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Vaginal versus oral E_2 administration: effects on endometrial thickness, uterine perfusion, and contractility

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Objective: To compare the effects of vaginal or oral E_2 administration on endometrial thickness, uterine perfusion, and contractility.

Design: Prospective, randomized, crossover study.

Setting: Assisted Reproduction Unit, Clamart, France.

Patient(s): Thirty-nine infertile women undergoing 78 E_2/P cycles.

Intervention(s): Women received micronized 17β-E_2, 2 mg/day orally (cycle days 1 to 28) and P, 300 mg/day vaginally (cycle days 15 to 28). After a menstrual cycle washout interval, women received a similar treatment except that 17β-E_2 was administered vaginally.

Main Outcome Measure(s): Endometrial thickness, mean uterine artery pulsatility index, endometrial blood flow, and uterine contraction frequency assessed in ultrasound scans on cycle days 14 and 18.

Result(s): On day 14, the endometrium was thicker (8.7 $\pm$ 0.6 vs. 7.1 $\pm$ 0.3 mm, $P<.0001$), pulsatility index values were lower (2.4 $\pm$ 0.1 vs. 3.0 $\pm$ 0.2, $P<.0002$), and endometrial blood flow tended to be increased in the vaginal E_2 cycles as compared to the oral E_2 cycles. On day 18, similar differences remained. However, P-induced decrease in contraction frequency was slighter in vaginal E_2 cycles (33% vs. 18%, $P<.0003$).

Conclusion(s): Vaginal E_2 administration improves endometrial proliferation and uterine perfusion, presumably because of combined local and systemic effects, but may interfere with P-induced uterine relaxation. (Fertil Steril® 2001;76:994–8. ©2001 by American Society for Reproductive Medicine.)

Key Words: Hormonal replacement cycle, E_2, P, ultrasound, endometrial receptivity, embryo implantation

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Adequate uterine estrogenization is a necessary condition for uterine receptivity (1–3). Estrogens partake in key mechanisms that regulate uterine preparation to embryo implantation, such as stimulation of endometrial proliferation (4, 5) and improvement of uterine (6, 7) and endometrial (8, 9) perfusion. In addition, estrogens are likely to stimulate myometrial contractile activity during the follicular phase of the menstrual cycle (12, 14). Subsequently, uterine contractility undergoes a significant decrease during the luteal phase as a result of P (12–14), presumably to assist in embryo implantation (10, 11).

In egg donation IVF (1–3) and in some modalities of frozen-thawed ET cycles (15, 16), satisfactory priming of the uterus with exogenous E_2 before ET is required. For this, either the oral (1–3, 16) or transdermal (15) routes have been used, with comparable efficacy (17). However, in a fraction of cases, both approaches fail to achieve proper uterine estrogenization and lead to defective endometrial thickness (4, 5, 18), uterine perfusion (7–9), and pregnancy rates (4, 5, 7–9, 18).

In these cases, vaginal E_2 administration may be an interesting alternative. A recent study by Tourgeman et al. (19) showed that vaginal E_2 administration induces serum and endometrial tissue E_2 concentrations, respectively, 10-fold and 70-fold higher than the oral route at similar doses (19). These data provide further support to the hypothesis of a preferential uterine absorption of vaginally administered drugs (20, 21). However, clinical documentation on possible beneficial effects of

vaginal E_2 administration on endometrial proliferation and uterine perfusion is still lacking.

In addition, possible overstimulation of uterine contractility by the high peripheral and local E_2 levels (12, 14) induced by vaginal E_2 administration, as well as the effectiveness of subsequent uterine relaxation mediated by P, remain to be investigated. This issue is of particular importance because high-frequency contractions at the time of ET are associated with poor implantation rates (11). The hypercontractility at the time of ET may be attributable to an incomplete uterine quiescent action of P in the presence of the supraphysiologic E_2 levels triggered by controlled ovarian hyperstimulation (COH) (22).

Therefore, we elected to investigate the possible uterine impact of E_2 administered vaginally on endometrial thickness, uterine perfusion, and uterine contractions before and after exposure to P.

MATERIALS AND METHODS

Patient Characteristics

We prospectively studied 39 infertile candidates undergoing 78 E_2/P replacement cycles with frozen-thawed ETs. To limit the possibility of confounding factors in the analysis of our results, we included only young (<38 years of age), nonsmoking, regularly ovulating, lean (body mass indexes ranging between 18 and 25) women, whose uteri were morphologically normal as confirmed by hysteroscopy and ultrasound (absence of fibroids, adenomyosis, or polyps). Women whose uterine position did not allow adequate visualization of the uterus by transvaginal ultrasonography were not included. In addition, women with a history of dilation and curettage or uterine surgery were also excluded, because of possible confounding effects of these conditions on uterine perfusion and endometrial morphology. Informed consent was obtained from all patients, and this investigation was approved by our Institutional Review Board.

Study Protocol

Each subject underwent two hormone replacement cycles. In cycle I, all 39 women received micronized 17β-E_2 tablets by the oral route (2 mg/day; Provamès, Cassenne Laboratories, Puteaux, France), in the evening, from cycle day 1 to 28. From day 15 to 28, vaginal micronized P was administered at doses of 100 mg in the morning and 200 mg in the evening (Utrogestan; Besins-Iscovesco Pharmaceuticals, Paris, France). Two or 3 days after P discontinuation, withdrawal bleeding occurred.

In cycle II, the same women received the same dose of micronized 17β-E_2 (2 mg/day) from cycle day 1 to 28, but E_2 tablets were placed in the vaginal fornix. Tablets of E_2 used in both the oral and the vaginal cycles were similar. From days 15 to 28, women received vaginal micronized P treatment as in cycle I.

To prevent the possibility that the effect of one cycle carried over to the other cycle, women were randomly allocated to start the protocol by cycle I or cycle II (19 women followed the cycle I–cycle II sequence, and 20 women followed the cycle II–cycle I sequence). In addition, between the two subsequent cycles, all women respected a washout interval of one menstrual cycle without hormonal treatment.

Ultrasonographic Monitoring

On days 14 and 18 of cycles I and II, ultrasound scans of a sagittal plane of the uterus were performed using a 4.5- to 7.2-MHz multifrequency transvaginal probe (Siemens Elegra, Siemens S.A.S., Saint-Denis, France) by one single operator (L.M.S.) at approximately 9:00 AM. The operator was not aware of the treatment schedule or the clinical outcome. Environmental conditions were standardized throughout the ultrasound examination.

To assess the endometrial thickness, endometrial borders were set arbitrarily as the outer limits of the hyperechogenic myometrium–endometrium interfaces. Endometrial thickness (truly double endometrial thickness) was calculated as the greatest distance between the outer limits of the proximal and distal endometrial–myometrial interfaces. Sensitivity of endometrial thickness calculation was 0.1 mm. Intra-analysis coefficient of variation of our measurements were <5%.

Uterine perfusion was assessed by uterine artery impedance (6, 7) and endometrial blood flow (8, 9) measurements. To evaluate uterine artery impedance, blood flow of the ascending branches of the right and left uterine arteries was detected by color Doppler and assessed with the mean pulsatility index calculation. Furthermore, the presence or absence of endometrial blood flow was evaluated by power Doppler. Power Doppler settings were standardized for adequate sensitivity using a high pass filter at 60 Hz, pulsed repetition frequency at 400 Hz, and moderate long persistence.

To assess uterine contraction frequency, 2-minute ultrasound scans of a sagittal plane of the uterus were digitized on-line using a two image/second rate with a computer-assisted image analysis system (IôTEC 3.1.2, IôDP, Paris, France), which assists in the quantification of frequency of myometrial contractile activity. The present study respected similar methodologic characteristics as previously described (11, 22). Briefly, uterine contraction frequency assessment was based on the analysis of time-dependent variation in the myometrial–endometrium interfaces and uterine cavity by using a three-dimension-derived methodology (11, 22).

Hormonal Monitoring

On days 14 and 18, women underwent blood samplings for serum E_2 and P measurements. Serum E_2 was determined by an immunometric technique using an Estradiol-60 Amerlite kit (Ortho Clinical Diagnostics, Strasbourg, France). The lower limit of detection was 14 pg/mL, and intra-assay and

TABLE 1

Serum E_2 and progesterone levels in oral and vaginal E_2 cycles.

		Oral E_2 cycle (n = 39)	Vaginal E_2 cycle (n = 39)	*P* value
Day 14 (E_2 alone)	Serum E_2 levels (pg/mL)	139 ± 9	1493 ± 96	<.0001
	Serum P[a] levels (ng/mL)	0.32 ± 0.04	0.31 ± 0.04	NS[b]
Day 18 (E_2 + progesterone)	Serum E_2 levels (pg/mL)	147 ± 9	1063 ± 95	<.0001
	Serum P levels (ng/mL)	13.0 ± 0.9	13.4 ± 0.9	NS

Note: Hormonal levels are expressed as means ± SE.

[a] Progesterone.

[b] Not significant.

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interassay coefficients of variation were 8% and 9% for E_2, respectively. Serum P was measured by RIA using an I^{125} Progesterone Coatria kit (Bio-Mérieux, Paris, France). The lower limit of detection was 0.05 ng/mL and intra-assay and interassay coefficients of variation were, respectively, 8% and 11% for P.

Statistics

Measures of central tendency used were means and measures of variability were standard errors. With the inclusion of 39 patients into this two-treatment, crossover study the probability is ≥90% that the study will detect a treatment difference at a two-sided .05 significance level, if the anticipated true difference between the treatments is 1 mm for endometrial thickness, 1 unit of pulsatility index, and 0.5 uterine contraction/minute. Because data distribution was parametric, statistical assessment of pairwise differences between cycles I and II was performed by using the paired Student's *t*-test. A *P* value of <.05 was considered statistically significant.

RESULTS

Hormonal Results

Consequences of oral or vaginal E_2 administration on serum E_2 and P levels are summarized in Table 1. On day 14, circulating E_2 levels in the vaginal E_2 cycles were approximately 10-fold as high as in the oral E_2 cycles, whereas serum P remained at very low levels in all women in both treatment cycles. On day 18, serum E_2 levels remained constant in the oral E_2 cycles, whereas they decreased slightly yet significantly in the vaginal E_2 cycles. As expected, vaginal P administration increased serum P to levels commonly observed in the midluteal phase of the menstrual cycle in both cycles. Furthermore, both modalities for E_2 administration were observed to be well tolerated by patients with no concurrent bleeding and no detectable side effects.

Ultrasound Results

Uterine effects of oral or vaginal E_2 administration observed in ultrasound scans are detailed in Table 2. Vaginal E_2 administration induced a remarkable increase in endometrial thickness as compared to oral E_2 administration on days 14 and 18 ($P<.0001$). After endometrial exposure to P, an additional increase in endometrial thickness occurred in both treatment cycles ($P<.001$).

Vaginal E_2 administration improved uterine perfusion as compared to oral E_2 administration, with significantly lower mean uterine artery pulsatility index values on days 14 and 18. Consistently, the presence of endometrial blood vessels in the endometrium detected by power Doppler tended to be more often observed in the vaginal E_2 cycles than in the oral E_2 cycles on days 14 and 18. However, these differences did not reach statistical significance.

The consequences of vaginal or oral E_2 on uterine contraction frequency are shown in Table 1. In contrast with the markedly different circulating E_2 levels, uterine contraction frequency remained similar in both cycles on day 14. As expected, after P administration, a significant reduction in the contraction frequency occurred from days 14 to 18 in both the vaginal and oral E_2 cycles ($P<.0001$, respectively).

Yet, the pace of decrease in uterine contraction frequency as a result of P administration differed in both cycles. Interestingly, when women received vaginal E_2, the decrease in uterine contraction frequency from days 14 to 18 was less than when they received E_2 orally (18% vs. 33%, $P<.0003$), thereby leading to higher contraction frequency levels in the vaginal E_2 cycles on day 18 ($P<.007$).

DISCUSSION

The present study compared the relative uterine effects of vaginal and oral routes for E_2 administration. To address this issue our investigation set stringent methodologic parameters. It selected only young, regularly ovulating, lean, nonsmoking women. Cases of morphologic uterine abnormalities or inadequate uterine visualization at ultrasound scans were excluded. In addition, the crossover design used for E_2 administration reduced confounding effects of individual attributes in the interpretation of results. Finally, ultrasound

TABLE 2

Uterine effects of oral and vaginal E_2 administration.

		Oral E_2 cycle (n = 39)	Vaginal E_2 cycle (n = 39)	*P* value
Day 14 (E_2 alone)	Endometrial thickness (mm)	7.1 ± 0.3	8.7 ± 0.6	<.0001
	Mean uterine artery PI[a]	3.0 ± 0.2	2.4 ± 0.1	<.0002
	Endometrial vascularization (%)[b]	36%	60%	NS[c]
	No. uterine contractions per minute	3.9 ± 0.2	3.7 ± 0.2	NS
Day 18 (E_2 + P)	Endometrial thickness (mm)	8.0 ± 0.3	9.6 ± 0.6	<.0007
	Mean uterine artery PI	3.3 ± 0.2	2.9 ± 0.2	<.0002
	Endometrial vascularization (%)	39%	61%	NS
	No. uterine contractions per minute	2.6 ± 0.2	3.0 ± 0.2	<.007

Note: Continuous variables are expressed as means ± SE.

[a] Pulsatility index.

[b] Expressed as % of cases in which presence of blood flow in the endometrium was detected by power Doppler.

[c] Not significant.

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scans were analyzed by an independent operator who was not aware of treatment modalities, and the assessment of uterine contraction frequency was assisted by a computerized system.

Our results showed improved endometrial proliferation, as reflected by thicker endometria, and increased uterine perfusion, as reflected by lower uterine artery pulsatility index values and enhanced endometrial vascularization, in the vaginal E_2 as compared to the oral E_2 cycles, at the same E_2 doses (2 mg/day). Moreover, uterine contraction frequency, which was similar during E_2 treatment in both cycles, underwent a less notable reduction after P administration in the vaginal E_2 than in the oral E_2 cycles.

The increased endometrial thickness and improved uterine vascularization observed after vaginal E_2 administration may be explained by at least two mechanisms. First, as supported by our own previous data (20) and those of other investigators (19, 21), it is possible that the vaginal route allows a direct, preferential transport of hormones to the uterus. The privileged uterine effects of drugs administered vaginally presumably are due to a direct in-between or through cell vagina-to-uterus diffusion or a more elaborate local transport mechanism involving either a countercurrent circulation system with venous to artery diffusion (20, 21).

Second, it is conceivable that the markedly high peripheral E_2 levels achieved with the vaginal route concurred with the direct uterine effects to foster endometrial proliferation and perfusion. Indeed, the 10-fold gradient between serum E_2 levels achieved by the vaginal as compared to the oral route observed in the present study is remarkably similar to that reported by Tourgeman et al. (19) and consistent with earlier observations (23–25). These discrepancies in serum E_2 levels probably are a consequence of the hepatic inactivation of orally administered E_2 (26).

The slight but significant decrease in serum E_2 levels observed from days 14 to 18 in the vaginal E_2 cycles may result from an interference of P administration in the absorption of vaginal E_2 tablets. Yet, it is noteworthy that this phenomenon did not exert measurable consequences on endometrial thickness or induced uterine bleeding. This issue needs, however, to be further clarified in women receiving oral P rather than vaginally. Incidentally, the uniformly low P levels observed on day 14 in all 78 cycles studied confirm that either oral and vaginal administration of micronized 17β-E_2 starting in the early follicular phase of the menstrual cycle (cycle day 1) are effective to prevent ovulation in patients with functioning ovaries (15, 16), even at doses as low as 2 mg/day.

Yet, in contrast with the differences observed in serum E_2 levels as well as in endometrial proliferation and perfusion, uterine contraction frequency remained similar in both groups on day 14. This observation agrees with our previous data that indicated that circulating E_2 levels and uterine contraction frequency in COH cycles are not correlated (11, 22). Furthermore, despite the supraphysiologic E_2 levels observed in vaginal E_2 cycles, uterine contraction frequency did not increase beyond the values commonly observed at the late follicular phase of the menstrual cycle (3–4 contractions/minute) (10, 14). Taken together, these results lead us to hypothesize that maximum uterine contractile activity is reached at physiologic E_2 levels and additional increases in circulating E_2 levels are ineffective to further stimulate uterine contractility.

Furthermore, the expected decrease in uterine contraction frequency as a result of P administration was milder in the vaginal E_2 cycles as compared to the oral E_2 cycles. This observation is in keeping with the fact that uterine contraction frequency remains high during the early luteal phase of

COH cycles, at the time of ET (11, 22), a phenomenon associated with poor pregnancy rates (11). The observed "uterine resistance" to P may result from persistent uterine stimulating effects of supraphysiologic E_2 levels that thwart the uterine quiescent action of P. Contrasting with the lack of measurable effects on uterine contraction frequency, it is possible that chronic uterine exposure to supraphysiologic E_2 levels in the vaginal E_2 cycles increases uterine excitability to levels that are scarcely counterbalanced by P. Our observation of a more profound uterine relaxation mediated by P in the presence of lower circulating E_2 levels (oral E_2 cycle) supports this hypothesis. Hence, increasing the exposure of the uterus to P to restore satisfactory uterine relaxation at the time of transfer of frozen/thawed or egg donation embryos should be considered. Yet, it is noteworthy that mean uterine contraction frequency after P administration remained below potentially harmful levels (≥5 contractions/minute) (11) in both treatment cycles, which is reassuring with regard to ET outcome.

In conclusion, the favorable proliferative and vascular effects induced by vaginal E_2 administration, concurring with the adequate tolerance by patients observed by the present investigation, encourage us to consider the use of the vaginal route for E_2 priming of the uterine receptivity in frozen-thawed ETs and in egg donation cycles. The observed improvement of endometrial thickness and uterine vascularization may be attributed to both the putative direct uterine effects of vaginally administered hormones (20, 21) or the high circulating E_2 levels achieved by vaginal E_2 administration.

Hence, this novel approach may be particularly useful in the cases of suboptimal endometrial thickness or unsatisfactory uterine perfusion not only in hormone replacement cycles, but potentially also in COH cycles. Possible beneficial effects of increasing uterine exposure to P to counterbalance the uterine exciting effects of supraphysiologic E_2 levels and further improve uterine quiescence at the time of ET deserve further investigation (27). Finally, prospective randomized studies are needed to ascertain that the beneficial proliferative and vascular uterine effects of vaginal E_2 administration will lead to a significant improvement of embryo implantation outcome in hormone replacement or COH cycles.

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