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# Peripheral progesterone (P) levels and endometrial response to various dosages of vaginally administered P in estrogen-primed women

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**Objective:** To compare the pharmacokinetics and pharmacodynamics of 100 mg/d, 200 mg/d, and 400 mg/d (200 mg two times per day) of P administered vaginally for 14 days to estrogen-primed postmenopausal women.

**Design:** Randomized, open-label, three-way crossover study.

**Setting:** Two university-based investigative sites.

**Patient(s):** Twenty healthy postmenopausal women with histologically normal endometria.

**Intervention(s):** Oral 17$\beta$-$E_2$ was given each day of a 28-day cycle; a P vaginal suppository was inserted daily according to the randomization schedule during days 15–28 of each cycle; blood samples were collected; an endometrial biopsy was obtained on day 25; and patients were crossed over to the next treatment cycle after a washout period of at least 30 days.

**Main Outcome Measure(s):** Mean P blood levels, endometrial dating/conversion.

**Result(s):** There was good vaginal absorption of P for all dosages. Endometrial conversion occurred in all 200- and 400-mg/d P–dosed cycles, whereas the 100-mg/d dosage failed to convert primed endometria consistently. There also was a significantly increased tendency for earlier bleeding and spotting with the 100-mg/d dosage.

**Conclusion(s):** Both the 200- and 400-mg/d dosage regimens consistently convert an estrogen-primed endometrium, and yield appropriate endometrial dating and bleeding patterns. However, the 400-mg/d dosage attains the highest sustained blood levels and may be the best dosage regimen for further study. (Fertil Steril® 1997;68:810–15. © 1997 by American Society for Reproductive Medicine.)

**Key Words:** Vaginal P, endometrial conversion, pharmacokinetics

Advances in assisted reproductive technology have led to the increased use of donor oocytes for women without ovaries, women with premature ovarian failure, and even menopausal women more than 50 years old (1–3). With oocyte donation, there is the need for exogenous hormonal endometrial support during early pregnancy. Progesterone administered orally, IM, and by vaginal suppository has been used successfully for this purpose in patients undergoing IVF for luteal phase insufficiency (4, 5).

Vaginal P suppositories have the advantage over oral P in that the latter has limited bioavailability (6, 7). Vaginal delivery of P avoids first-pass metabolism in the liver, which theoretically provides sustained blood levels and subsequently higher endometrial concentrations of P with lower dosages (7–9).

In addition, the administration of a vaginal P suppository is less painful than IM administration, and

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allows for variable administration because the patient or spouse can administer the product (10, 11). However, vaginal P is not available on the market. Many physicians, therefore, presently use ersatz preparations. The delivery of P with these "informal" systems is unpredictable because the wide varieties of formulations are difficult or impossible to control, and their safety and effectiveness also are unpredictable. In addition to the lack of quality control in the preparation of vaginal suppositories, there also is a lack of clinical data on their bioavailability, safety, and effectiveness in maintaining pregnancy (11, 12).

The purpose of this study was to compare the pharmacokinetics and pharmacodynamics of daily dosages of 100, 200, and 400 mg (200 mg two times per day) of $P_4$ administered vaginally for 14 days to estrogen-treated postmenopausal women. The dosage form proposed in this study has been manufactured in a validated, controlled manner.

This dose-response trial was designed to help determine the safety and efficacy of three different dosages of P vaginal suppositories for pregnancy maintenance in women undergoing IVF primary ovarian failure or luteal phase defects.

Postmenopausal women were used to avoid any potential confounding endogenous P contribution from the ovary. The suitability of postmenopausal patients for this study is well supported by the literature, which demonstrates that a properly primed postmenopausal endometrium can support a pregnancy to term (3, 12, 13).

This was a multicenter, randomized, open-label, pharmacokinetic/pharmacodynamic study with a three-way crossover design. The study was conducted at two investigative sites: UMDNJ—Robert Wood Johnson Medical School and the University of Alabama-Birmingham.

## MATERIALS AND METHODS

The protocol was reviewed and approved by the UMDNJ-Robert Wood Johnson Medical School's and University of Alabama School of Medicine's IRB's. An informed consent was obtained from each subject.

### Subject Selection

Postmenopausal women (no vaginal bleeding for >6 months) in good health, <70 years of age, with an intact uterus, receiving no hormonal therapy for at least 30 days before dosing, and within 40% of their ideal body weight (Metropolitan Weight Table) were recruited through various advertisements and referrals by area physicians. Women with medical histories associated with accepted contraindications to hormone replacement therapy were excluded.

### Screening

The 25 subjects who met the selection criteria underwent further screening, including a physical examination and Papanicolaou smear, a complete blood count with differential, a chemistry profile, a urinalysis, an $E_2$ level, a P level ($P_4$), a follicle-stimulating hormone (FSH) level, an endometrial biopsy, a transvaginal ultrasound (US) examination, and a mammogram. Two women were excluded because of ovarian pathology detected on US. One woman was excluded because of FSH screening levels below the testing laboratory's (SmithKline Beecham, Van Nuys, California) acceptable range for postmenopausal women (>34.3 mIU/mL). One woman was excluded because of a markedly proliferative endometrium. One woman elected not to participate in the study despite adequate screening. Three women with screening endometrial biopsies consistent with moderate hyperplasia were treated with 10 days of medroxyprogesterone acetate (Provera; Pharmacia-Upjohn, Kalamazoo, Michigan) and had subsequent normal repeated biopsies.

### Treatment

Twenty subjects were entered into the study and randomly assigned to one of three P vaginal treatment sequences, all of which included 100-, 200-, and 400-mg/d (200 mg two times per day) dosing regimen cycles (Table 1). The treatment schedule was as follows.

On day 1 of their treatment cycle, subjects were given 17$\beta$-$E_2$ (Estrace; Bristol-Myers Squibb, Princeton, New Jersey) with the following dosing schedule: 1 mg/d on days 1–5, 1 mg every 12 hours on days 6–9, 1 mg every 8 hours on days 10–14, and 1 mg every 12 hours on days 15–28. Periodic $E_2$ levels were monitored to ensure compliance with the dosing regimen.

On day 15, after a transvaginal US, subjects were admitted to a clinical research center, where a baseline blood sample was drawn. After a vaginal exami-

**Table 1** Progesterone Vaginal Treatment Sequences

| Sequence I | Sequence II | Sequence III |
|---|---|---|
| 100 mg/d | 200 mg/d | 400 mg/d |
| ⇓ | ⇓ | ⇓ |
| washout | washout | washout |
| 200 mg/d | 400 mg/d | 100 mg/d |
| ⇓ | ⇓ | ⇓ |
| washout | washout | washout |
| 400 mg/d | 100 mg/d | 200 mg/d |

nation, a $P_4$ vaginal suppository was inserted by the clinician according to the subject's treatment sequence dosage. Blood was drawn for determination of $P_4$ and $E_2$ levels at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hours after insertion. On days 16, 19, 22, and 25, blood was drawn for determination of $P_4$ and $E_2$ levels, and then was drawn again 2 hours after the $P_4$ vaginal suppository was inserted.

On day 25, a vaginal examination and endometrial biopsy also were performed. On day 28, the schedule for day 15 was repeated. On day 29, a blood sample for determination of $P_4$ and $E_2$ levels was obtained. On day 35, an interval history and vital signs were taken.

All subjects completed a menstrual bleeding calendar for each treatment cycle. After each treatment cycle, subjects underwent a drug-free interval lasting between 28 and 45 days. Each subject then was crossed over to another randomized treatment regimen. Thus, each subject who completed the entire protocol received all three treatment dosages (Table 1).

Progesterone ($P_4$) levels for each regimen were tabulated. Mean $P_4$ blood levels were used to calculated half-life, time to peak concentration, peak plasma concentration, and area under the plasma concentration time curve.

All endometrial biopsies were collected using a Gynosampler (Gynetics Inc., Somerville, New Jersey) and were sent for interpretation to the same designated blinded pathologist for interpretation. All endometrial specimens were dated according to the criteria developed by Noyes et al. (14). Dosage-related conversion to a secretory endometrium was evaluated histologically. Differences between apparent dates and the chronologic dates of the endometrium were compared for each dosage group.

An analysis of variance was used to compare the three treatment groups for pharmacokinetic analysis. Comparisons between bleeding patterns and endometrial dating were interpreted graphically. Determination of the optimal vaginal dosage(s) for endometrial maturation was based on the pharmacokinetic profiles, as well as the histologic determination of endometrial conversion and overall safety profiles associated with each dosage regimen.

## RESULTS

One subject dropped out of the study in the middle of her first cycle (approximately day 18 of 21) because of intolerance of venipunctures. Another subject dropped out in the middle of her first cycle because of scheduling conflicts. Neither pharmacokinetic nor endometrial sampling data from these two subjects were included for analysis.

One subject moved away from the area after completing one cycle (400 mg/d). Although pharmacokinetic data from this subject were included for analysis of the 400-mg/d dosage, her endometrial sampling data were not.

One subject repeated the 200-mg/d treatment sequence twice, and another subject repeated the 100-mg/d treatment sequence twice. For purposes of pharmacokinetic analysis, only the data from the first of the duplicate dosages for these two subjects were used. However, the endometrial sampling data were not.

Thus, the pharmacokinetic analyses are based on comparisons among the 18 subjects who completed the 400-mg/d sequence and the 16 subjects who completed the 200- and 100-mg/d sequences, respectively. The endometrial sampling data are based on the 15 subjects who successfully completed all three treatment cycles. All 20 subjects initially entered in the study were included in the safety analyses, because they all received at least one dose of medication.

The 20 subjects randomly assigned to the treatment sequences were predominantly white (82%), with a mean (±SD) age of 52 years (range, 35–69 years), and a mean (±SD) weight of 152 lbs (range, 124–195). The 15 subjects who successfully completed all three treatment cycles were predominantly white (80%), with a mean (±SD) age of 52 years and a mean (±SD) weight of approximately 153 lbs.

### Pharmacokinetics/Pharmacodynamics

*Time to Peak Concentration*

The maximum concentrations after P administration were reached in 4.3 ± 4.0 hours.

*Peak Plasma Concentration*

The levels of P produced by the P vaginal suppositories in all dosage groups exceeded the levels expected during the normal menstrual cycle. The average peak plasma concentrations for the 100-, 200-, and 400-mg/d dosages were 9.46 ± 4.99, 14.8 ± 5.4, and 19.2 ± 6.0 ng/mL, respectively.

*Area Under the Plasma Concentration Time Curve*

Average (±SD) steady state concentration (Css) were 5.75 ± 3.89, 10.26 ± 5.30, and 15.26 ± 4.94 ng/mL, respectively. Analysis of variance demonstrated that the dose-normalized area under the curve was dose-dependent ($P < 0.05$). This implies that although suppository P bioavailability is dose-dependent, the absorption of P may be saturated with increasing dosages. The average (±SD) area under the

curve values for the 100-, 200-, and 400-mg/d dosages were 138.11 ± 112.02, 246.22 ± 127.12, and 187.2 ± 60.1 ng/mL, respectively.

*Half-Life*

The average apparent half-life of the 100-, 200-, and 400-mg/d regimens, based on apparent elimination rate constants, was 10.91 ± 5.66, 18.39 ± 16.26, and 17.62 ± 8.30 hours, respectively, with an overall average of 15.2 ± 12.0 hours. The mean (±SD) estimated values for absorption and elimination were 0.09 and 0.32 hours$^{-1}$, respectively. These correspond to an absorption and elimination half-life of 7.5 and 2.2 hours, respectively.

In summary, in all dosage groups, Css values were at or above the expected levels during the normal menstrual cycle. The estimated absorption half-life was 7.5 hours, and the elimination half-life was 2.2 hours. The elimination half-life was consistent with that reported in the literature, and there was no evidence for saturable elimination (8, 9). These observations (noncompartmentalized and compartmentalized) indicate that the bioavailability of the higher dosages of P (200 and 400 mg/d) is relatively less than that of the 100-mg/d dosage.

**Endometrial Dating**

Individual subject transvaginal US results demonstrated endometrial thickening and indicated that all subjects had sufficient endometrium for biopsy during each treatment period (mean [±SD] endometrial thickness: pretreatment = 5.04 mm, treatment = 9.27 mm). A total of 18 subjects underwent endometrial biopsies. One subject underwent a biopsy only during the 400-mg/d dosage regimen. One subject had insufficient tissue to evaluate the endometrium during the 100-mg/d phase of the study; subsequent endometrial biopsies during the 200- and 400-mg/d dosage regimens showed secretory endometrium. One subject manifested complex endometrial hyperplasia (no atypia) with secretory changes while receiving the 100-mg/d dosage. One subject manifested proliferative endometrium with some hyperplastic features without secretory transformation while receiving the 100-mg/d dosage.

Biopsies for both these patients during the 200- and 400-mg/d dosage regimens showed secretory endometrium. Two subjects did not receive all three dosage regimens, but rather received two dosage regimens, one of which was repeated; all dosage regimens showed secretory endometrium.

For the 12 remaining subjects, no meaningful differences were observed between treatment groups with respect to these biopsy results. Similar endometrial maturation was associated with each dosage regimen. Further, the biopsy results obtained with each dosage regimen were consistently representative of fully developed secretory endometrium.

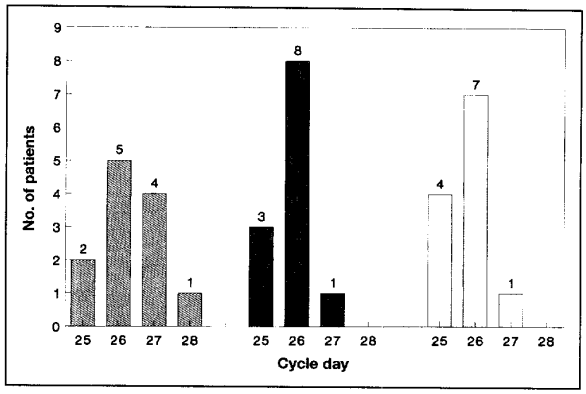


**Figure 1** Endometrial dating of the day 25 biopsy (completed subjects: $n$ = 12) for the 100-mg/d dosage (*striped bars*), the 200-mg/d dosage (*cross-hatched bars*), and the 400-mg/d dosage (*stippled bars*).

Endometrial dating was performed on the 12 subjects who had satisfactory biopsies during all three treatment sequences (Fig. 1). All endometrial biopsies were taken on day 25. Individual subject endometrial dating was based on the criteria published by Noyes et al. (14). All 12 subjects were evaluated as falling between days 25 and 28 for the 100- and 200-mg/d dosages, and between days 25 and 27 for the 400-mg/d dosage; thus, there was extremely small within-group variability.

Most of the biopsies were considered to correlate best with day 26 of the natural menstrual cycle. The calculated mean (±SD) number of cycle days for the 100-, 200-, and 400-mg/d dosage groups were 26.33, 25.83, and 25.75 days, respectively. A statistically significant difference in the average number of cycle days was detected between the 100-mg/d dosage and the 200-mg/d dosage ($P < 0.04$), and between the 100-mg/d dosage and the 400-mg/d dosage ($P < 0.02$).

**Bleeding Patterns**

The amount and duration of blood flow after completion of P dosing were evaluated in the 15 subjects who completed all three dosage regimens using subject menstruation diary data (Fig. 2). Overall, these results indicated that >90% of the subjects in each dosage group displayed bleeding or spotting. There was a tendency, however, for bleeding or spotting to occur earlier in the lowest (100-mg/d) dosage group. By day 28, 67% of the subjects in the 100-mg/d group experienced bleeding or spotting, whereas 40% and 30% of the subjects in the 200- and 400-mg/d dosage

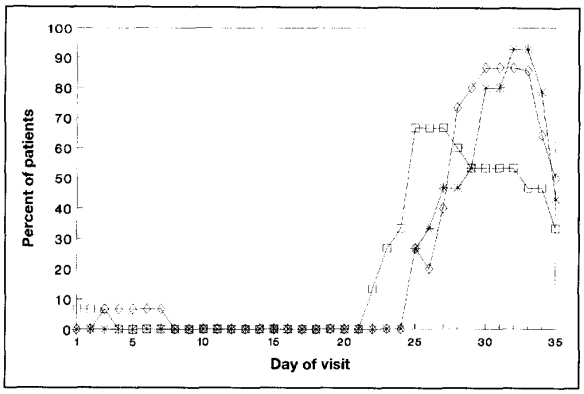


**Figure 2** Bleeding/spotting profiles (completed subjects: $n = 15$) for the 100-mg/d dosage (□), the 200-mg/d dosage (◇), and the 400-mg/d dosage (*).

groups, respectively, experienced bleeding or spotting during the same period. The onset of bleeding was examined further in completed subjects by both P dosage and plasma level.

When evaluated by dosage, no patients receiving either 200 or 400 mg/d reported any incidence of bleeding before day 25 (the day of the endometrial biopsy). However, 26.67% of patients receiving the 100-mg/d dosage reported the onset of bleeding before day 25. The data demonstrate a shift to the right in the onset of bleeding with increased dosage. Similarly, a trend in delay of the onset of bleeding is evident with higher plasma levels of P.

### Safety

No significant adverse events were reported. There was an apparent dosage-related increase in reported headaches in this study: one with the 100-mg/d dosage, four with the 200-mg/d dosage, and five with the 400-mg/d dosage. There also was a higher incidence of vaginitis with the 400-mg/d dosage: two cases with the 100-mg/d dosage, no cases with the 200 mg/d dosage, and six cases with the 400-mg/d dosage. No clinically significant findings on physical examination were noted during the study.

## DISCUSSION

In the present study, the pharmacokinetic profiles observed after administration of the 100-, 200-, and 400-mg/d dosages of P were similar to previous reports in the literature (8–10). In this study, all dosages of P were absorbed rapidly by the vaginal tissue, and measurable plasma levels were attained readily. Further, the steady-state concentrations after each of the dosage regimens used in this trial reached levels that were consistent with the physiologic range of P seen during the secretory phase of a normal menstrual cycle. Most of the pharmacokinetic parameters displayed the expected linear dosage relation; however, the bioavailability of the higher dosages was limited. Possible explanations for this might include saturation of P receptors or the limited vaginal surface area where absorption could occur.

A similar degree of endometrial maturation was seen with each of the three P vaginal dosage regimens for the 12 of 15 subjects who had suitable biopsy tissue for dating. However, the other 3 subjects had inadequate samples for endometrial dating with the 100-mg/d dosage. Two of these subjects' biopsies demonstrated a lack of adequate endometrial conversion on the 100-mg/d dosage. All 3 of these subjects' biopsies showed a mature, secretory endometrium after both the 200-mg/d and 400-mg/d dosage regimens. In addition, there was a significantly increased tendency for earlier bleeding and spotting with the 100-mg/d dosage.

Although the pharmacokinetic data may be supportive, the endometrial dating and bleeding patterns observed in this study demonstrate the inability of the 100-mg/d dosage of P vaginal suppositories to convert consistently an estrogen-primed endometrium. The pharmacokinetic and endometrial results obtained in this study suggest that either the 200- or the 400-mg/d (200 mg two times per day) dosage of P vaginal suppositories may be sufficient to establish a viable endometrium, suitable for successful implantation and support of a fertilized ovum.

The comparative safety profiles suggest that the 400-mg/d dosage may be associated with a slightly higher risk of vaginitis symptoms than the 100- or 200-mg/d dosage. However, this may be due more to the fact that subjects inserted vaginal suppositories (200 mg) two times per day during the 400-mg/d sequence than to the dose of P itself. Overall, the incidence of adverse effects was similar at all dosages, was not serious, and was not considered to be clinically significant. Of interest, there were no reports of somnolence, which has been reported with oral P (15).

Progesterone blood levels observed during pregnancy reportedly are considerably higher than the levels associated with the secretory phase of the normal menstrual cycle. Consequently, IM P has been used to produce the higher blood levels of P that were thought to be required to maintain early pregnancies until the placenta can adequately take over P production.

Recent important evidence reported by Miles et al. (8), however, suggested that the endometrial P

levels obtained after vaginal administration of P are significantly (approximately eightfold) higher than those seen after IM administration. These data further suggested that endometrial P levels may be a more important determinant of endometrial viability than blood levels, and that vaginal P administration therefore may maintain pregnancy adequately because of the high regional absorption and distribution into local tissues. However, endometrial P levels were not determined in this study.

Pharmacokinetic evidence that the 400-mg/d vaginal P dosage may maintain a pregnancy best is based primarily on the observation that this dosage attained the highest sustained blood levels. The expectation is that this also represents maximal sustained endometrial levels.

Given the comparable safety profiles, the optimal vaginal P dosage to establish and maintain a viable pregnancy should be based primarily on the ability to convert an estrogen-primed endometrium. The endometrial dating and bleeding pattern data from this study indicate that both the 200-mg/d and the 400-mg/d dosages may be appropriate. Overall, the pharmacokinetic and pharmacodynamic data support the selection of the 400-mg P vaginal suppository for further study.

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