routinely by a board-certified veterinary pathologist (J.D.V.).

Vaginal irritation was scored based on the rabbit vaginal irritation method described by Eckstein et al.¹⁶ For each animal, 3 vaginal regions including the portion adjacent to the cervix (cranial), the middle portion (mid), and the portion at the level of the urethra (uro) were scored separately for 4 parameters (epithelial damage, vascular congestion, edema, and leukocyte infiltration) with each parameter receiving a score of 0 (normal) to 4 (marked).

Statistical Analysis

Statistical analysis of data was limited to calculation of descriptive statistics, including means, standard deviations, relative standard deviations, group size for each group and period (continuous endpoints), and either medians or incident counts for each group and period (categorical endpoints).

Results

In Vitro Release of E_2 and P

Release rates of E_2 and P over the 28-day test period are characterized by the rate over day 1, from day 2 to day 28, and the rate on day 28. Table 1 shows the data collected in this manner from the different 160/4 and 160/8 IVRs. The *in vitro* release profiles of both drugs are typical of a matrix-type delivery system with a relatively rapid release of drug followed by a period of slower release, which can be seen in Figure 1. The total amount of E_2 over 28 days from the 160/4 and 160/8 IVRs was 4.1 mg and 4.3 mg, respectively. The total

Table 1
Release Rates of E_2 and P From the 160/4 and 160/8 IVRs

Test Time Points	160/4 IVR		160/8 IVR	
	E_2 ($\mu\text{g}/\text{d}$)	P (mg/d)	E_2 ($\mu\text{g}/\text{d}$)	P (mg/d)
0–24 h	742 \pm 6.2 ^a	30 \pm 3.7	762 \pm 5.6	58.7 \pm 0.8
2–28 d	122 \pm 6.9	4.1 \pm 2.7	119 \pm 3.0	7.9 \pm 0.9
28 d	70.3 \pm 5.5	2.2 \pm 2.6	67.3 \pm 2.8	4.2 \pm 1.1

^a All data are means \pm SD ($n = 6$).

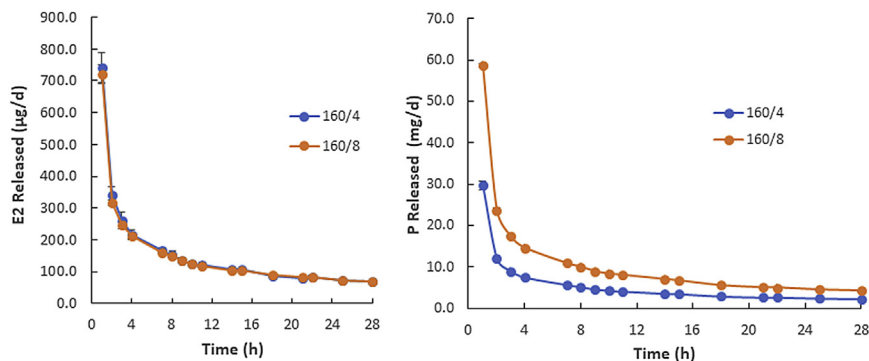


Figure 1. *In vitro* release profiles of E₂ (Panel A) and P (Panel B) from IVRs (160/4) or 8 (160/8). Values are mean values ($n = 6$) \pm SD.

amount of P released over 28 days from the 160/4 and 160/8 IVRs was 127 mg and 251 mg, respectively.

Pharmacokinetics

After removal of the IVRs on day 29, analysis of the residual E₂ and P showed that all rings were within $\pm 10\%$ of the theoretical mass balance of both hormones, with the exception of the ring obtained from the group 4 animal that was released on day 18. For instance, the average amount of E₂ released *in vivo* over 28 days from the 160/4 IVR was 4.6 mg (or 107% of that released *in vitro*). The average amount of P released *in vivo* from the 160/8 IVRs was 236 mg (or 94% of that released *in vitro*).

The plasma concentrations of E₂ from the 2 comparator IVRs (Femring, 50 $\mu\text{g/d}$ and 100 $\mu\text{g/d}$) and from the 160/4 and 160/8 IVRs are shown in Figure 2. PK parameters of E₂ from these 4 IVRs groups over the 28-day release period are presented in Table 2. Peak E₂ plasma concentrations after 160/4 and 160/8 IVR insertion on day 1 were observed at a median of 4 h in group 3 and at 2 h after insertion in group 4, respectively. Maximum observed E₂ plasma concentrations (C_{max}) values were 149 ± 21.3 pg/mL and 158 ± 54.6 pg/mL in groups 3 and 4, respectively. E₂ concentrations remained at a quantifiable level at day 29 after IVR insertion in all animals. The mean E₂ AUC_{0–672 h} value in group 3 was $17,400 \pm 2120$ pg \cdot h/mL lower than that observed in group 4 ($21,000 \pm 3540$ pg \cdot h/mL). The average plasma concentration over the entire dosing interval (C_{AVG}) was also numerically lower in group 3 than in group 4 (25.9 ± 3.16 pg/mL and 31.3 ± 5.26 pg/mL, respectively).

Animals administered comparator IVRs (Femring 50 $\mu\text{g/d}$ or 100 $\mu\text{g/d}$ E₂ release rates) had peak E₂ plasma concentrations observed at 2 h after IVR insertion on day 1 for both comparator ring groups. The mean C_{max} values were comparable between the 2 dose groups; despite the 2-fold difference in theoretical E₂ release rates, there was only a 1.2-fold increase in C_{max} between group 1 (164 ± 26.8 pg/mL) and group 2 (197 ± 29.6 pg/mL). By contrast, the AUC_{0–672 h} values increased proportionally with increasing dose; a 2-fold increase in dose resulted in a 2-fold increase in AUC_{0–672 h} values, from 9690 ± 1750 pg \cdot h/mL in group 1 to $19,000 \pm 1170$ pg \cdot h/mL in group 2. Similarly, the average plasma concentration over the entire dosing interval (C_{AVG}) saw a 2-fold increase from 14.4 ± 2.6 pg/mL in group 1 to 28.2 ± 1.75 pg/mL in group 2.

The calculated *in vivo* release rate (R_0) can be determined using the PK data and the equation $R_0 = C_{\text{AVG}} \times \text{CL}$, where C_{AVG} is obtained individually from each animal in groups 3 and 4 and CL is the total clearance obtained with intravenous administration (Table 3). From 160/4 IVRs (group 3) and the 160/8 IVRs (group 4) E₂ *in vivo* release rates in this sheep model were 55.9 ± 6.8 $\mu\text{g/d}$ and 67.3 ± 11.3 $\mu\text{g/d}$, respectively. These estradiol values bracket the value obtained from the group 2 comparator administration, at 60.8 ± 3.8 $\mu\text{g/d}$.

Across the different IVRs tested (the comparator silicone Femring IVRs vs. EVA-derived 160/4 and 160/8 IVRs), the release rates and hence AUCs for E₂ differed, with the release rates being lower for the 160/4 and 160/8 IVRs despite higher E₂ concentrations being loaded into the EVA matrix. This lower release is presumed because of a different IVR construct that contributes to the release characteristics of the IVR (e.g., EVA vs. silicone for the comparator).

For the animals receiving the 160/4 or 160/8 IVRs, the median peak P plasma concentrations were observed 4 h after insertion for both dose groups (4 mg/d and 8 mg/d). P was quantifiable up to 21 days (2 animals) or 28 days (3 animals) after IVR insertion in group 3 and up to 28 days after IVR insertion in group 4. Plasma concentrations of P over time from the 160/4 and 160/8 IVRs are shown in Figure 3.

Mean P C_{max} and AUC_{0–672 h} values increased with increasing dose (Table 4). The C_{max} values in groups 3 and 4 were 1590 ± 272 pg/mL and 2400 ± 322 pg/mL, respectively. There was a 2-fold increase in AUC_{0–672 h} values ($240,000 \pm 7510$ pg \cdot h/mL and $485,000 \pm 63,200$ pg \cdot h/mL, respectively). A 2-fold increase was also then observed between dose groups for C_{AVG} , with mean values of 357 ± 11.2 pg/mL in group 3 and 722 ± 94.1 pg/mL in group 4.

Group 5 ($n = 3$) received a single intravenous bolus administration of 160 μg E₂ and 10 mg P. Data from 1 animal were considered aberrant, because of an unexplained second peak in E₂ and P concentrations 3 h after dose, and were excluded. In the remaining animals, the mean E₂ AUC_{Inf} was 1790 pg \cdot h/mL; for P, the mean AUC_{Inf} was 20,000 pg \cdot h/mL (Table 3).

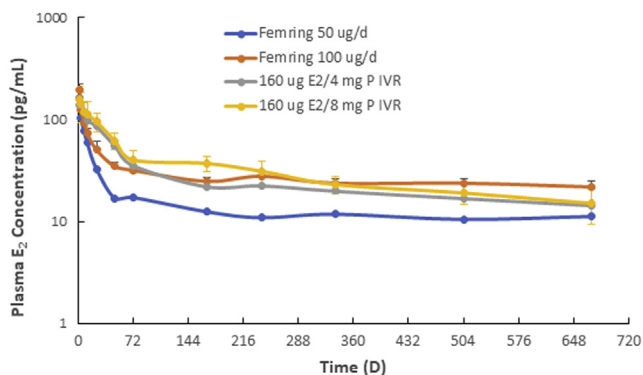


Figure 2. Plasma concentration-time profiles of E₂ after a single intravaginal insertion of comparator IVRs Femring (50 or 100 $\mu\text{g/d}$ E₂) or the 160/4 and 160/8 IVRs in female sheep. Data are means ($n = 5$) \pm SD intravaginal ring.

Table 2
E₂ PK Parameters From Intravaginal Rings

Treatment	Group 1		Group 2		Group 3		Group 4	
	50 µg/d Comparator Ring		100 µg/d Comparator Ring		160/4 IVR		160/8 IVR	
PK Parameter	Mean (SD)	CV%	Mean (SD)	CV%	Mean (SD)	CV%	Mean (SD)	CV%
C _{max} (pg/mL) ^a	164 (26.8)	16.3	197 (29.6)	15.0	149 (21.3)	14.3	158 (54.6)	34.6
AUC _{0–672 h} (pg·h/mL) ^b	9690 (1750)	18.0	19,000 (1170)	6.19	17,400 (2120)	12.2	21,000 (3540)	16.8
C _{avg} (pg/mL) ^c	14.4 (2.6)	18.0	28.2 (1.75)	6.19	25.9 (3.16)	12.2	31.3 (5.26)	16.8
R ₀ (µg/d) ^d	31.0 (5.6)	18.1	60.8 (3.8)	6.25	55.9 (6.8)	12.2	67.3 (11.3)	16.8

^a C_{max}, maximum observed plasma concentration.^b AUC_{0–672 h}, area under the plasma concentration–time curve from 0 to 672 h.^c C_{avg}, average plasma concentration over the entire dosing interval.^d R₀, calculated *in vivo* release rate.

The *in vivo* release rates from the PK data were similarly calculated for P from the 2 IVRs as described above for E₂. The *in vivo* release rates were 3.5 and 7.1 mg/d for groups 3 and 4, respectively, similar to the labeled release rates of 4 and 8 mg/d.

Animals—Observations

Observations collected during the treatment period were unremarkable for abnormal physical findings. Enlarged udders were observed in several animals (including some animals with enlargement before administration of study treatment), but because all animals had a history of lactation, these observations were not considered to be related to the study treatment. A clear vaginal discharge was observed in 1 animal from group 4, but discharge findings are common during studies with the intravaginal route. Red discoloration of the vulva was observed in some animals across all treatment groups. Mean body weight was increased by approximately 10% relative to 1 week before treatment in all groups (1 to 4) on day 29.

After administration of the IVRs, daily vaginal ring checks confirmed that the IVRs remained in place until the time of removal (day 29) in all but 1 animal. For 1 animal in group 4, the ring was found to have been expelled on day 16. After a visual inspection for damage, the ring was washed and reinserted, but was expelled again on day 18 and could not be located. A new ring was inserted and remained in place until day 29. Blood samples from this animal were not used in the PK analyses.

In-life Vaginal Irritation Scoring

Evaluations of external vaginal irritation performed on days 1 through 29 of the treatment period comprised assessments of erythema and edema on a scale of 0 to 4 (Table 5). The number of assessments showing well-defined erythema and eschar was low

and comparable across the 4 treatment groups. Erythema developed on day 2 in all animals and was most severe at the outset of treatment. Very slight erythema (scores of 1) persisted longer in groups 3 and 4 than in groups 1 and 2; the incidence of observations of very slight erythema/eschar was greatest in the group 4 animals, with most animals experiencing very slight erythema for most of the study period (data not shown).

Evaluation of the external vagina for edema showed no scores greater than 1 (very slight edema) in any animal. The incidence of very slight edema was greatest in group 2, the high-dose comparator ring group. In both of the 160/4 and 160/8 IVR groups, the incidence of very slight edema was comparable with that in group 1.

Internal vaginal assessment on day 29 after removal of IVRs showed little or no irritation in both comparator ring groups. The incidence of observations of erythema was greater in the 160/4 and 160/8 IVR groups than in the comparator ring groups and was higher in the 8 mg/d progesterone group than the 4 mg/d dose (Table 5).

Postmortem Assessments

Postmortem macroscopic assessment of the vagina showed treatment-related changes in the 160/4 and 160/8 IVR-treated animals; red foci were observed in the cranial vagina, near the cervix, of those animals which had erythema in the day 29 in-life internal examinations (2 in group 3, and 5 in group 4).

Microscopic observation showed changes in the 160/4 and 160/8 IVR-treated animals, which were more frequent in the cranial vagina (Table 6). The changes observed showed a degree of correlation with the P dose. In the cranial vagina, focal areas showing minimal atrophy were observed in 2 animals in group 3, and 4 animals in group 4; these areas were characterized by focal areas of

Table 3
E₂ and P PK Parameters After a Single Intravenous Administration

Treatment	Group 5	
	E ₂ (n = 2 ^a)	P (n = 2)
Intravenous dose	160 µg	10 mg
PK Parameter	Mean	Mean
AUC _{INF} (pg·h/mL) ^b	1790	20,000
CL (L/h) ^c	89.6	500
V _z (L) ^d	287	1980
T _{1/2} (h) ^e	2.22	2.75

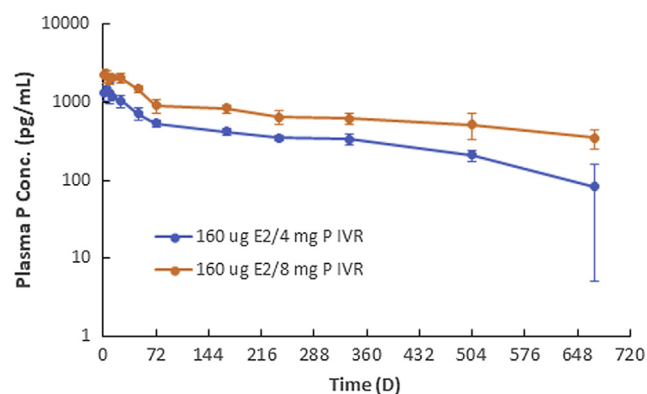
^a Standard deviations and coefficients of variation were not calculated since n = 2.^b AUC_{INF}, area under the plasma concentration–time curve from time zero extrapolated to infinity.^c CL, total body clearance.^d V_z, volume of distribution based on terminal elimination phase.^e T_{1/2}, terminal half-life.**Figure 3.** Plasma concentration–time profiles of P after a single intravaginal insertion of the 160/4 or 160/8 IVRs in female sheep. Data are means (n = 5) ± SD intravaginal ring.

Table 4
P Pharmacokinetic Parameters From 160/4 and 160/8 IVRs

Treatment	Group 3		Group 4	
	160/4 IVRs		160/8 IVRs	
P dose (mg/d)	4		8	
PK Parameter	Mean (SD)	CV%	Mean (SD)	CV%
C _{max} (pg/mL)	1590 (272)	17.1	2400 (322)	13.4
AUC _{0–672 h} (pg·h/mL)	240,000 (7510)	3.13	485,000 (63,200)	13.0
C _{avg} (pg/mL)	357 (11.2)	3.13	722 (94.1)	13.0
R ₀ (μg/d)	3523 (112)	3.18	7132 (923)	12.9

thinning epithelium, with minimal mononuclear cell infiltration in the underlying tissue. Ulceration was observed in the cranial vagina of 1 animal in each of the 160/4 and 160/8 IVR groups (1 minimal, 1 mild). For both animals, infiltration of monocytes and neutrophils was reported, the extent of which correlated with the severity of the ulceration. Areas of atrophy and ulceration generally correlated with the red foci that were observed macroscopically.

Epithelial hyperplasia was observed at all regions of the vagina in groups 1, 2, and 3 and considered minimal to mild (Tables 6 and 7). The incidence and severity of hyperplasia were lower in the group 3 (160/4 IVR animals) than in the comparator ring groups, with no epithelial hyperplasia observed in the group 4 (160/8 IVR animals) at any region of the vagina. Similarly, within the cervix, squamous metaplasia was observed in all treatment groups, but was more frequent and more severe in the comparator ring groups than the 160/4 or 160/8 IVR groups, consistent with unopposed estrogen treatment. As is expected, all (ovariectomized) groups treated with exogenous estradiol treatment had minimal uterine hypertrophy when compared with ovariectomized control animals. These changes would be consistent with the general stimulation associated estrogenic treatment in ovariectomized animals (data not shown).

Figure 3 shows photomicrographs of the cranial vaginal epithelium. Panel A shows tissue from an animal that did not receive any treatment. The remaining images (B, C, and D) were obtained from

Table 5
Vaginal Irritation Scoring

	Treatment			
	Group 1	Group 2	Group 3	Group 4
External irritation, days 1 through 29				
Erythema and eschar score				
0	74	108	88	29
1	64	29	48	107
2	7	8	9	9
3	0	0	0	0
4	0	0	0	0
Edema score				
0	76	51	73	79
1	69	94	72	66
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
Internal irritation, day 29				
Erythema and eschar score				
0	4	5	3	0
1	1	0	1	1
2	0	0	1	4
3	0	0	0	0
4	0	0	0	0
Edema score				
0	5	5	5	4
1	0	0	0	1
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0

animals treated with Femring (50 μg/d), the 160/4 IVR, and the 160/8 IVR, respectively. There was an increase in epithelial thickness in the animal receiving Femring and the 160/4 IVR, where the animal receiving the 160/8 IVR appears similar to the untreated animal.

Discussion

When estrogens are administered at the doses recommended for treatment of VMS, unopposed endometrial exposure is associated with an increase in the risk of endometrial hyperplasia and cancer.¹⁷ Coadministration of a progestogen is recommended in women with an intact uterus to reduce the risk of endometrial hyperplasia and cancer.¹⁸ The choice of the route of HT is based on the patient risk profile and preference, but selection should also take into consideration potential advantages offered by nonoral routes such as vaginal administration. Such routes bypass the first-pass hepatic effect and, in theory, reduce the hypercoagulability and clotting risk associated with estrogen therapy⁴ and avoid the increase in sex hormone-binding globulin concentrations seen with oral E₂.¹⁹ Among the various vaginal treatment modalities, evidence suggests a patient preference for the use of IVRs over cream or tablets, and hence an improvement in adherence to treatment.²⁰

Development of the EVA-based 160/4 and 160/8 IVR products target release rates for E₂ and P combined to offer additional dosing options to the 50 and 100 μg/d doses provided by existing Femring IVR product. Based on the relative plasma concentrations of E₂ in this sheep study, the 160/4 or 160/8 IVRs should be effective in treatment of VMS because the plasma levels of E₂ were similar to those of Femring, which is effective in managing VMS.^{21–23}

The present study was conducted to evaluate the comparative bioavailability of EVA 160/4 and 160/8 IVRs in a relevant *in vivo* model, with marketed E₂ IVRs (Femring) as comparators. Inclusion of the intravenous-administration treatment group allowed for the calculation of systemic clearance in a PK control group. The ovariectomized sheep model was chosen for a number of positive attributes: device retention, ease of IVR placement and assessment, anatomic correctness, physiologic similarities to a human postmenopausal state, and histologic tissue similarity.^{10,24} More specifically, a comparison of the anatomic and physiologic feature of the sheep versus human reveal that cervical diameter is 1–1.5 cm versus 2.3–3.3 cm, vaginal length is 9–13 cm versus 8–12 cm, vaginal epithelial layer thickness is 86–114 mm versus 175–284 mm, vaginal epithelial cell layers are 8–13 versus 22–29 and vaginal pH 7.5–8.5 versus >6.0 in postmenopausal women, respectively.²⁴ In addition, on histology evaluation from biopsy, the sheep vaginal and cervical epithelium consists of stratified squamous epithelium overlying dense vascular submucosa, thinner than but similar in structure to human epithelium.²⁴

The calculated *in vivo* E₂ release rates for the EVA IVR groups 3 and 4 were similar to, and bracket, the release rate calculated for the high-dose comparator ring. Sustained release of E₂ was observed throughout the administration period, with E₂ concentrations of 14.4 and 15.4 pg/mL observed on day 29 in the EVA IVR-treated sheep (in group 3 and group 4, respectively). By day 2, relatively continuous release of E₂ was achieved. E₂ plasma concentrations and *in vivo* release rates from EVA IVR were similar to those observed with the high-dose comparator IVR (100 μg/d) in this animal model, demonstrating a product that may provide efficacy over 28 days despite the fact that Femring (a reservoir device) releases 17β-estradiol acetate at a relatively constant rate over 3 months.²⁵

Examination of the external vagina and the postmortem evaluations demonstrate the local safety and tolerability of the EVA IVRs in the sheep model. Daily clinical observations showed no treatment-related effects other than changes to the vulva, with a

Table 6
Histologic Findings of Uterus, Cervix, and Cranial Vagina^a

Treatment			Group 1	Group 2	Group 3	Group 4
E ₂ dose (µg/d)			50	100	160	160
P dose (mg/d)			-	-	4	8
Uterus	Hypertrophy/hyperplasia	-Minimal	5	5	5	5
	Cyst	-Mild	1	2	2	1
Cervix	Metaplasia, squamous	-Minimal	0	0	1	0
		-Mild	5	2	3	2
		-Moderate	0	3	0	0
		-Moderate	0	1	0	0
Vagina, cranial	Inflammation	-Minimal	0	0	2	4
	Atrophy	-Minimal	5	5	5	5
	Increased keratinization	-Minimal	1	1	5	4
	Infiltration, mononuclear cell	-Minimal	0	0	0	1
		-Mild	0	1	0	0
	Hyperplasia, epithelial	-Minimal	1	3	2	0
		-Mild	4	2	0	0
		-Moderate	0	0	1	0
	Ulcer	-Minimal	0	0	0	1
		-Mild	0	0	0	0
		-Moderate	1	0	4	2
		-Mild	0	1	0	1
	Infiltration, neutrophil	-Moderate	0	1	0	0
		-Minimal	0	1	0	0
		-Mild	0	1	0	0
	Degeneration	-Minimal	0	1	0	0

^a n = 5 animals in all groups.

clear vaginal discharge in 1 animal. External examinations showed low levels of edema and erythema that were not unexpected; because vaginal redness and swelling are commonplace in sheep in the presence of E₂, these were considered likely to be a hormonally mediated change. Minor differences observed between treatment groups (a tendency toward greater edema in the high-dose comparator ring group and greater erythema in the 160/8 IVR group) were not considered significant given that both conditions rarely had a severity greater than “very slight.”

Internal assessments conducted at the end of the treatment period showed almost no irritation in the comparator ring animals. In the EVA IVR animals, erythema was observed in 2 animals in group 3 and in all 5 animals in group 4; these animals had more severe erythema, suggesting a dose-dependent P effect.

Postmortem macroscopic evaluation showed red foci in the cranial vagina near the cervix, which was observed only in those animals with erythema observed on day 29. The observation of atrophy and ulceration is thought to be related to the physical

presence of the IVR. In groups 3 and 4, with the lower level of epithelial hyperplasia (i.e., thinner epithelium) is thought to have contributed to this, with thinner epithelium rendering the vagina more susceptible to the mechanical damage caused by the presence of a foreign body.

The extent of epithelial hyperplasia observed in the vaginas of EVA IVR groups was lower than that in the comparator groups, with vaginal hyperplasia completely absent in the high P-dose group (see Fig. 4). Because PK data indicate similar E₂ exposure in the high-dose comparator ring group and the 160/4 or 160/8 IVR groups (despite the different nominal release rates), this histological difference could reflect a physiological action of the progesterone.

Currently, low-dose E₂ IVRs are already approved for the treatment of VMS associated with menopause; however, the recommendation is still to administer a progesterone supplement aiming to protect the endometrium of females with intact uteri when E₂ therapy is recommended. The coadministration of both hormones together in the same IVR that delivers a consistent and reliable

Table 7
Histologic Finding in the Vagina (Mid and Uro)^a

Treatment			Group 1	Group 2	Group 3	Group 4
E ₂ dose (µg/d)			50	100	160	160
P dose (mg/d)			-	-	4	8
Vagina, mid	Atrophy	-Minimal	1	0	1	1
	Cyst	-Mild	1	0	0	1
	Increased keratinization	-Minimal	4	4	5	4
		-Mild	1	1	0	0
	Infiltration, mononuclear cell	-Minimal	2	2	2	4
		-Mild	0	0	0	1
	Hyperplasia, epithelial	-Minimal	1	2	4	0
		-Mild	4	3	0	0
	Ulcer	-Mild	0	0	0	1
	Infiltration, neutrophil	-Mild	0	0	0	1
		-Minimal	0	1	1	3
	Infiltration, mononuclear cells	-Minimal	2	2	1	1
Vagina, uro	Hemorrhage	-Minimal	1	0	0	0
	Hyperplasia, epithelial	-Minimal	1	2	3	0
		-Mild	3	3	1	0
	Cyst	-Mild	3	0	0	1
	Increased keratinization	-Minimal	4	4	5	5
		-Mild	0	1	0	0

^a n = 5 animals in all groups.

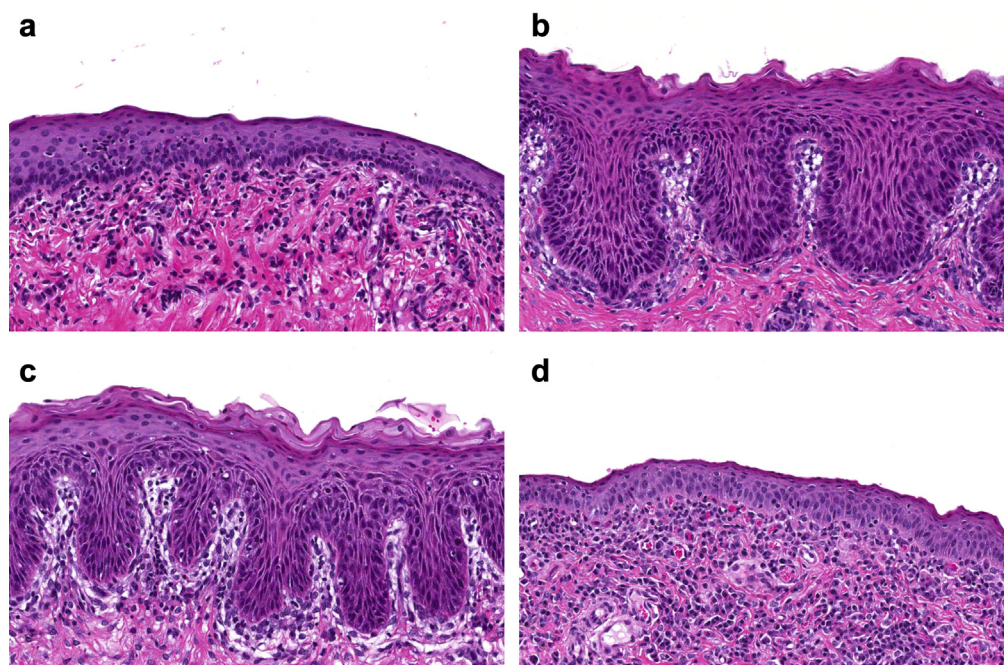


Figure 4. Photomicrographs of the cranial vagina epithelium (hematoxylin and eosin stain) at the same magnification. (a): Untreated animal; (b): comparator IVR (Femring 50 µg/d); (c): 160/4 IVR; and (d): 160/8 IVR.

nonoral dose over a 28-day period would be a valuable option for the treatment of VMS and urogenital symptoms of menopause.

Conclusions

This study demonstrated the safety and tolerability of the novel EVA E₂/P combination IVR in a relevant animal species. The EVA-based IVRs were well tolerated with demonstrable release and concentration-time profiles of both E₂ and P that are projected to be efficacious and with comparable bioavailability to currently marketed products. These results, in a representative mammalian model, support the progression of this combination product into human clinical studies.

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