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Progesterone Pharmacokinetics and Pharmacodynamics With 3 Dosages and 2 Regimens of an Effervescent Micronized Progesterone Vaginal Insert

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Context: Progesterone vaginal insert (PVI), an effervescent delivery system, dissolves rapidly, is absorbed through the vaginal epithelium, and achieves higher endometrial tissue concentrations than those achieved with progesterone in oil (PIO) given im.

Objective: Our objective was to examine the pharmacokinetics and pharmacodynamics of PVI compared with PIO.

Design, Setting, and Participants: Fifty-eight healthy premenopausal women were randomized to 50, 100, or 200 mg PVI once daily; 100 or 200 mg PVI twice daily; or 50 to 100 mg PIO via im injection once daily for 10 days. Serum samples were obtained after the first dose; serum and endometrial tissue were obtained after the last dose.

Main Outcome Measures: Maximum observed serum concentration (C_{max}), time to C_{max}, and area under the serum-concentration time curve over the dosing interval were calculated after correcting for baseline progesterone concentrations. ANOVA and paired *t* test were used to compare results across and within groups.

Results: A higher C_{max} was observed after PIO than PVI administration. Endometrial tissue progesterone concentrations were higher for PVI regimens. Time to C_{max} was 7.3 hours after PIO and 3.3 to 5.9 hours after PVI. Steady state was achieved within 24 and 48 hours for PVI and PIO regimens, respectively. The area under the curve increased with increasing PVI dosage; however, the increase was not proportional to the increase in dosage. Downregulation of estrogen and progesterone receptors was observed in secretory biopsy specimens.

Conclusion: The PVI system consistently allowed for rapid progesterone absorption and achieved higher endometrial tissue concentrations and lower systemic exposures than observed after im PIO. (*J Clin Endocrinol Metab* 99: 4241–4249, 2014)

Progesterone produced by the corpus luteum during the luteal phase is necessary to promote secretory transformation of endometrial tissue in preparation for embryo implantation (1–3). Administration of GnRH agonists or antagonists to patients undergoing in vitro fertilization (IVF) may adversely affect luteal function and endometrial transformation (4, 5). GnRH agonists or antagonists have

been shown to prematurely initiate luteolysis and limit the duration of the luteal phase (5, 6). Consequently, pregnancy rates during IVF are diminished in the absence of luteal phase support (6). Therefore, optimal luteal phase support is vital, especially when GnRH agonists or antagonists are used. The use of exogenous progesterone to establish a secretory endometrium has been shown to pre-

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Abbreviations: AE, adverse event; AUC, area under the serum-concentration time curve; BMI, body mass index; CL/F, apparent clearance; C_{max}, maximum observed serum concentration; IVF, in vitro fertilization; PD, pharmacodynamics; PIO, progesterone in oil; PK, pharmacokinetics; PVI, progesterone vaginal insert; T_{max}, time (in hours) at which C_{max} occurred; TVU, transvaginal ultrasonography.

pare the uterus for embryo implantation and pregnancy support (7, 8).

Progesterone products are available for oral, rectal, im, and vaginal administration (9). Progesterone administered orally undergoes extensive first-pass metabolism in the liver, which limits its efficacy for luteal support (8, 10). Administration of progesterone by im injection or vaginally avoids first-pass metabolism and achieves higher concentrations in endometrial tissue (10–12). Serum progesterone concentrations achieved with im administration are higher than those achieved with vaginal administration; however, serum concentrations have not been shown to correlate with endometrial transformation or the ability to establish and support a pregnancy (3, 8, 13). Currently, im and vaginal administration of progesterone are the primary routes used for luteal phase support in patients undergoing assisted reproductive technology (9, 13). Both the im and vaginal routes of administration appear to result in similar pregnancy rates (7, 11, 13–17). When administered vaginally, progesterone is preferentially absorbed by uterine endometrial tissue, whereas a small percentage is distributed into the systemic circulation (18). Miles and colleagues (18) were the first to identify the preferential uptake of progesterone by endometrial tissue after vaginal administration. Concentrations of progesterone were approximately 10 times higher in endometrial tissue after vaginal administration of micronized progesterone capsules when compared with progesterone given im. De Ziegler (32) and colleagues were the first to describe preferential extraction of progesterone by uterine tissues after vaginal administration as the first uterine pass effect. Bulletti and colleagues (19) later confirmed accumulation of progesterone in uterine and vaginal tissues using a human *ex vivo* perfusion model with intact uteri obtained at hysterectomy. Uptake of radiolabeled progesterone in endometrial tissue and myometrium was greater when tissue was obtained during the secretory phase than in the proliferative phase of the menstrual cycle (19). Differences in tissue vascularity, number of progesterone receptors in endometrial tissue, and the pattern of uterine contractions may account for a dissimilarity in uptake of progesterone by endometrial tissue in the secretory compared with the proliferative phases (19). Preferential absorption of progesterone into endometrial tissue with relatively low associated serum concentrations was further confirmed by Cicinelli and colleagues (12) in endometrial tissue obtained from women who received vaginal progesterone before hysterectomy.

When comparing im vs vaginally administered progesterone, other factors are also important to consider, such as the inconvenience of daily painful injections and potential dermal inflammation/infections, including sterile

abscesses at the injection site (3, 7, 20). When surveyed, most women prefer vaginal administration of progesterone over im injections (1, 20–22). Several dosage forms of progesterone are available for vaginal administration including a gel, tablets, extemporaneously compounded suppositories, and a unique micronized progesterone vaginal insert (PVI) (Ferring Pharmaceuticals, Inc) (23). However, unlike vaginal gel, which accumulates in the vagina with repeat administration and slowly releases progesterone, PVI disintegrates, allowing for rapid absorption of progesterone (7, 24, 25).

PVI contains progesterone in a base containing lactose monohydrate, adipic acid, and sodium bicarbonate (23). When PVI encounters vaginal moisture, an acid-base reaction takes place; the insert disintegrates, generating water and sodium bicarbonate, which releases carbon dioxide (25, 26). Carbon dioxide has been shown to enhance absorption of drugs across epithelium (27). Thus, this unique formulation allows for both rapid dissolution and rapid absorption of progesterone from PVI.

Rapid absorption of progesterone has been demonstrated previously with administration of 50- and 100-mg doses of PVI in non-estrogen-primed postmenopausal women (28). Blake and colleagues (29) compared the pharmacokinetics (PK) of PVI to 8% progesterone vaginal gel. PVI reached a higher maximum observed serum concentration (C_{max}), greater systemic exposure (area under the serum-concentration time curve from time 0–24 hours [AUC_{0-24}]), and achieved steady state faster than the gel (29).

The objective of this study was to fully characterize the PK and pharmacodynamics (PD) of 50-, 100-, and 200-mg doses of PVI administered either once or twice daily compared with progesterone in oil (PIO) at 50 mg/ml administered im once daily.

Subjects and Methods

This was a randomized, open-label PK and PD study of a unique formulation of micronized PVI in healthy premenopausal women conducted at 2 U.S. sites. The study included 4 phases: screening, downregulation, estrogen priming, and randomization (Figure 1A). This study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, in compliance with the approved protocol, good clinical practice guidelines, and applicable regulatory requirements. The protocol was approved by the Essex Institutional Review Board, Lebanon, NJ, and the Institutional Review Board of the University of Miami, Miami, FL. Study personnel obtained written informed consent directly from all women before their entry into the study.

Healthy, premenopausal women 18 to 40 years of age with an intact uterus; regular menstrual cycles (24–35 days), and a body mass index (BMI) of 18 to 28 kg/m² were eligible for entry into

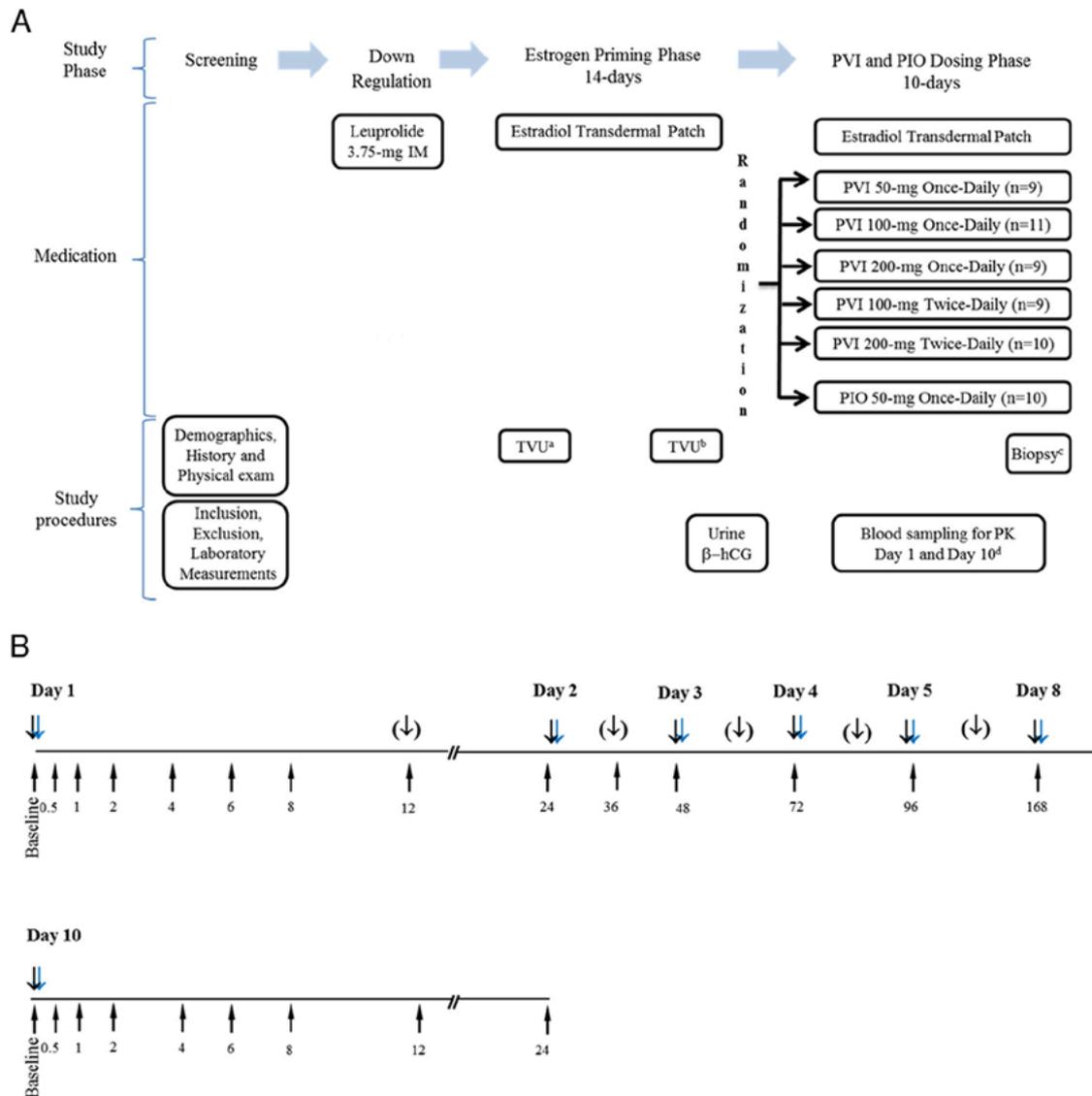


Figure 1. A, Study phases and randomization schema: ^a TVU at end of downregulation; ^b TVU at end of estrogen priming; ^c endometrial biopsy on day 10; ^d overnight stay required for blood sampling for PK on day 1 (first dose) and day 10 (last dose at end of study). B, Timing of PK sampling. Numbers indicate the time in hours. Upward arrows indicate the time (in hours) of blood sampling. Downward arrows indicate timing of PVI once daily (downward arrow) and twice daily (downward arrow in parentheses) or PIO (blue downward arrow) administration.

the study. Patients were screened based on inclusion/exclusion criteria, medical history, findings on physical examinations, gynecological examinations, and vital sign measurements. Clinical safety laboratory tests performed included urine drug screening, HIV testing, hepatitis B and C testing, and a urine pregnancy test. Transvaginal ultrasonography (TVU) and concomitant medication reviews were performed at screening and at specified times during the study (Figure 1A). For simplicity, women received a single im injection of 3.75 mg leuprolide acetate (depot formulation) approximately 18 to 23 days after the first day of the start of menses to suppress endogenous hormonal production. This is dissimilar from the current treatment practice of administering a lower daily dose of immediate-acting leuprolide acetate or GnRH antagonists for pituitary downregulation to women undergoing cycles of assisted reproductive technology in the United States. However, depot GnRH agonist formulations are frequently used in other parts of the world. Approximately 2 to 4

days after menstrual bleeding began, women returned to the study center for blood sampling and TVU. Estrogen priming of the endothelium was initiated if a patient's serum estradiol concentration was ≤ 50 pg/mL, progesterone concentration was ≤ 2 ng/mL, and endometrial lining was ≤ 7 mm in thickness. For estrogen priming, estradiol transdermal patches (Climara 0.1 mg; Bayer Healthcare Pharmaceuticals, Inc), with increasing concentration of estradiol, were applied for 14 days (1 patch for 4 days, 2 patches for 5 days, and 3 patches for 5 days).

To be eligible for randomization, women were required to have a serum progesterone concentration of ≤ 1 ng/mL and endometrial thickness ≥ 7 mm. Patients were randomly assigned to 1 of 5 PVI dosing groups (50, 100, or 200 mg once daily or 100 or 200 mg twice daily) or to the im PIO dosing group (50 mg/mL) during the 10-day dosing phase. Study site personnel instructed patients on proper administration technique for im injection of PIO or vaginal insertion of PVI depending on their randomization group.

All randomized patients continued to apply 1 estradiol transdermal patch per week during the 10-day PVI and PIO dosing period. Compliance was monitored throughout the study by the return of empty, partially used, and unused PVI blister packs or vials of PIO at each visit. Any discrepancies with the drug accountability log were discussed with the study participant at the time of the return of the blister packs or vials.

Blood samples collected for PK analysis of serum progesterone concentrations were obtained before the first dose of PVI or PIO and at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 168 hours after the dose (Figure 1B). On day 10, blood samples were obtained immediately before the last dose of study drug and at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after the dose (Figure 1B). Serum samples were immediately frozen at -20°C and shipped in dry ice to a central laboratory for determination of serum progesterone concentration by RIA (18). Endometrial tissue samples collected for progesterone concentration measurement were obtained during biopsy on day 10 of the dosing phase (Figure 1). After homogenization of the endometrial tissue, RIA was used to determine endometrial tissue progesterone concentration.

PK parameters derived on day 1 (after the first PVI or PIO dose) and day 10 (last PVI or PIO dose) included maximum observed serum concentration within the dosing interval, time (in hours) at which Cmax occurred (Tmax), AUC_{0-T} (where T is time), computed using the linear trapezoidal rule over a dosing interval (AUC₀₋₁₂ or AUC₀₋₂₄, depending on whether the dosing was twice daily or once daily, respectively), and the apparent clearance [apparent clearance (CL/F), liters per hour].

PD parameters examined included endometrial progesterone and estrogen receptor content and endometrial thickness. On day 10 ± 1 of the dosing phase (final visit), endometrial tissue was obtained using procedures outlined in a biopsy manual provided by DCL Medical Laboratories, Inc. The cervix was swabbed carefully to avoid contamination of the curette with residual progesterone. The tip of the curette was sectioned prox-

imal to its distal opening before endometrial tissue was expressed into the collection container. TVU was used to determine endometrial thickness. Endometrial estrogen and progesterone receptor content was analyzed by immunohistochemistry.

Statistical analysis

Dosing group comparisons for demographics, baseline characteristics, and endometrial tissue concentrations were analyzed using descriptive statistics, Fisher’s exact test, and one-way ANOVA. ANOVA was performed on the natural logarithm of Cmax and AUC. Outcomes for each dosing group were compared with outcomes from the PIO reference group based on Cmax, AUC₀₋₁₂ (twice-daily dosing), and AUC₀₋₂₄ (once-daily dosing) analysis. All statistical analyses were performed using SAS version 8.2 software (SAS Institute, Inc).

Results

Patient demographics and disposition

Patient demographic characteristics were similar across dosing groups; no statistical differences were found among dosing groups for age, race, BMI, or smoking history. A significant difference was found in the mean height among groups (P < .02), but this was not considered to be clinically meaningful (Table 1). Most women were Hispanic (84%), the mean age was 31.1 year, and the mean BMI was 24.3 kg/m². Most women had never smoked (95%).

Of the 58 women who were enrolled in the study, all but 1 completed the study. This woman, who was in the 100-mg twice-daily dosing group, discontinued on day 1

Table 1. Demographic Characteristics

Characteristic	PVI Once Daily			PVI Twice Daily		PIO im, 50 mg/mL (n = 10)	P Value
	50 mg (n = 9)	100 mg (n = 11)	200 mg (n = 9)	100 mg (n = 9)	200 mg (n = 10)		
Age, y							
Mean ^a	32.2 (7.81)	30.4 (6.58)	33.2 (5.80)	29.1 (7.17)	31.3 (6.46)	30.7 (5.12)	.812 ^b
Min, Max	19, 40	18, 38	22, 40	19, 39	21, 40	24, 38	
Race, n (%)							
Hispanic	6 (67)	10 (91)	9 (100)	7 (78)	9 (90)	8 (80)	.630 ^c
Caucasian	2 (22)	1 (9)	0 (0)	2 (22)	1 (10)	2 (20)	
Black	1 (11)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
BMI, kg/m ²							
Mean ^a	25.3 (3.09)	24.4 (3.52)	24.4 (2.60)	23.0 (3.19)	24.2 (2.78)	24.4 (3.00)	.783 ^b
Min, Max	21, 28	20, 28	20, 28	19, 29	21, 28	19, 28	
Height, cm							
Mean ^a	165.1 (9.07)	159.6 (4.07)	156.6 (5.96)	161.1 (4.42)	157.2 (5.99)	157.5 (5.35)	0.02 ^b
Min, Max	157, 180	152, 168	147, 165	152, 168	145, 165	150, 170	
Has subject ever smoked?							
No	8 (89)	9 (82)	9 (100)	9 (100)	10 (100)	10 (100)	.381 ^c
Yes	1 (11)	2 (18)	0 (0)	0 (0)	0 (0)	0 (0)	

Abbreviations: Max, maximum; Min, minimum.

^a Values are mean (SD).

^b P value from one-way ANOVA.

^c P value from Fisher’s exact test.

due to an adverse event (AE) (leg pain) not considered by the investigator to be related to the study drug. Mean compliance ranged from 97.5% to 100%, with an individual range from 78% in the 200-mg once-daily dosing group to 100% in the 100-mg once-daily dosing group.

Pharmacokinetics

On day 1, serum progesterone concentrations increased to a C_{max} of 8.06 ± 2.43 , 8.29 ± 2.87 , 11.5 ± 3.9 , 8.06 ± 3.57 , and 11.3 ± 4.0 ng/mL in the 50-, 100-, and 200-mg once-daily dosing groups and 100- and 200-mg twice-daily PVI groups, respectively (Table 2). In the PIO group, C_{max} was 20.0 ± 5.3 ng/mL on day 1 (Table 2). The PVI 200-mg once-daily and twice-daily dosing groups had an approximately 50% higher C_{max} (Table 2) and mean serum progesterone concentration (Figure 2A) when compared with the 50- and 100-mg once-daily dosing groups and the 100-mg twice-daily dosing group. The C_{max} (Table 2) and mean serum progesterone concentration at each time point were also higher for the PIO group when compared with all PVI groups (Figure 2). The maximum serum progesterone concentrations were reached for all dosing groups from 8 to 12 hours (T_{max}) after administration, with a steady decline over the next 12 to 24 hours (Figure 2, A and B). Mean T_{max} values after the first dose ranged from 7.3 hours for the 100-mg PVI twice-daily dosing group to 12.0 hours for the 200-mg once-daily dosing group (Table 2). For the PIO group, T_{max} was 8.2 hours (Table 2).

When the PVI groups were compared, peak serum progesterone concentrations were not dose proportional after the first dose of progesterone. The mean C_{max} on day 1 for the 50-mg dose group was approximately the same as for the 100-mg once-daily dose group and 100-mg twice-daily dose group (Table 2). The mean C_{max} value for the 200-mg once-daily dose group and 200-mg twice-daily dose group was only approximately 40% greater than the

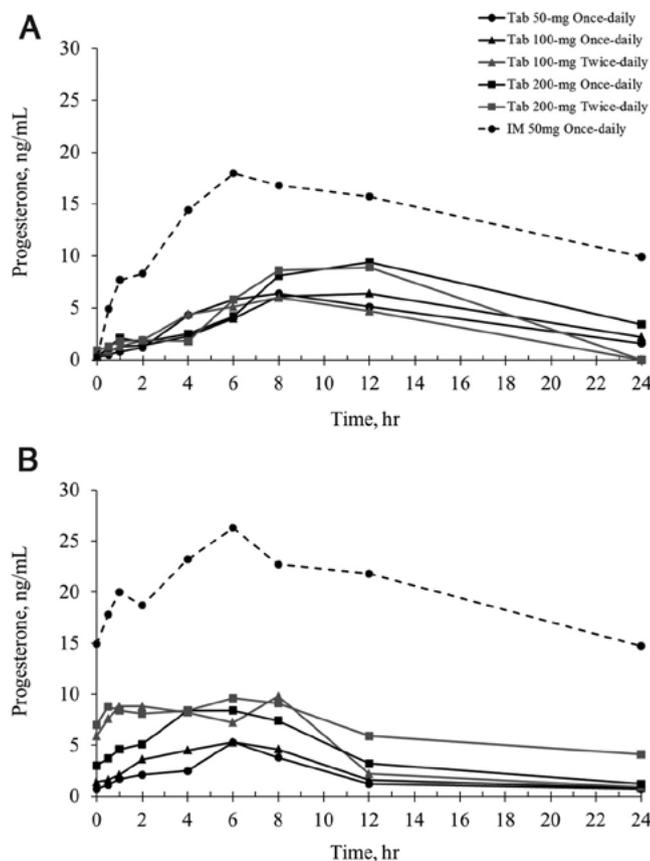


Figure 2. Mean serum progesterone concentration vs time curve, A, First dose. B, Last dose on day 10.

C_{max} for the 100-mg dose (Table 2). The lack of dose proportionality could not be explained by endogenous progesterone production. Mean baseline endogenous serum progesterone concentrations ranged from 0.28 to 0.87 ng/mL for all 6 dosing groups on day 1 and 0.26 to 0.29 ng/mL on day 10. C_{max} and AUC were corrected for the day-1 and -10 baseline serum progesterone concentration.

Mean AUCs ranged from 50.5 ng·h/mL for the 100-mg twice-daily dose group to 138 ng·h/mL for the 200-mg

Table 2. Serum Progesterone PK Parameters^a

Parameter	PVI Once Daily			PVI Twice Daily		PIO im, 50 mg/mL (n = 10)
	50 mg (n = 9)	100 mg (n = 11)	200 mg (n = 9)	100 mg (n = 9)	200 mg (n = 10)	
Single-dose PK parameters, d 1						
C_{max} , ng/mL	8.06 ± 2.43	8.29 ± 2.87	11.5 ± 3.9	8.06 ± 3.57	11.3 ± 4.0	20.0 ± 5.3
T_{max} , h	7.56 ± 2.96	10.7 ± 4.9	12.0 ± 4.9	7.33 ± 3.16	10.2 ± 2.4	8.20 ± 2.74
AUC_{0-T} , ng·h/mL	92.3 ± 20.7	98.8 ± 33.8	138 ± 35	50.5 ± 20.7	64.1 ± 27.9	320 ± 67
Multiple-dose PK parameters, d 10						
C_{max} , ng/mL	6.59 ± 2.39	7.70 ± 1.92	12.5 ± 2.8	13.2 ± 8.3	13.0 ± 4.5	30.3 ± 7.6
T_{max} , h	5.44 ± 2.07	5.45 ± 2.21	4.73 ± 3.08	3.31 ± 3.43	5.88 ± 4.13	7.15 ± 2.23
AUC_{0-T} , ng·h/mL	45.7 ± 11.8	58.8 ± 19.3	102 ± 25	89.7 ± 45.7	99.6 ± 44.8	485 ± 72
CL/F, L/h ^b	1367 ± 348	2512 ± 2083	2283 ± 799	1432 ± 664	3218 ± 3384	106 ± 15

^a All data are presented as mean \pm SD.

^b Apparent clearance.

once-daily dose group. For the PIO dosing regimen, the AUC was 320 ng·h/mL. AUCs showed a less-than-dose-proportional increase in drug exposure with increasing dose. For the 50-, 100-, and 200-mg once-daily dose groups, AUC ratios were 1:1.33:2.43 compared with their dosing ratios of 1:2:4. For the twice-daily dose groups, AUC ratios were 1:1.11 compared with their dosing ratios of 1:2.

Based on AUC_{0-T} (AUC from time 0 to end of the dosing interval) comparisons, bioavailability of PVI ranged from about 4% to 8%, with no consistent pattern of lower bioavailability being associated with either higher or lower doses of progesterone or with once-daily vs twice-daily dosing.

PK at steady state (24 hours) was determined for all groups on day 10 of the study. Tmax was attained earlier on day 10 than on day 1 for the PVI and PIO groups (Figure 2 and Table 2). For the PIO group, serum progesterone concentrations increased rapidly during the first 4 hours after administration of progesterone; median peak concentrations with PVI were reached by most groups 2 to 6 hours earlier on day 10 than on day 1.

Pharmacodynamics

At the end of the priming phase, the mean estradiol concentration in most groups was approximately 200 pg/mL or greater (ranging from 170–290 pg/mL); in the 200-mg once-daily dose group, the mean estradiol concentration was approximately 90 pg/mL (Table 3). At the

end of priming, endometrial thickness was not significantly different across treatment groups (*P* = .429) (Table 3). Endometrial thickness from the end of priming to day 10 significantly decreased in the 100- and 200-mg once-daily dose groups and 200-mg twice-daily PVI group and significantly increased in the PIO group Table 3.

Mean endometrial tissue concentrations were significantly higher in the PVI groups (*P* < .001) than in the PIO group (Figure 3). The mean endometrial tissue progesterone concentration was 75.9 ng/mg protein in the once-daily and 51.6 ng/mg protein in the PVI twice-daily dosing groups compared with 0.71 ng/mg protein in the PIO im group (Figure 3).

Endometrial progesterone and estrogen receptor content was analyzed by ChromaVision (Clariant, Inc). ChromaVision is an image analysis system that creates digital images of tissue slides and performs quantitative analyses based on the size and staining properties of selected cells. Suppression of progesterone and estrogen receptors was observed in all secretory endometrial tissue.

Safety

Across all PVI groups (n = 48), the most common AEs, regardless of their relatedness to study drug, were headache and dysmenorrhea, each reported by 4 women; nausea was reported by 3 women. The most common AE in the PIO group (n = 10) was headache, reported by 3 women. AEs considered as possibly related to the study drug were mild headache and a mild hot flush in 1 woman in the

Table 3. Estradiol Serum Concentrations and Endometrial Thickness at the End of the Priming Phase and on Day 10 of PVI and PIO Dosing Phase

Parameter	PVI Once Daily			PVI Twice Daily		PIO im, 50 mg/mL (n = 10)
	50 mg (n = 9)	100 mg (n = 11)	200 mg (n = 9)	100 mg (n = 9)	200 mg (n = 10)	
End of priming						
Estradiol, pg/mL						
Mean ^a	254.2 (205.3)	197.8 (176.5)	88.4 (49.7)	289.6 (97.5)	170.3 (50.4)	209.2 (133.0)
Median	199.9	136.5	82.0	315.0	168.0	187.1
Min, Max	93.0, 717.0	32.0, 613.0	32.0, 190.0	142.0, 410.0	98.0, 232.5	49.0, 492.0
Endometrial thickness, mm						
Mean ^a	8.8 (3.1)	9.2 (3.2)	9.3 (1.8)	10.9 (3.2)	11.2 (3.5)	10.3 (3.2)
Median	9.0	7.9	9.1	10.0	11.3	9.6
Min, Max	3.9, 13.1	5.8, 15.0	5.8, 12.0	5.5, 17.1	6.4, 16.3	6.8, 18.0
PVI and PIO dosing phase, d 10						
Estradiol, pg/mL						
Mean ^a	85.2 (51.6)	56.0 (26.9)	102.0 (27.0)	156.6 (104.5)	147.1 (103.9)	60.3 (40.5)
Median	69.0	51.0	93.0	140.0	135.0	60.0
Min, Max	29.0, 190.0	10.0, 110.0	61.0, 150.0	5.0, 310.0	32.0, 390.0	12.0, 140.0
Endometrial thickness (mm)						
Mean ^a	7.5 (3.7)	6.2 (2.0)	7.2 (2.3)	8.4 (3.6)	10.2 (4.1)	12.4 (4.2)
Median	6.3	6.1	7.0	7.5	9.7	12.1
Min, Max	3.2, 14.4	2.0, 9.4	3.4, 11.0	5.1, 16.8	4.9, 15.9	4.4, 19.5

Abbreviations: Max, maximum; Min, minimum.

^a Values are mean ± SD.

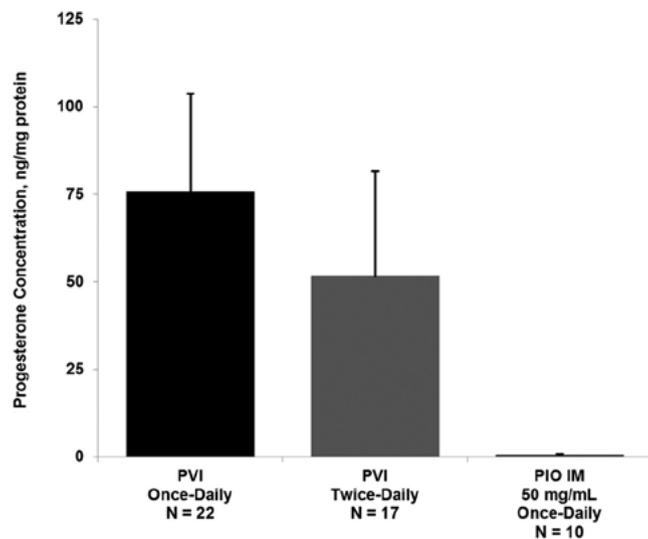


Figure 3. Mean endometrial tissue progesterone concentrations (ng/mg protein) for PVI once or twice daily and im PIO once daily dosage regimens. *, $P < .001$ from paired t test.

200-mg twice-daily dose group and a moderate headache reported by 1 woman in the PIO group. No serious AEs were reported during the study.

Discussion

This study showed that serum concentrations of progesterone after PVI administration rapidly reach the systemic circulation, with detectable levels measured in as little as 30 minutes. Furthermore, progesterone was systemically bioavailable in all 6 dosing groups throughout the 10 days of dosing. The mean C_{max} , T_{max} , and AUC derived for PVI on day 1 (after the first 100-mg dose) in this study were similar to those observed by Blake et al (29) for the same dosage. The rate of progesterone absorption into the systemic circulation was slower on the first day of PVI administration when compared with absorption noted on day 10. This suggests that vaginal progesterone itself, the pharmaceutical vehicle, or fluctuations in other endogenous hormone concentrations, may have an effect on the vaginal tissue with respect to progesterone absorption. Likewise, accumulation of progesterone in endometrial tissue and serum, occupation of the available binding sites, and downregulation of receptors in endometrial tissue with repeated dosage may account for a more rapid entry of progesterone into the systemic circulation on day 10 when compared with day 1 with PVI. Increasing doses of PVI did not proportionally increase systemic progesterone concentrations. Disproportionate absorption has been observed with other vaginally administered progesterone preparations, and Archer et al (30) posited the absorption of progesterone by the vaginal epithelium may be a rate-limited process.

Assuming that the 50-mg PIO administered im is 100% bioavailable, PVI demonstrated an approximately 4% to 8% relative systemic bioavailability. However, this low systemic bioavailability is not indicative of a reduced biologic effect. Experiments have shown a uterine first-pass effect with vaginal administration, whereby progesterone is preferentially absorbed by the endometrium before entering the general circulation (12, 31). The amount of progesterone in the systemic circulation appears to be less relevant, especially when implantation, pregnancy, and live birth outcomes appear similar for im and vaginal administration (7, 11, 14–17). An accurate estimate of the fraction of administered dose that reaches the systemic circulation after vaginal administration is difficult. Therefore, CL/F was calculated for all PVI regimens. Mean CL/F for PVI ranged from approximately 1400 to 3200 L/h. This result implies that only a fraction of the administered PVI dose reached the systemic circulation. The apparent clearance of PIO given im may indicate 100% bioavailability.

Similar to previous studies comparing endometrial tissue concentrations of progesterone after vaginal and im administration, this unique formulation of micronized progesterone in a vaginal insert achieved significantly higher endometrial tissue concentrations than those seen after PIO im (18, 19, 32, 33). The mean progesterone concentration in endometrial tissue was significantly higher after PVI was given once ($P < .001$) or twice daily ($P < .001$) when compared with PIO im given once daily (Figure 3). However, the mean progesterone concentration in endometrial tissue was not dose proportional. Endometrial biopsies were performed on day 10 of progesterone administration; however, the timing of endometrial tissue sampling was not strictly controlled relative to the administration of PVI or im PIO. Contamination of the endometrial tissue with residual progesterone during biopsy was unlikely; therefore, the lack of dose proportionality in endometrial tissue concentrations may reflect variations in timing of endometrial tissue sampling relative to the time of dosage administration. It is also possible that absorption of vaginal progesterone is limited by individual patient variability and that at these high doses, additional progesterone administration (such as twice-daily) does not appreciably affect the amount that is absorbed.

A clear cutoff for an optimal endometrial thickness has not been established. Most clinicians consider endometrial thickness of >7 to 8 mm acceptable. However, acceptable pregnancy rates have also been achieved in women with an endometrial thickness <6 mm (34). In this study, all dosing groups achieved a mean endometrial thickness of >7 mm at the end of the estrogen priming phase, which is consistent with the support of good preg-

nancy rates. These measurements also substantiate that the progesterone absorption experiments were performed in a clinically relevant setting.

Endometrial biopsy specimens obtained during the secretory phase showed a suppression of progesterone receptors. This is consistent with the action of progesterone in the suppression of its own receptor in the endometrium during the secretory phase (35). Nearby stromal cells continue to express progesterone receptors and are thought to produce stromal-derived growth factors that act on uterine luminal and superficial glandular epithelia during the peri-implantation period (35).

Substantial estrogen receptor downregulation was seen in the secretory endometrial biopsy specimens. Estrogen receptors are self-regulated by estrogen, and normally estrogen receptors are at the highest levels during the late proliferative phase (days 10–14) and decline during the secretory phase (36). This estrogen-receptor result is consistent with expected results from endometrial biopsy specimens obtained during the secretory phase (35).

Progesterone administered by the oral route has a short serum half-life (approximately 5 minutes); it is rapidly metabolized to 17-hydroxyprogesterone during its first pass through the liver (37). Progesterone administered by the im route avoids extensive first-pass hepatic metabolism; consequently, endometrial tissue concentrations of progesterone achieved with im administration are higher when compared with oral administration (10–12). However, the highest concentrations of progesterone in endometrial tissue are achieved with vaginal administration (12, 18).

Few AEs and no serious AEs were reported in the participating groups in this study. However, it should be duly noted that this PK/PD study was not designed to inspect safety or efficacy of progesterone for women undergoing fertility treatments. One patient in the 200-mg twice-daily dosing group reported mild hot flush and mild headache, considered by investigators as probably related to the study drug. PIO injections have been shown to have the potential for serious AEs. However, AEs reported during a 10-day PK/PD study are not likely representative of AEs expected when PIO is given for longer periods during a typical IVF cycle. These AEs may include (as reported) injury to the sciatic nerve, with resulting sensory and motor dysfunction, injection-site/inflammatory reactions, abscesses, allergic reactions to the oil vehicle, and patient discomfort (1, 21, 38).

To date, this is the most comprehensive study of PK and PD with im and vaginally administered progesterone. Our data showed that all tested regimens of PVI provided higher endometrial tissue concentrations and produced lower systemic progesterone exposures than did the im-

administered dosing regimen. Progesterone rapidly reached the systemic circulation after PVI was administered, with detectable levels measured in as little as 30 minutes, achieving steady state within 24 hours. Increasing doses of PVI did not proportionally increase systemic progesterone concentrations. Progesterone and estrogen receptor downregulation was evident in endometrial tissue specimens in the secretory phase. PK and endometrial tissue concentrations were similar after once-daily and twice-daily administration of PVI. Whereas this observation does not imply similar clinical efficacy, it does suggest that future studies should address whether multiple daily dosing regimens are clinically superior to single doses.

Conclusion

PVI is a unique vaginal delivery system that dissolves upon contact with vaginal moisture. Thus, PVI allows rapid absorption of progesterone across the epithelium and achieves a high concentration of progesterone in endometrial tissue while limiting systemic exposure.

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