

Vaginal versus oral E₂ administration: effects on endometrial thickness, uterine perfusion, and contractility

Renato Fanchin, M.D., Claudia Righini, M.D., Luca Maria Schönauer, M.D.,
François Olivennes, M.D., João Sabino Cunha Filho, M.D., and René Frydman, M.D.

Department of Obstetrics and Gynecology and Reproductive Endocrinology, Hôpital Antoine Béchère,
Clamart, France

Objective: To compare the effects of vaginal or oral E₂ 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 E₂/P cycles.

Intervention(s): Women received micronized 17β-E₂, 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-E₂ 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 ± 0.6 vs. 7.1 ± 0.3 mm, $P < .0001$), pulsatility index values were lower (2.4 ± 0.1 vs. 3.0 ± 0.2 , $P < .0002$), and endometrial blood flow tended to be increased in the vaginal E₂ cycles as compared to the oral E₂ cycles. On day 18, similar differences remained. However, P-induced decrease in contraction frequency was slighter in vaginal E₂ cycles (33% vs. 18%, $P < .0003$).

Conclusion(s): Vaginal E₂ 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, E₂, P, ultrasound, endometrial receptivity, embryo implantation

Received February 21,
2001; revised and
accepted May 24, 2001.

This paper was pre-
selected for the Society for
Reproductive
Endocrinology and
Infertility Prize at the
Annual Meeting of the
American Society for
Reproductive Medicine,
San Diego, California,
October 21–26, 2000.

Reprint requests: Renato
Fanchin, M.D., Department of
Obstetrics and Gynecology and
Reproductive Endocrinology,
Hôpital Antoine Béchère,
157, rue de la Porte de
Trivaux, 92141, Clamart,
France (FAX: 0033 1
45374980; E-mail:
renato.fanchin@abc.ap-
hop-paris).

0015-0282/01/\$20.00
PII S0015-0282(01)02841-2

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 E₂ 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 E₂ administration may be an interesting alternative. A recent study by Tourgeman et al. (19) showed that vaginal E₂ administration induces serum and endometrial tissue E₂ 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 E₂ administration on endometrial proliferation and uterine perfusion is still lacking.

In addition, possible overstimulation of uterine contractility by the high peripheral and local E₂ levels (12, 14) induced by vaginal E₂ 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 E₂ levels triggered by controlled ovarian hyperstimulation (COH) (22).

Therefore, we elected to investigate the possible uterine impact of E₂ 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 E₂/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 17 β -E₂ 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 17 β -E₂ (2 mg/day) from cycle day 1 to 28, but E₂ tablets were placed in the vaginal fornix. Tablets of E₂ 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 (I6TEC 3.1.2, I6DP, 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 E₂ and P measurements. Serum E₂ 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 E₂ and progesterone levels in oral and vaginal E₂ 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.

Fanchin. Uterine effects of vaginal E₂. *Fertil Steril* 2001.

interassay coefficients of variation were 8% and 9% for E₂, respectively. Serum P was measured by RIA using an I¹²⁵ 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 E₂ administration on serum E₂ and P levels are summarized in Table 1. On day 14, circulating E₂ levels in the vaginal E₂ cycles were approximately 10-fold as high as in the oral E₂ cycles, whereas serum P remained at very low levels in all women in both treatment cycles. On day 18, serum E₂ levels remained constant in the oral E₂ cycles, whereas they decreased slightly yet significantly in the vaginal E₂ 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 E₂ 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 E₂ administration observed in ultrasound scans are detailed in Table 2. Vaginal

E₂ administration induced a remarkable increase in endometrial thickness as compared to oral E₂ 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 E₂ administration improved uterine perfusion as compared to oral E₂ 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 E₂ cycles than in the oral E₂ cycles on days 14 and 18. However, these differences did not reach statistical significance.

The consequences of vaginal or oral E₂ on uterine contraction frequency are shown in Table 1. In contrast with the markedly different circulating E₂ 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 E₂ 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 E₂, the decrease in uterine contraction frequency from days 14 to 18 was less than when they received E₂ orally (18% vs. 33%, *P*<.0003), thereby leading to higher contraction frequency levels in the vaginal E₂ cycles on day 18 (*P*<.007).

DISCUSSION

The present study compared the relative uterine effects of vaginal and oral routes for E₂ administration. To address this issue our investigation set stringent methodologic parameters. It selected only young, regularly ovulating, lean, non-smoking women. Cases of morphologic uterine abnormalities or inadequate uterine visualization at ultrasound scans were excluded. In addition, the crossover design used for E₂ administration reduced confounding effects of individual attributes in the interpretation of results. Finally, ultrasound

TABLE 2

Uterine effects of oral and vaginal E₂ 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.

Fanchin. Uterine effects of vaginal E₂. *Fertil Steril* 2001.

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 E₂ as compared to the oral E₂ cycles, at the same E₂ doses (2 mg/day). Moreover, uterine contraction frequency, which was similar during E₂ treatment in both cycles, underwent a less notable reduction after P administration in the vaginal E₂ than in the oral E₂ cycles.

The increased endometrial thickness and improved uterine vascularization observed after vaginal E₂ 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 E₂ 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 E₂ 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 E₂ levels probably are a consequence of the hepatic inactivation of orally administered E₂ (26).

The slight but significant decrease in serum E₂ levels observed from days 14 to 18 in the vaginal E₂ cycles may result from an interference of P administration in the absorption of vaginal E₂ 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-E₂ 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 E₂ 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 E₂ levels and uterine contraction frequency in COH cycles are not correlated (11, 22). Furthermore, despite the supraphysiologic E₂ levels observed in vaginal E₂ 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 E₂ levels and additional increases in circulating E₂ 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 E₂ cycles as compared to the oral E₂ 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 E₂ 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 E₂ levels in the vaginal E₂ 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 E₂ levels (oral E₂ 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 E₂ administration, concurring with the adequate tolerance by patients observed by the present investigation, encourage us to consider the use of the vaginal route for E₂ 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 E₂ levels achieved by vaginal E₂ 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 E₂ 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 E₂ administration will lead to a significant improvement of embryo implantation outcome in hormone replacement or COH cycles.

References

- Lutjen P, Trounson A, Leeton J, Findlay J, Wood C, Renou P. The establishment and maintenance of pregnancy using in vitro fertilization and embryo donation in a patient with primary ovarian failure. *Nature* 1984;307:174-5.
- Navot D, Laufer N, Kopolovic J, Rabinowitz R, Birkenfeld A, Lewin A, et al. Artificially induced endometrial cycles and establishment of pregnancies in the absence of ovaries. *N Engl J Med* 1986;314:806-11.
- Li TC, Cooke ID, Warren MA, Goolamallee M, Graham RA, Aplin JD. Endometrial responses in artificial cycles: a prospective study comparing four different oestrogen dosages. *Br J Obstet Gynaecol* 1992;99:751-6.
- Rabinowitz R, Laufer N, Lewin A, Navot D, Bar I, Margalioth EJ, et al. The value of ultrasonographic endometrial measurement in the prediction of pregnancy following in vitro fertilization. *Fertil Steril* 1986;45:824-8.
- Gonen Y, Casper RF, Jacobson W, Blankier J. Endometrial thickness and growth during ovarian stimulation: a possible predictor of implantation in in vitro fertilization. *Fertil Steril* 1989;52:446-50.
- Sterzik K, Grab D, Sasse V, Hutter W, Rosenbusch B, Terinde R. Doppler sonographic findings and their correlation with implantation in an in vitro fertilization program. *Fertil Steril* 1989;52:825-8.
- de Ziegler D, Bessis R, Frydman R. Vascular resistance of uterine arteries: physiological effects of estradiol and progesterone. *Fertil Steril* 1991;55:775-9.
- Steer CV, Campbell S, Tan SL, Crayford T, Mills C, Mason BA, et al. The use of transvaginal color flow imaging after in vitro fertilization to identify optimum uterine conditions before embryo transfer. *Fertil Steril* 1992;57:372-6.
- Yang JH, Wu MY, Chen CD, Jiang MC, Ho HN, Yang YS. Association of endometrial blood flow as determined by a modified colour Doppler technique with subsequent outcome of in-vitro fertilization. *Hum Reprod* 1999;14:1606-10.
- IJland MM, Evers JL, Dunselman GA, Volovics L, Hoogland HJ. Relation between endometrial wavelike activity and fecundability in spontaneous cycles. *Fertil Steril* 1997;67:492-6.
- Fanchin R, Righini C, Olivennes F, Taylor S, de Ziegler D, Frydman R. Uterine contractions at the time of embryo transfer alter pregnancy rates after in-vitro fertilization. *Hum Reprod* 1998;13:1968-74.
- Csapo AI, Pinto-Dantas CR. The cyclic activity of the nonpregnant human uterus. A new method for recording intrauterine pressure. *Fertil Steril* 1966;17:34-8.
- Martinez-Gaudio M, Yoshida T, Bengtsson LP. Propagated and non-propagated myometrial contractions in normal menstrual cycles. *Am J Obstet Gynecol* 1973;115:107-11.
- Lyons EA, Taylor PJ, Zheng XH, Ballard G, Levi CS, Kredentser JV. Characterization of subendometrial myometrial contractions throughout the menstrual cycle in normal fertile women. *Fertil Steril* 1991;55:771-4.
- Schmidt CL, de Ziegler D, Gagliardi CL, Mellon RW, Taney FH, Kuhar MJ, et al. Transfer of cryopreserved-thawed embryos: the natural cycle versus controlled preparation of the endometrium with gonadotropin-releasing hormone agonist and exogenous estradiol and progesterone (GEEP). *Fertil Steril* 1989;52:609-16.
- Lelaider C, de Ziegler D, Gaetano J, Hazout A, Fernandez H, Frydman R. Controlled preparation of the endometrium with exogenous estradiol and progesterone: a novel regimen not using a gonadotrophin-releasing hormone agonist. *Hum Reprod* 1992;7:1353-6.
- Steingold KA, Matt DW, de Ziegler D, Sealey JE, Fratkin M, Reznikov S. Comparison of desferal to oral estradiol administration on hormonal and hepatic parameters in women with premature ovarian failure. *J Clin Endocrinol Metab* 1991;73:275-80.
- Hassan HA, Saleh HA. Endometrial unresponsiveness: a novel approach to assessment and prognosis in in vitro fertilization cycles. *Fertil Steril* 1996;66:604-7.
- Tourgeman DE, Gentzchein E, Stanczyk FZ, Paulson RJ. Serum and tissue hormone levels of vaginally and orally administered estradiol. *Am J Obstet Gynecol* 1999;180:1480-3.
- Fanchin R, de Ziegler D, Bergeron C, Righini C, Torrisi C, Frydman R. Transvaginal administration of progesterone. *Obstet Gynecol* 1997;90:396-401.
- Cicinelli E, de Ziegler D, Bulletti C, Matteo MG, Schonauer LM, Galantino P. Direct transport of progesterone from vagina to uterus. *Obstet Gynecol* 2000;95:403-6.
- Fanchin R, Ayoubi JM, Olivennes F, Righini C, de Ziegler D, Frydman R. Hormonal influence on the uterine contractility during controlled ovarian hyperstimulation. *Hum Reprod* 2000;15:90-100.
- Schiff I, Tulchinsky D, Ryan KJ. Vaginal absorption of estrone and 17beta-estradiol. *Fertil Steril* 1977;28:1063-6.
- Rigg LA, Milanese B, Villanueva B, Yen SS. Efficacy of intravaginal and intranasal administration of micronized estradiol-17beta. *J Clin Endocrinol Metab* 1977;45:1261-4.
- Shushan A, Simon A, Reubinoff BE, Lewin A, Schenker JG, Laufer N. The use of vaginal tablets as a vehicle for steroid replacement in a donor oocyte program. *Fertil Steril* 1994;62:412-4.
- Yen SS, Martin PL, Burnier AM, Czekala NM, Greaney MO Jr, Callantine MR. Circulating estradiol, estrone and gonadotropin levels following the administration of orally active 17beta-estradiol in postmenopausal women. *J Clin Endocrinol Metab* 1975;40:518-21.
- Fanchin R, Righini C, de Ziegler D, Olivennes F, Lédée N, Frydman R. Effects of vaginal progesterone administration on uterine contractility at the time of embryo transfer. *Fertil Steril* 2001;75:1136-40.