made the incision and then tried to measure the deepest portion of the wound, which tended to be the middle of the wound. We measure the thickness vertically with the knife handle.

REFERENCE

1. Gallup DG. Modifications of celiotomy techniques to decrease morbidity in obese gynecologic patients. Am J Obstet Gynecol 1984;150:171-8.

Initial and steady-state pharmacokinetics of a vaginally administered formulation of progesterone

David F. Archer, MD,[a] George E. Fahy, MD,[a] Amabel Viniegra-Sibal, MD,[a] Freedolph D. Anderson, MD,[a] Wallace Snipes, PhD,[b] and Robin G. Foldesy, PhD[c]

Norfolk, Virginia, State College, Pennsylvania, and Somerville, New Jersey

OBJECTIVE: The pharmacokinetics of a 100 mg vaginal progesterone suppository was evaluated on days 1 and 7 and a 200 mg suppository on day 14. All the volunteers were given oral 17β-estradiol during the study.

STUDY DESIGN: Ten postmenopausal women volunteered for this study. Progesterone was given as a vaginal suppository. Peripheral venous samples were obtained at appropriate intervals and analyzed for 17β-estradiol and progesterone levels. Area under the curve for progesterone was assessed by the trapezoidal method. Statistical analysis was performed by a one-way analysis of variance.

RESULTS: Serum 17β-estradiol levels ranged from 22 to 182 pg/ml. Maximal serum progesterone levels ranged from 5.7 to 20.9 ng/ml, with the mean maximal levels 13.97, 16.09, and 12.68 ng/ml (not significantly different) and a mean area under the curve of 168.13, 207.64 and 227.71 ng/ml per hour on days 1, 7, and 14 (not statistically different).

CONCLUSIONS: These data indicate that vaginal absorption of progesterone is efficient. The lack of difference in the area under the curve for both doses suggests that the vaginal mucosa or the total surface area of the vagina may limit the absorption of progesterone from the vagina. (Am J Obstet Gynecol 1995;173:471-8.)

Key words: Progesterone, pharmacokinetics, vaginal

Progesterone has been used therapeutically for a variety of medical indications. It has been recommended for use in luteal phase inadequacy, for repetitive miscarriage, and for the treatment of premenstrual syndrome.[1, 2] Progesterone has been administered as part of hormone replacement therapy in the menopausal woman and after embryo transfer in assisted reproduction.[3-5] Women with premature ovarian failure who are undergoing embryo transfer with donor oocyte protocols have received progesterone by intramuscular, oral, or vaginal routes with reported successful pregnancies.[4, 5] The administration of progesterone to postpartum nursing women has been advocated as a means of prolonging the physiologic anovulation that is associated with lactation.[6-10]

For many of these therapeutic indications progesterone has been administered intramuscularly because of its poor bioavailability from the gastrointestinal tract.[3, 11-13] The intramuscular route is uncomfortable and requires daily administration to maintain an appropriate serum level. Oral administration of progesterone requires large doses, an appropriate vehicle, and an increased surface area (micronization) to achieve reasonable blood levels.[3, 12] The pharmacokinetic profile of orally administered progesterone is one of rapid absorption with a short half-life.[3, 12] The short half-life is related to the rapid metabolic clearance of progesterone. Because of this, twice-daily dosing, or more, is

From the Clinical Research Unit of the Contraceptive Research and Development Program, Department of Obstetrics and Gynecology, Eastern Virginia Medical School,[a] Zetachron, Inc.,[b] and GynoPharma, Inc.[c]
Supported in part by the Contraceptive Research and Development Program, Eastern Virginia Medical School, under a Cooperative Agreement (DPE-2044-A-00-6063-00) with the United States Agency for International Development, GynoPharma, Inc., Somerville, New Jersey, and Zetachron, Inc., State College, Pennsylvania.
Presented at the Fifty-seventh Annual Meeting of The South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 28-31, 1995.
The views expressed by the author(s) do not necessarily reflect the view of the Agency for International Development.
Reprint requests: David F. Archer, MD, CONRAD, Clinical Research Unit, The Jones Institute for Reproductive Medicine, 601 Colley Ave., Norfolk, VA 23507.

0002-9378/95 $3.00 + 0 6/6/65562

Table I. Demographic information and body mass index of postmenopausal women

Patient No.	*Age (yr)*	*Weight (lb)*	*Height (in)*	*BMI*	*LMP*
001	54	157	67	24.6	1989
002	55	157	65.5	27.7	1981
003	56	144	61	34.6	1985
004	59	155	66	25.0	1975
005	46	166	66	26.9	1985
006	62	190	68	29.0	1980
007	49	133	63	23.4	1988
008	55	124	62	22.7	1976
009	61	132	61	26.7	1980
010	57	127	65.5	22.4	1986

BMI, Body mass index; *LMP*, last spontaneous menstrual period.

Table II. Serum 17β-estradiol (mean ± SE) levels on days 1, 7, and 14 of 10 postmenopausal women receiving oral 17β-estradiol (Estrace) 1.0 mg daily

Time	*Day 1*	*Day 7*	*Day 14*
Baseline	72.9 ± 7.64	79.0 ± 11.96	76.3 ± 13.44
0.5	71.9 ± 7.76	70.3 ± 53	66.8 ± 11.82
1.0	72.6 ± 7.21	76.1 ± 10.65	69.2 ± 12.40
1.5	82.76 ± 8.3	85.1 ± 11.15	71.1 ± 12.37
2.0	80.3 ± 6.78	87.9 ± 10.98	85.2 ± 14.11
2.5	81.4 ± 7.21	88.0 ± 12.04	82.7 ± 12.21
3.0	80.4 ± 7.58	97.9 ± 13.95	78.9 ± 11.79
4.0	79.3 ± 6.60	79.1 ± 11.82	77.5 ± 13.39
6.0	70.9 ± 8.19	73.9 ± 10.10	73.0 ± 12.91
8.0	70.11 ± 8.68	75.4 ± 12.66	71.5 ± 10.55
12.0	80.3 ± 9.23	75.8 ± 10.94	76.3 ± 10.29
24.0	79.6 ± 10.78	78.7 ± 11.42	73.4 ± 8.97

Each woman took oral 17β-estradiol at different times of day beginning 14 days before day 1 and stopping on day 14 of study. All 17β-estradiol serum values in picograms per milliliter.

required. Oral progesterone also has been found to have a significant sedative action, possibly because of a metabolite that is produced in the gastrointestinal tract or liver.[14]

The vaginal route of administration has certain advantages. These are a surface (vaginal epithelium) that readily transfers progesterone, avoidance of first-pass metabolism in the gastrointestinal tract and liver, and a more sustained delivery from the vaginal progesterone formulation (suppository), which results in extended progesterone serum levels.[11, 12, 15-17]

This study was designed to evaluate the pharmacokinetics of two doses of a progesterone formulation for vaginal administration. A 100 mg vaginal progesterone suppository was used to determine steady-state pharmacokinetics. This was followed after 1 week with one 200 mg progesterone vaginal suppository to evaluate the dose response. Postmenopausal women were selected for the study to circumvent endogenous progesterone secretion from the ovary. All the volunteers were given oral 17β-estradiol before vaginal progesterone, to optimize absorption.[18]

Material and methods

Volunteers. Ten postmenopausal women were asked to volunteer for this study. The protocol had previously been approved by the Institutional Review Board of the Eastern Virginia Medical School. Each volunteer was apprised of the study by an appropriate informed consent, which was obtained before enrollment in the study. Each volunteer was required to have undergone a complete history and physical examination to meet specific inclusion and exclusion criteria before enrollment. The demographic profile of the volunteers is shown in Table I. Postmenopausal status was assigned if the volunteer had been amenorrheic for at least 6 months. Each volunteer was also required to have follicle-stimulating hormone level ≥40 mIU/ml. All the volunteers were required to be free of all hormonal medications for ≥1 month before initiating the oral 17β-estradiol (see below).

17β-Estradiol administration. Each volunteer was started on a regimen of oral 17β-estradiol, 1.0 mg daily (Estrace, Mead Johnson Pharmaceuticals, Evansville, Ind.) for 14 days before the insertion of the vaginal suppositories and for the duration of the study.

Vaginal progesterone. Progesterone in polyethylene glycol was produced as a vaginal suppository in concentrations of 100 and 200 mg. The vaginal progesterone formulations were provided by Zetachron, State College, Pennsylvania. All the suppositories for each volunteer were packaged in an individual plastic container.

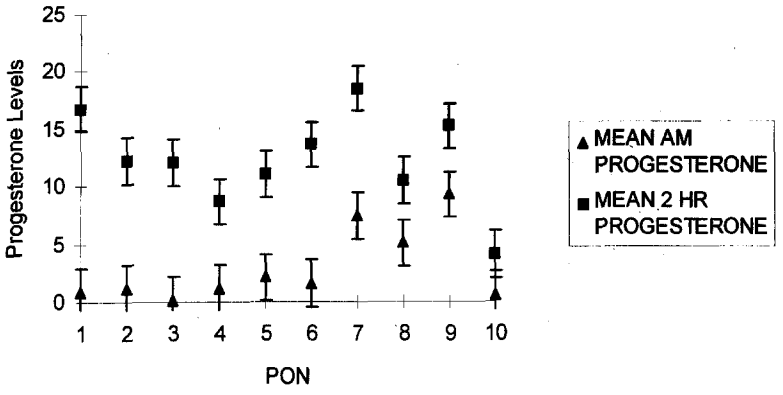

Fig. 1. Serum progesterone levels (mean ± SD) at baseline and 2 hours after insertion of 100 mg progesterone vaginal suppository in 10 volunteers over 7-day period. *PON,* patient order number.

Table III. Serum progesterone levels (nanograms per milliliter) by day of treatment and for each volunteer during 7 days of therapy at zero time and 2 hours after insertion of vaginal suppository (mean ± SD)

Day	*Baseline**	*2 hr†*	*Patient order No.*	*Baseline*	*2 hr‡*
1	0.20	11.27	001	0.87	14.68
2	2.52	10.40	002	1.87	13.36
3	3.98	9.98	003	0.23	12.08
4	3.91	12.18	004	2.35	9.51
5	4.30	13.35	005	2.24	11.20
6	3.56	13.32	006	1.57	13.29
7	2.71	13.67	007	7.81	16.97
			008	5.13	9.61
			009	7.42	14.69
			010	0.783	4.85

Progesterone levels are indicated by day of study and by individual volunteers (patient order number). Mean progesterone levels between volunteers and between days demonstrated a highly significant difference by two-way analysis of variance.

*Mean values statistically different, $p \leq 0.0053$ between day 1 and other 6 days.

†Mean values not statistically different between days, $p = 0.31$.

‡Mean values statistically different between baseline and 2 hours for each volunteer, $p \leq 0.001$.

The suppositories were stored at room temperature until used. Each volunteer was requested, under supervision, to insert one suppository high into the vagina daily between 7 and 9 AM. The volunteer was then requested to lie prone for 30 minutes after insertion of the suppository. The 100 mg progesterone suppositories were inserted daily for 7 days. There was a break of 7 days with no progesterone administration. On the fourteenth day a 200 mg progesterone suppository was inserted vaginally in the same manner.

Peripheral venous sampling for pharmacokinetics. Peripheral blood samples of 10 ml were obtained from an antecubital vein. The samples were obtained immediately before insertion of each vaginal suppository and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, and 24.0 hours after insertion of the vaginal suppository. The 24-hour blood sample was obtained at the same time of day as the initial baseline sampling from the first pharmacokinetic day, and this schedule was maintained throughout the study. Each volunteer, once the initial time of suppository insertion was determined, maintained that time for repeat suppository placement throughout the course of the study. A venous blood sample was obtained each day before placement and 2 hours after placement of the 100 mg vaginal progesterone suppository in each of the volunteers on days 2, 3, 4, 5, and 6.

No blood samples were obtained for the succeeding 24 hours after the last 100 mg progesterone suppository, but the same protocol was used for the 200 mg progesterone suppository. The 200 mg progesterone suppository was administered only once, and blood sampling was performed at the same intervals during the next 24 hours.

Each venous blood sample was allowed to clot at room temperature for 5 minutes and was then centrifuged at 3000 revolutions/min for 15 minutes. The resulting serum supernatant was drawn off and placed in a polytetrafluoroethylene-coated storage vial. The individual storage vials were then stored at −20° C until analyzed for estradiol and progesterone.

Quantitation of 17β-estradiol and progesterone. Se-

Table IV. Maximum serum progesterone levels, the greatest change between baseline and maximum concentration, time to maximum concentration, and area under curve for progesterone in 10 postmenopausal women

	Day 1: Progesterone 100 mg					*Day 7: Progesterone 100 mg*				
Volunteer No.	*Baseline progesterone (ng/ml)*	*C_{max} progesterone (ng/ml)*	*Change (ng/ml)*	*Time to C_{max} (hr)*	*AUC (ng/ml/hr)*	*Baseline progesterone (ng/ml)*	*C_{max} progesterone (ng/ml)*	*Change (ng/ml)*	*Time to C_{max} (hr)*	*AUC (ng/ml/hr)*
001	0.2	20.7	20.5	2.0	270	0.7	18.1	17.4	2.5	202
002	0.2	14.7	14.5	3.0	192	0.9	19.6	19.1	3.0	185
003	0.2	16.9	16.7	3.0	83	0.2	12.2	12.0	1.5	56
004	0.2	7.8	7.5	1.5	43	1.2	17.7	16.5	3.0	246
005	0.2	13.5	13.3	4.0	164	4.5	13.7	9.5	2.5	193
006	0.2	20.9	20.7	3.0	188	1.5	20.4	18.9	4.0	213
007	0.2	16.5	16.3	3.0	232	3.1	19.7	16.6	2.0	256
008	0.2	12.4	12.2	6.0	199	2.7	11.9	9.2	6.0	245
009	0.2	14.3	4.1	3.0	222	0.7	24.4	14.7	3.0	381
010	0.2	7.3	7.1	3.0	63	2.7	9.6	6.9	2.5	96

Data derived from day of pharmacokinetic profile of 100 mg vaginal progesterone suppositories on days 1 and 7 and 200 mg vaginal progesterone suppository on day 14 in 17β-estradiol–treated postmenopausal women. *C_{max}*, Maximum concentration; *AUC*, area under curve.

Table V. Occurrence of bleeding in postmenopausal women pretreated with 17β-estradiol 1.0 mg for 14 days, who used 100 mg progesterone vaginal suppository for 7 days followed by 200 mg progesterone vaginal suppository

Patient No.	*Bleeding*	*Severity*	*Duration (days)*	*Endometrial biopsy results*	*Side effects*
001	Yes	Mild	3	Prolif.	Local warmth, tired
002	Yes	Mild	2	Prolif.	Local warmth, tired, cramps
003	Hyst.	–	–	–	None
004	Hyst.	–	–	–	Tired
005	Hyst.	–	–	–	None
006	Yes	Mild	3	Not done	Cramps
007	Yes	Mild	3	Prolif.	Tired, cramps
008	Hyst.	–	–		None
009	Yes	Mild	4	Prolif.	Breast tenderness, tired
010	Yes	Mild	1	Insuff.	Breast tenderness, anxiety

Endometrial histologic studies are reported for five of six women who had a uterus and who had bleeding. Other reported adverse effects are listed. *Prolif.*, Proliferative endometrium; *Hyst.*, hysterectomy; *Insuff.*, insufficient tissue for diagnosis.

rum 17β-estradiol and progesterone were estimated with radioimmunoassay kits obtained from ICM Biomedical, Costa Mesa, California.

Statistical analysis. All data were analyzed by a one- or two-way analysis of variance or Student *t* test by use of STATISTICA (CSS:STATISTICA, Statsoft, Tulsa, Okla.). A p value of ≤ 0.05 was accepted as indicating significant differences.

Diary cards. Each volunteer was asked to maintain a daily diary card throughout the study. Any symptoms were recorded on these cards.

Endometrial biopsy. The women who had a uterus and who had vaginal or uterine bleeding were asked to undergo an endometrial biopsy, which was performed with a Pipelle curette (Unimar, Wilton, Conn.). The tissue obtained was immediately fixed in formalin and then sectioned and interpreted by a gynecologic pathologist.

Results

Demographics. The demographics of the volunteers are given in Table I. There were 10 postmenopausal women whose ages ranged from 46 to 62 years. The duration of the postmenopausal state ranged from 4 to 12 years. Seven of the women had previously received estrogen or estrogen plus progestin replacement therapy. The volunteers' individual weight, height, and body mass index are shown in Table I.

Serum 17β-estradiol. All the volunteers were requested to take 17β-estradiol (Estrace) at a specific time of day that was convenient for her, and because of this the time of day that the medication was taken was variable. The mean serum 17β-estradiol levels in the volunteers at baseline and at the described intervals throughout the three pharmacokinetic study days 1, 7 and 14 are shown in Table II. The mean serum 17β-estradiol values were between 70 and 100 pg/ml on the

Day 14: Progesterone 200 mg				
Baseline progesterone (ng/ml)	*C_{max} progesterone (ng/ml)*	*Change (ng/ml)*	*Time to C_{max} (hr)*	*AUC (ng/ml/hr)*
0.2	17.3	17.1	12.0	214
0.3	13.9	13.5	8.0	230
0.2	17.3	17.1	4.0	168
0.2	19.5	19.3	4.0	185
0.2	13.7	13.5	3.0	230
0.2	20.4	20.2	3.0	281
0.2	11.8	11.6	3.0	199
0.2	14.4	14.2	8.0	299
0.2	12.9	12.7	8.0	263
0.2	5.7	5.5	3.0	59

three pharmacokinetic days. Individual 17β-estradiol levels for the volunteers ranged from 22 to 182 pg/ml (data not shown). No statistical analysis was performed on these data, because the time of oral administration of 17β-estradiol was variable among volunteers.

Serum progesterone. Serum progesterone values (mean ± SEM) at 24 hours after insertion of the vaginal suppository (daily baselines) were 2.52 ± 0.77, 3.98 ± 1.23, 3.91 ± 1.46, 4.30 ± 1.33, 3.56 ± 1.02, and 2.71 ± 0.88 ng/ml on days 2, 3, 4, 5, 6, and 7, respectively (Fig. 1). The mean 2-hour serum progesterone levels ranged between 10.8 and 13.9 ng/ml (Fig. 1, Table III). The baseline and 2-hour serum progesterone levels in each volunteer by day of treatment were analyzed by a two-way analysis of variance. The progesterone levels varied significantly among volunteers and with time. The interaction between the two variables (volunteer and time) was highly significant.

A comparison between the serum progesterone levels on days 1 and day 14 of the pharmacokinetic study for each volunteer indicates the following. Serum levels were comparable on days 1 and day 14 in volunteers 005, 006, 008, 009, and 010 (data not shown). Levels were lower on day 14 compared with day 1 in volunteers 001, 002, and 007, whereas levels were higher on day 14 in volunteers 003 and 004 (data not shown). These results do not appear to be related to suppository placement or physical activity of the individual.

The mean time to maximum concentration varied among volunteers, but the mean for progesterone was 3 hours on days 1 and 7 but between 3 and 8 hours on day 14 (Fig. 2, Table IV). The mean maximal serum progesterone concentration on each of the 3 days (1, 7, and 14) was 13.97, 16.09, and 12.68 ng/ml, respectively (Fig. 2). The absolute change in progesterone from baseline to maximum concentration for each volunteer was variable and not statistically significant ($p = 0.98$) (Table IV). The mean serum progesterone levels for the 10 volunteers were similar at each time interval during the pharmacokinetic studies on days 1, 7, and 14 (Fig. 2). The elevated baseline mean progesterone value of 2.4 ng/ml resulted in an increased maximum concentration value for progesterone on day 7. The mean area under the curve for progesterone was 168.13, 207.64, and 222.71 ng/ml per hour on days 1, 7, and 14 and was not statistically different ($p = 0.35$). The elevated baseline progesterone value on day 7 was adjusted for in the calculations of the area under the curve. Each volunteer was found to have a unique progesterone area under the curve that was different from those of the other volunteers ($p = 0.0027$) (Table IV).

Side effects. Two of the women (001 and 002) within 30 minutes of inserting the progesterone vaginal suppository had a local warmth in the vagina, which lasted for 20 to 30 minutes (Table V). Visual inspection of the vaginal mucosa with a speculum 24 hours later did not demonstrate any evidence of local irritation. Three of the women (002, 006, and 007) complained of mild low abdominal cramps, similar to what they experienced when having a menstrual flow in the past, at some time during the study; these women had vaginal bleeding associated with cramps (see below). Five of the volunteers (001, 002, 004, 007, 009) complained of feeling "tired" at some time during the study (Table V). It should be noted that the patients who complained of feeling tired did so on the day of the use of the 200 mg vaginal progesterone suppository. The feeling of being "tired" disappeared within 2 hours after insertion of the suppository. Two of the women (009 and 010) had breast tenderness during the course of the study without evidence of breast lumps or masses.

Vaginal bleeding. Six of the women (001, 002, 006, 007, 009, 010), who had not had a hysterectomy, had vaginal bleeding during or after the use of the vaginal progesterone (Table V). The other four women had previously had a hysterectomy. The vaginal bleeding lasted between 2 to 5 days and was characterized by the volunteers as minimal. Two volunteers (001 and 010) had spotting that did not require sanitary protection. The spotting or bleeding occurred after initiating the vaginal progesterone on days 8 to 10 in five of the women (002, 006, 007, 009, and 010) and on day 5 in one woman (001).

An endometrial biopsy was obtained after the completion of the study in five of the six women who had vaginal spotting or bleeding (001, 002, 007, 009, and 010). One volunteer (006) did not have a biopsy. The tissue was interpreted as proliferative endometrium in four of the cases (001, 002, 007, and 009) and insufficient tissue for interpretation (cervical glands and mucus) in one volunteer (010) (Table V).

Comment

The use of the vagina as a repository for medication is not new. The advantage of the absorption of steroids

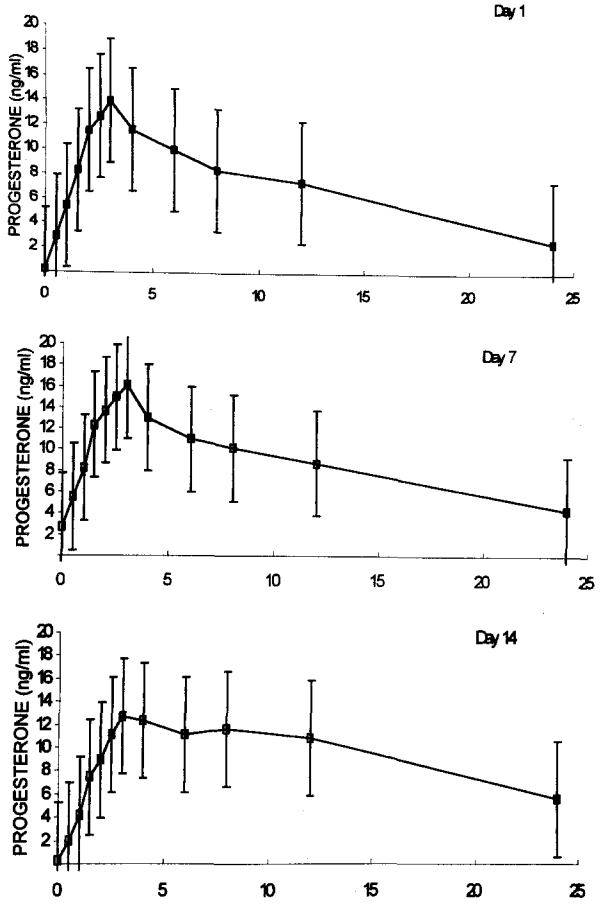

Fig. 2. Serum progesterone levels (mean ± SD) on days 1, 7, and 14 in 10 postmenopausal women. Vaginal progesterone suppository, 100 mg, was administered daily for 7 days, followed by no medication for 7 days and 200 mg progesterone suppository on day 14. All women were taking oral 17β-estradiol, 1.0 mg, throughout study.

and other compounds across the vagina is twofold. The avoidance of the first-pass effect of the gastrointestinal tract and liver on progesterone metabolism is apparent. The second advantage is a prolonged, sustained serum progesterone level. Previous studies have shown that a relatively large amount of progesterone must be administered by mouth to achieve luteal blood levels.[3, 19] Although there is rapid absorption of progesterone from the gastrointestinal tract, metabolic clearance is rapid, thus resulting in a short half-life in serum.[19]

Metabolites of progesterone are produced after oral administration that could account for some of the adverse effects, notably somnolence, that have been reported.[14] Metabolites of progesterone were not identified in this study. The volunteers in this study reported only a "tired" feeling, not somnolence, as a side effect (Table V).

The current study demonstrates a rapid absorption of progesterone after vaginal administration. The maximum concentration of serum progesterone was reached within 3.0 hours in the majority of instances. These findings indicate that the vaginal route may be a preferred route of administration, because we were able to reach luteal levels in the serum with a lower dose than required by the oral route. That the levels reached with vaginal progesterone administration are physiologic is attested to by the successful pregnancies in assisted reproductive programs and changes in endometrial histologic features.[4, 20, 21] The bioavailability of progesterone appears to be greater when it is administered through the vagina compared with the oral route, when the area under the curve for progesterone is used for comparison.[21]

Progesterone is rapidly cleared from the peripheral circulation because of its short metabolic half-life. The concentration of progesterone is maintained in peripheral sera for a prolonged period after vaginal administration (Fig. 2). By 24 hours postinsertion on each day of the progesterone 100 mg suppository, there were still significant elevations of serum progesterone over the initial baseline value. The reason for this is not clear, but similar findings of a prolonged serum level without an initial rapid peak have been found in women using a different formulation of vaginal progesterone.[12] This prolonged serum level could be a result of the reservoir effect of the vaginal suppository or of the vagina itself. The fluid volume in the vagina has been found to be approximately 0.2 ml. It is possible that the vaginal suppository is slowly dissolved, thus resulting in a progesterone reservoir contained in the suppository base.

Our data do not show any difference in the area under the curve between 100 and 200 mg vaginal suppositories (Table IV). The vaginal epithelium may function as a rate-limiting membrane allowing a finite amount of progesterone to be absorbed. Serum levels could be a function of the surface area of the vaginal epithelium exposed to progesterone. It should be noted that there is a highly variable absorption within the same volunteer (≥20%) of the same vaginal dose (Table III, area under the curve, and Table IV). The reason for the individual difference in absorption of progesterone is not known. Another possibility is that other factors in the vaginal epithelium (dermis) contribute to the absorption.[22] There is no available information that demonstrates a correlation between surface area of the vagina and serum progesterone levels. Previous studies of transdermal 17β-estradiol absorption have suggested that dermal surface area is important, but definitive information is lacking.[22] We have found interindividual variation to be present with the transdermal administration of estradiol.[22-24] Oral contraceptive steroids have been found to have significant differences in oral absorption in the same woman at different times.[25] Genetic differences and dietary changes have been hypothesized to explain this difference.[25] The actual cause of this discrepancy in pharmacokinetic profiles in the same individual under similar conditions at two separate study times is not known.

The hormonal status of the volunteers could also play a role in the efficiency of the vaginal absorption of progesterone. Lack of estrogen could affect absorption. In this study a 14-day pretreatment with oral 17β-estradiol was used to ensure that the vaginal epithelium was well estrogenized. Mean serum 17β-estradiol levels, although variable between volunteers, are in a range compatible with the follicular phase of the cycle (Table II). Thus we feel that we have maximized the absorption of progesterone by the use of exogenous 17β-estradiol.

In summary, these data indicate that a simple formulation of progesterone in polyethylene glycol can achieve luteal-phase progesterone levels. We did not demonstrate dose proportionality of the 100 and 200 mg vaginal suppositories. We believe that absorption of vaginal progesterone is a function of both the vaginal mucosa surface area and the reservoir effect of the vaginal suppository.

We thank Ms. Eling Gaines for her assistance with data management and Ms. Pauline M. Clynes for her editorial assistance.

REFERENCES

1. Jones GS. The historic review of the clinical use of progesterone and progestin. In: Bardin CW, Milgröm E, Mauvais-Jarvis P, eds. Progesterone and progestin. New York: Raven Press, 1983:189-202.
2. Backstrom T. Premenstrual tension syndrome. In: Bardin CW, Milgröm E, Mauvais-Jarvis P, eds. Progesterone and progestin. New York: Raven Press, 1983:203-17.
3. Whitehead MI, Townsend PT, Gill DK, Collins WP, Campbell S. Absorption and metabolism of oral progesterone. BMJ 1980;280:825-9.
4. Bourgain C, Devroey P, VanWaesberghe L, Smitz J, Van Steirteghem AC. Effects of nocturnal progesterone on the morphology of the endometrium in patients with primary ovary failure. Hum Reprod 1990;5:537-43.
5. Navot D, Scott RT, Droesch K, Veeck LL, Liu HC, Rosenwaks Z. The window of embryo transfer and the efficiency of human conception in vitro. Fertil Steril 1991;515:114-8.
6. Victor A, Jackanicz TM, Johansson EDB. Vaginal progesterone for contraception. Fertil Steril 1978;30:631-5.
7. Diaz S, Jackanicz TM, Herros C. Fertility regulation in nursing women, VIII: progesterone plasma levels and contraceptive efficacy of a progesterone-releasing vaginal ring. Contraception 1985;32:603-22.
8. Diczfalusy E. New developments in oral, injectable and implantable contraceptives, vaginal rings and intrauterine devices: a review. Contraception 1986;33:7-22.
9. Matlin SA, Belenquer A, Hall PE. Progesterone-releasing vaginal ring for use in postpartum contraception, I: in vitro release rates of progesterone from core-loaded rings. Contraception 1992;45:329-41.
10. Landrgen BM, Hall PE, Cekan SZ. Progesterone-releasing vaginal rings for use in postpartum contraception, II: pharmacokinetic profiles in women. Contraception 1992; 45:343-9.
11. Nillius SJ, Johansson EDB. Plasma levels of progesterone after vaginal, rectal, or intramuscular injection of progesterone. Am J Obstet Gynecol 1971;110:470-7.
12. Norman TR, Morse CA, Dennerstein L. Comparative bioavailability of orally and vaginally administered progesterone. Fertil Steril 1991;56:1034-9.
13. Chakmakjian ZH, Zachariah NY. Bioavailability of progesterone with different modes of administration. J Reprod Med 1987;32:443-8.
14. Arafat ES, Hargrove JT, Maxson WAS, Desiderio DM, Wentz AC, Andersen RN. Sedative and hypnotic effects of oral administration of micronized progesterone may be mediated through its metabolites. Am J Obstet Gynecol 1988;159:1203-9.
15. Kimzey LM, Grimes GJ Jr, Gumowski J, Nelsen LM, Merriam GR. Absorption of micronized progesterone from a nonliquefying vaginal cream. Fertil Steril 1991;56: 995-6.
16. Price JH, Ismail H, Gorwill R, Sarda IR. Effect of the suppository base on progesterone delivery from the vagina. Fertil Steril 1983;39:490-3.
17. Fulper LD, Cleary RW, Harland EC, Hikal AA, Jonos AB. Comparison of serum progesterone levels in dogs after administration of progesterone by vaginal tablet and vaginal suppository. Am J Obstet Gynecol 1987;156:253-6.
18. Villánueva B, Casper RF, Yen SSC. Intravaginal administration of progesterone: enhanced absorption after estrogen treatment. Fertil Steril 1981;35:433-7.
19. Simon JA, Rocci ML, Robinson DE, et al. The absorption of oral micronized progesterone: the effect of food, dose proportionality, and comparison with intramuscular progesterone. Fertil Steril 1993;60:26-33.
20. Miles RA, Press MF, Paulson RJ, Dahmoush L, Lobo RA, Sauer MV. Pharmacokinetics and endometrial tissue levels of progesterone after administration by intramuscular and vaginal routes: a comparative study. Fertil Steril 1994;62: 485-90.
21. Cornet D, Alvarez S, Antoine JM, et al. Pregnancies following ovum donation in gonadal dysgenesis. Hum Reprod 1990;5:291-3.
22. Simon JA, Hodgen GD, Archer DF. Are there any significant pharmacokinetic differences between patch and gel cutaneous estradiol therapy? In: Tenazzani AR, Petraglia F, Volpe A, Facchinetti F, eds. Recent research on gynecological endocrinology. Park Ridge, New Jersey: Parthenon, 1988:317-24.
23. Scott RT, Ross B, Anderson C, Archer DF. Pharmacokinetics of percutaneous estradiol: a crossover study using a gel and a transdermal system in comparison with oral micronized estradiol. Obstet Gynecol 1991;77:758-64.
24. Smith RD, Tomai TP, Robinson DE, et al. Effects of vehicle supplementation on total estradiol absorption from a transdermal estradiol delivery system. Fertil Steril 1991; 56:1029-33.
25. Goldzieher JW. Pharmacokinetics and metabolism of ethinyl estrogen. In: Goldzieher JW, Fotherby K, eds. Pharmacology of the contraceptive steroids. New York: Raven Press, 1994:127-51.

Discussion

Dr. William J. Butler, Charleston, South Carolina (Official Guest). The use of natural progesterone has been advocated for therapeutic intervention in a wide range of gynecologic and other pathologic disorders. It is of prime importance in the preparation of a receptive endometrium before transfer of embryos conceived from donor oocytes. That exogenous progesterone administration is effective in this regard is demonstrated by a success rate of 31.3% pregnancies per retrieval in donor oocyte cycles reported in the American Fertility Society–Society for Assisted Reproductive Technology registry from 1992.[1] Vaginal administration of progesterone may have an advantage in bioavailability, which may translate into a clinical advantage, particularly in donor oocyte assisted reproductive technique cycles.[2, 3]

A possible mechanism of this bioadvantage is a reservoir effect proposed by Archer et al. as a unique effect of vaginal administration and one of the limiting factors

Table I. Randomized complete block analysis

	Sums of squares	*Degrees of freedom*	*Mean square*
Days	13,323.27	2	6,661.63
Patients	119,572.03	9	13,285.78
Error	46,938.07	18	2,607.67
TOTAL	179,833.37	29	

in absorption of progesterone from the vagina. A corollary to this is the authors' interpretation that the data indicate the vaginal mucosal surface area is a rate-limiting membrane allowing only a finite amount of progesterone to be absorbed. The statistical analysis of the data presented indicates no difference in absorption between the 100 and 200 mg vaginal suppositories. The study design obtains an accurate representation of progesterone absorption over a 24-hour period, but a closer look at the data is warranted.

A visual analysis of the graphs indicating the serum progesterone levels over the 24-hour periods of days 1, 7, and 14 appears to show a much larger area under the curve encompassed on day 14 (200 mg suppository) compared with day 1 (100 mg suppository), with the day 7 levels intermediate. The peak serum levels are comparable, but from 4 hours on the median day 14 serum levels are uniformly higher. As the authors state, the day 7 levels are measuring a different biologic condition—steady-state pharmacokinetics—compared with the initial pharmacokinetics measured on days 1 and 14 for the differing suppository dosages. No steady-state pharmacokinetic data are presented for 200 mg suppositories.

Statistical analysis of this data can be done in several ways, but a traditional one-way analysis of variance does not take into account that some measurements were made on the same individuals. Because 10 subjects each supplied area under the curve measurements on three different days, one way to take this into account is a randomized complete block analysis. The corresponding analysis of variance is shown in Table I.

The test statistic comparing the 3 days if $F = 2.5546$ (with 2 and 18 degrees of freedom) with an associated p value of 0.1055. This is still not quite significant but does quite clearly differ from the p value of 0.3538 obtained with the authors' statistical analysis.

A more physiologically relevant analysis would be a comparison of the initial pharmacokinetics for the 100 and 200 mg suppositories on days 1 and 14. Visual inspection of the graphs of only days 1 and 14 does seem to indicate a difference. The appropriate statistical analysis here would be a paired Student t test, which was also used for analysis of similar data in several of the authors' cited references.[2, 4] This analysis obtains a p value of 0.0414, statistically significant if a threshold of $p \leq 0.05$ is accepted. The correlation coefficient is 0.631, also confirming a significant trend to higher total progesterone absorption with the 200 mg suppository. The authors admit they do not demonstrate dose proportionality of the 100 and 200 mg suppositories, but it may be premature for them to state that they did not show any difference in the area under the curve for progesterone. I believe the data demonstrate a dose-dependent reservoir effect on progesterone absorption. This is still compatible with initial absorption, having vaginal surface area as its rate-limiting parameter.

Studies such as this are important in defining the parameters for ideal hormone replacement for a variety of medical conditions. I would like to ask the authors if they have initial pharmacokinetic data for the commonly used 50 mg progesterone vaginal suppositories and or steady-state pharmacokinetics for 50 mg daily or twice-daily 200 mg or intermediate progesterone suppository dosages.

I thank Philip Rust, PhD, for his assistance in the statistical analysis.

REFERENCES

1. Assisted reproductive technology in the United States and Canada: 1992 results generated from The American Fertility Society/Society for Reproductive Technology registry. Fertil Steril 1994;62:1121-8.
2. Miles AR, Paulson RJ, Lobo RA, Press MF, Dahmoush L, Sauer MV. Pharmacokinetics and endometrial tissue levels of progesterone after administration by intramuscular and vaginal routes: a comparative study. Fertil Steril 1994;62: 485-90.
3. Weckstein LN, Jacobson A, Galen D, Hampton K, Ivani K, Andres J. Improvement of pregnancy rates with oocyte donation in older recipients with the addition of progesterone vaginal suppositories. Fertil Steril 1993;60:573-5.
4. Simon JA, Robinson DE, Andres MC, et al. The absorption of oral micronized progesterone: the effect of food, dose proportionality, and comparison with intramuscular progesterone. Fertil Steril 1993;60:27-33.

Dr. Archer (Closing). We have not really evaluated steady-state levels for other formulations of vaginal progesterone, but the Contraceptive Research and Development program (CONRAD) has a current clinical trial in Santiago, Chile, where we are evaluating both 100 and 200 mg progesterone suppositories clinically for their contraceptive efficacy in postpartum nursing women; we hope to see some data next year.