Direct Transport of Progesterone From Vagina to Uterus

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***Objective*: To compare progesterone concentrations in serum and endometrial tissue from hysterectomy specimens after vaginal or intramuscular (IM) administration of progesterone gel.**

***Methods*: This was a randomized open study of 14 postmenopausal women undergoing transabdominal hysterectomies. Participants received either vaginal progesterone gel, 90 mg, or IM progesterone, 50 mg, at 8:00 AM and 8:00 PM on the day before surgery and at 6:00 AM on the day of surgery. Venous blood samples for progesterone measurement were collected at 8:00 AM on the day before surgery (baseline) and during surgery. After removal of the uterus, the endometrium was sampled from the anterior and posterior walls. Results were expressed as ratios of endometrial to serum progesterone concentrations × 100.**

***Results*: Ratios of endometrial to serum progesterone concentrations were markedly higher in women who received vaginal progesterone (14.1 median, 8.5–59.4 range; 95% confidence interval [CI] 9.89, 38.79) compared with IM injections (1.2 median, 0.5–13.1 range; 95% CI −0.48, 7.39) ($P < .005$).**

***Conclusion*: Ratios of endometrial to serum progesterone concentrations were higher after vaginal administration of progesterone than after IM injections. Our findings in endometrial tissue specimens from hysterectomies excluded the possibility of contamination by progesterone that remained in the vagina. (Obstet Gynecol 2000;95:403–6. © 2000 by The American College of Obstetricians and Gynecologists.)**

The advent of assisted reproductive treatments in which synthetic progestins are prohibited has revived interest in physiologic progesterone treatments to replace or supplement production by the corpus luteum. Progesterone was administered vaginally before the advent of new fertility treatments,[1,2] but formal proof of efficacy was missing, and this led to the preference for daily intramuscular (IM) injections. Since then, efforts have been made to validate the efficacy of other delivery routes. Oral progesterone cannot be used because of its excessive metabolism during the first liver pass, which greatly limits efficacy.[3–5] Transdermal administration is not an option either because of the large dose needed to match midluteal production (25 mg/24 hours), poor skin permeability, and the presence of 5α-reductase in skin, which inactivates progesterone. Hence, vaginal administration seems the best remaining option for delivering progesterone nonorally while avoiding the inconvenience of IM injections.

Yet the relatively low circulating levels of progesterone with vaginal administration have caused concern.[6] Originally, it was not foreseen that the uterine effects of vaginal progesterone could exceed those expected based on the relatively modest blood levels achieved.[7–9] Experience with vaginal progesterone showed a discrepancy between the low circulating level and reliable, complete predecidualization of the endometrium[9,10] associated with high uterine tissue concentrations.[8] This finding led us to hypothesize a direct vagina-to-uterus transport or "first uterine pass effect" as an underlying mechanism for this paradox.[7]

The present study was designed to confirm a direct vagina-to-uterus "portal" transport. The experimental model was set up to refute the objection made that data reported by Miles et al,[8] showing higher uterine tissue concentrations after vaginal administration, resulted from contamination of endometrial samples by progesterone still in the vagina. To refute this objection, we compared ratios of endometrial to serum progesterone concentrations after vaginal or IM administration of progesterone. We avoided the possibility of contamina-

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tion of endometrial tissue by progesterone still present in the vagina by obtaining tissue specimens from hysterectomies. A practical aim of this study was to reassure physicians concerned about the relatively low levels of serum progesterone obtained with vaginal administration, particularly because the controlled and sustained-release gel (Crinone 8%; Wyeth Lederle, Aprilia, Italy) allowed fewer daily dosings and a reduced daily amount of progesterone.[10,11]

Materials and Methods

Fourteen postmenopausal women undergoing transabdominal hysterectomies for uterine prolapse or pelvic floor repair consented to participate. This was a randomized, open study approved by our institutional review board. Subjects were healthy with normal body mass indexes (BMI less than 25 kg/m^2) and had been in spontaneous menopause for longer than 12 months. Subjects recruited from a single center represented approximately 15% of women who had abdominal hysterectomies at our institution. Serum FSH and estradiol (E2) levels were within the menopausal range (FSH greater than 40 mIU/mL; E2 less than 30 pg/mL). We excluded women with vaginal infections, other vaginal treatments, leiomyomas larger than 3 cm, and cancer or other contraindications to hormones. The targeted number, seven per group, was based on estimates drawn from previous work by Miles et al.[8]

To avoid alterations in progesterone absorption due to vaginal atrophy, each subject received E2 from transdermal systems (Estraderm TTS 50; Ciba Pharmaceuticals, Origgio, Varese, Italy) delivering 0.05 mg of E2 per day for 2–4 weeks before surgery. They were subsequently randomized using a computer-generated list (blocks of 2) to receive either vaginal progesterone gel, 90 mg (Crinone) or IM progesterone injections, 50 mg (Prontogest; Amsa Pharmaceuticals, Rome, Italy) at 8:00 AM and 8:00 PM on the day before surgery and at 6:00 AM on the day of surgery. The vaginal gel provides controlled, sustained release of progesterone through its bioadhesive properties linked to its inert polycarbophil base. This product was approved for progesterone replacement and supplementation in various forms of infertility treatments. The respective doses of progesterone administered in the vaginal gel and IM injections were selected because approximately half of the progesterone in the vaginal gel is absorbed.[9] The true amounts delivered are not crucial because the end points studied were the ratios of endometrial tissue to serum concentrations.

Venous blood samples for progesterone assay were drawn at 8:00 AM on the day before surgery (baseline) and during the operation just before removing the uterus. After removal, the uterus was opened and examined. We avoided contact between the tissue specimens collected and progesterone gel still present in the vagina. Macroscopic evidence of endometrial hyperplasia or cancer was excluded in all participants. Endometrium was scraped from the anterior and posterior upper parts of the cavity, and samples were snap-frozen in liquid nitrogen.

Serum progesterone was measured in duplicate using a nonextractive ^{125}I radioimmunologic technique (DI RIA-PROG; Sorin Biomedica, Saluggia, Italy) with a lower limit of detection of 0.06 ng/mL and a specificity for progesterone of 97.5%. At low concentrations, the interassay and intra-assay coefficients of variation are less than 8%.

The tissue concentration of progesterone was measured by radioimmunoassay with adjustment for recovery. The procedure differed from that of Miles et al[8] because we added a chromatographic step to improve separation. Approximately 30-mg aliquots of thawed tissue samples were transferred into glass vials with 0.5 mL of cold phosphate buffer 0.1 mol/L, pH 7.2, and were homogenized using Ultraturrax (Ika, Staufen, Germany). Specimens were extracted in 4 mL of hexane for 30 minutes under rotatory vortex. The hexane fraction was transferred into 10-mL conic tubes and dried under nitrogen. Extracts were further purified on Sephadex LH 20 (mobile phase hexane/benzene/methanol 85:10:5), and fractions between the seventh and 11th mL were collected and dried under nitrogen. Mean recovery was 72%.

Results are expressed as ratios of endometrial to serum progesterone concentrations $\times$ 100. Ratios were compared between the vaginal and IM groups using two-tailed Wilcoxon rank-sum test; 95% confidence interval (CI) was also calculated for the observed mean differences. $P < .05$ was considered statistically significant.

Results

The two groups were similar with respect to age, parity, age at onset of menopause, and weight (Table 1). Recruitment lasted 7 months, with approximately two hysterectomy cases included each month.

The mean ($\pm$ standard deviation) baseline progesterone levels were less than 0.27 $\pm$ 0.05 and 0.30 $\pm$ 0.13 ng/mL for the vaginal and IM groups, respectively. Serum and tissue progesterone levels during surgery are illustrated in Figure 1. The ratios of endometrial to serum progesterone concentrations were markedly higher at a median of 14.1 (range 8.5–59.4; 95% CI 9.89, 38.79) in women who received vaginal rather than IM

Table 1. Demographic Characteristics

Characteristic	Vaginal	Intramuscular
Subjects (no.)	7	7
Age (y)	61.28 ± 5.64	59.86 ± 5.37
Nulliparous	1	1
Parous	6	6
Age at menopause (y)	50.86 ± 1.86	50 ± 4.58
Years since menopause (y)	10.42 ± 4.85	9.86 ± 5.67
Body mass index (kg/m^2)	23.21 ± 1.35	23.64 ± 1.02

Data are presented as *n* or mean ± standard deviation.

progesterone, in whom they reached a median of 1.2 (range 0.5–13.1; 95% CI −0.48, 7.39) ($P < .005$).

Discussion

Progesterone has been administered vaginally because this appeared to be the best option for avoiding painful IM injections. The reliability of endometrial biopsies[10] and pregnancy rates in donor egg recipients[11] far exceeded the expectations drawn from the relatively low serum levels achieved.[9–11] Our data showed that the discrepancy between reliable endometrial effects and relatively low plasma progesterone levels resulted from preferential delivery of the hormone to the uterus and higher uterine tissue concentrations of progesterone. This finding confirms the work by Miles et al[8] who used a similar method, although with higher doses and longer vaginal progesterone treatment. Our findings refute the objection that the high tissue levels observed by Miles et al[8] might have resulted from contamination of their biopsy samples by high progesterone concentrations remaining in the vagina. Whereas Miles et al[8] collected their endometrial samples from transvaginal biopsies, our tissue samples were obtained at hysterectomy. Blood levels obtained after the third administration of vaginal and IM progesterone at the time of endometrial sampling served for the calculations of endometrial/serum progesterone concentrations used in the comparison. Hence, the observed higher ratios of endometrial to serum progesterone concentrations after vaginal administration of progesterone show a local direct transport or functional "portal" system from the vagina to the uterus.

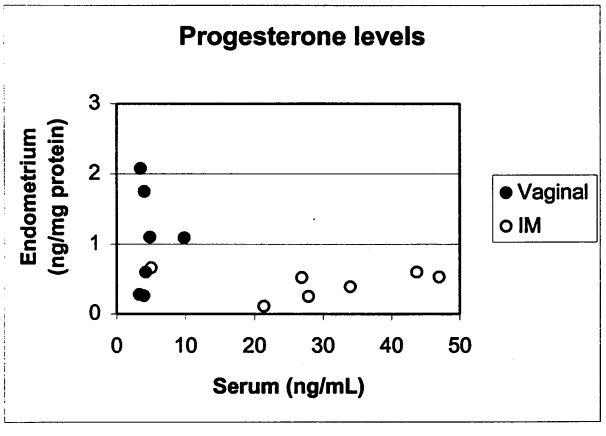

Figure 1. Endometrial and serum levels of progesterone in women treated with vaginal progesterone (*closed circles*) and intramuscular (IM) progesterone (*open circles*). After vaginal progesterone, mean endometrial and serum levels of progesterone were 1.05 ± 0.67 ng/mg protein and 4.82 ± 2.25 ng/mL, respectively. After IM progesterone, mean endometrial and serum levels of progesterone were 0.43 ± 0.19 ng/mg protein and 29.42 ± 14.14 ng/mL, respectively.

Various plausible mechanisms can explain the direct vagina-to-uterus transport, including direct diffusion through tissues, intraluminal (transcervical) passage, and transport through the venous or lymphatic circulatory systems. Another possibility is facilitated diffusion with countercurrent artery-to-vein exchange, in which progesterone diffuses from the uterovaginal lymph vessels or veins to the uterine arterial system. This type of exchange mechanism is known to take place between two tubes such as blood vessels that share a common (exchange) surface and have flows running in opposite directions. Substances in high concentrations in venous and lymphatic vessels can diffuse to nearby arteries, where their concentrations will rise above those of other organs. The higher progesterone concentration in uterine arterial blood as compared with the peripheral circulation (radial artery) after vaginal administration of progesterone strongly supports the latter mechanism.[12] Numerous other examples of countercurrent exchange exist in the body, including in the kidney (Henle's loop) and for heat exchange in the sinus cavernosus between the carotid and venous flow draining the nasopharyngeal area.

The total amount of progesterone absorbed from the vaginal gel can be approximated to be about 50% based on serum concentrations achieved at steady state.[9] The progesterone amounts administered vaginally and by IM injections were comparable, but the true amounts administered are not crucial because the end points studied were the endometrial/serum progesterone concentrations.

The preferential delivery to the uterus of substances administered vaginally through a "first uterine pass effect" was unveiled with vaginal administration of progesterone. This finding implies that serum levels do not reflect the efficacy of vaginal progesterone treatment. Our data should reassure clinicians who may be puzzled by the low levels of progesterone observed after daily use of the controlled, sustained-release vaginal progesterone gel Crinone. With this product, maximum benefit is drawn from the uterine trophicity of vaginal progesterone. Progesterone remains in the va-

gina, where it is continuously transferred to the uterus. Besides its use in infertility, vaginal progesterone may be clinically advantageous in hormone replacement therapy when synthetic progestins are feared to antagonize the beneficial effects of estrogens on the cardiovascular system.[13] Vaginal progesterone does not seem to cause the side effects seen with synthetic progestins.[14]

Teleologically, the finding of a local vagina-to-uterus transport system raises questions about its physiologic significance. It is reasonable to speculate that the primary function of the vagina-to-uterus transport is for uterine delivery of prostaglandins present in sperm. This would activate uterine contractility for facilitating sperm transport without raising circulating levels of prostaglandins, therefore avoiding side effects.

The "first uterine pass effect" confirmed by the present work offers exciting prospects for the future use of the vaginal route. Potential applications include the administration of various other uterotropic substances, notably uterorelaxing products. Maximal benefit would be observed from substances that are directly effective on the uterus but whose clinical use is limited because of serum-dependent side effects.

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