Title:

Vaginal micronized progesterone versus intramuscular progesterone for luteal support in women undergoing in vitro fertilisation-embryo transfer

Authors:

Mohamed F Mitwally, Michael P Diamond and Mostafa Abuzeid

Journal:

Fertil Steril 2010

Vaginal micronized progesterone versus intramuscular progesterone for luteal support in women undergoing in vitro fertilization–embryo transfer

Mohamed F. Mitwally, M.D., H.C.L.D.,[a] *Michael P. Diamond, M.D.,*[b] *and Mostafa Abuzeid, M.D.*[c]

[a] Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology and Women's Health, University of Minnesota, Minneapolis, Minnesota; [b] Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Wayne State University, Detroit, Michigan; and [c] Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Michigan State University, East Lansing, Michigan

Objective: To study the outcome of IVF-ET in women who used vaginal P (vaginal P_4) versus those who used P in oil via IM injection (IM-P_4) for luteal support.
Design: Retrospective cohort.
Setting: Tertiary referral infertility center.
Patient(s): A cohort of 544 women.
Intervention(s): In 145 women, vaginal P_4 was used, while in 399 women, IM-P_4 was used for luteal support.
Main Outcome Measure(s): The primary outcome was ongoing pregnancy rate. Secondary outcomes included other IVF-ET outcomes: rates of clinical pregnancy and pregnancy loss (chemical and miscarriage) and serum P levels during the luteal phase and early pregnancy.
Result(s): Women who used vaginal P_4 for luteal support had ongoing pregnancy rates (odds ratio [OR], 1.0675; 95% confidence interval [CI], 0.7587–1.5020) and rates of total pregnancy loss (OR, 1.0775; 95% CI, 0.7383–1.5727) that were not statistically different from those who used IM-P_4. During the luteal phase, women who used vaginal P_4 had mean serum P levels that were not statistically different from those who used IM-P_4. However, during early pregnancy, mean P levels in pregnant women who used vaginal P_4 were statistically significantly higher.
Conclusion(s): In women undergoing IVF-ET according to the GnRH agonist long protocol, luteal support with vaginal P_4 was associated with treatment outcomes that were no different from those associated with IM-P_4 luteal support. (Fertil Steril® 2010;93:554–69. ©2010 by American Society for Reproductive Medicine.)

Key Words: IM-P_4, in vitro fertilization, luteal support, micronized vaginal progesterone

IVF-ET has become the mainstay of infertility treatment, with the number of IVF clinics increasing worldwide. Women undergoing IVF-ET treatment almost always receive controlled ovarian hyperstimulation (COH) medications (exogenous injectable gonadotropins) to stimulate the formation of multiple mature ovarian follicles to retrieve several oocytes (1–7). With COH and the formation of several mature ovarian follicles, E_2 levels are expected to rise to supraphysiological values particularly during the follicular phase. In many patients, high E_2 levels may result in a premature endogenous LH surge and premature release of the oocytes unless such endogenous LH surge is prevented. Prevention of premature endogenous LH surge is usually achieved by adding GnRH analogs (GnRH agonist or GnRH antagonist) to the COH protocol (1–3).

Received July 23, 2008; revised January 24, 2009; accepted February 14, 2009; published online April 10, 2009.
M.F.M. has nothing to disclose. M.P.D. has nothing to disclose. M.A. has nothing to disclose.
Ferring Pharmaceuticals has provided financial support for conducting and writing this study.
Reprint requests: Mohamed F. Mitwally, M.D., H.C.L.D., Toronto Center for Advanced Reproductive Technology (TCART), University of Toronto, 150 Bloor Street West, Suite 210, Toronto, Ontario M5S 2X9 Canada (FAX: 416-972-0036; E-mail: mmitwally@yahoo.com).

After ovulation, the major function of the corpus luteum is the production of sex hormones (particularly P) and other possible factors that are mandatory for supporting pregnancy. This is particularly crucial during the time of implantation and early pregnancy weeks before the establishment of the placenta. At that time, P is known to be a crucial factor required for successful implantation and continuation of early pregnancy. The role of P in the maintenance and support of pregnancy has been established for more than three decades since the classic experiments of Csapo et al. (8, 9). They demonstrated that P secretion by the corpus luteum was absolutely essential for the success of early human pregnancy. Surgical removal of the corpus luteum before 7 weeks' gestation (calculated from the last menstrual period) uniformly precipitated an abrupt decrease in serum P levels followed by pregnancy loss. Furthermore, they found that removal of the corpus luteum after 8 weeks' gestation resulted in a slight and transient decrease in P serum levels with continuation of pregnancy (8). Interestingly, the investigators found that exogenous P replacement after early removal of the corpus luteum (before 7 weeks' gestation) prevented an otherwise inevitable pregnancy loss. These findings demonstrated obviously the crucial role of P in the success and maintenance of early pregnancy and that such P is primarily derived from the

0015-0282/10/$36.00
doi:10.1016/j.fertnstert.2009.02.047

corpus luteum before 7 weeks' gestation. On the other hand, P is believed to be derived almost entirely from the trophoblast beyond 9 weeks' gestation, while in the interval between 7 and 9 weeks of gestation P is produced from both sources to a varying extent during an interval known as the luteal-placental shift (9).

After ovulation in women undergoing COH, multiple follicular development results in the formation of several corpora lutea in the ovaries; each is expected to secrete P. However, despite the presence of several corpora lutea, P production has been believed to be inadequate, that is, there is not enough to support the implantation and maintenance of early pregnancy, and the luteal phase has been described as inadequate in women undergoing COH for IVF-ET (10–15). Inadequate corpus luteum functioning is thought to be due to several reasons (15). This includes the use of GnRH analogs that suppress endogenous gonadotropins, in particular, LH, which is mandatory for driving an adequately functioning corpus luteum (16, 17). Commonly used during COH protocols for IVF-ET, GnRH agonists have an initially agonistic effect on the pituitary to stimulate gonadotropin release directly. However, after continued stimulation, ultimately they result in down-regulation of the pituitary GnRH receptors leading to suppression of gonadotropin secretion and prevention of endogenous LH surge. After down-regulation, the anterior pituitary function is slow to recover and does not return to normal (production of endogenous gonadotropins, FSH, and LH) until 2–3 weeks after GnRH agonist treatment ends (17). The dependence of a normal function of the corpus luteum on the presence of endogenous LH stimulation makes some form of luteal phase support prudent, if not essential, to ensure the adequate P production that is crucial for the success of implantation and continuation of early pregnancy (10, 17, 18). Other reasons that have been postulated for inadequate corpus luteum function associated with COH for IVF-ET include the use of exogenous injectable gonadotropins as well as the supraphysiological levels of E_2 associated with multiple follicular development. In addition, other unknown factors associated with COH and infertility diagnosis have been suggested (19). Finally, the trauma inflicted on the ovarian follicles during oocyte retrieval (bleeding during follicular wall puncturing and aspirating granulosa cells) has been hypothesized to be associated with a reduced steroidogenesis capacity of the corpora lutea. However, this hypothesis has been challenged (20).

The consensus regarding inadequately functioning corpora lutea has led to the universal practice of administering large amounts of exogenous P to support the luteal phase in women undergoing IVF-ET treatment (10–12). Clinicians have been adopting practices that ensure the achievement of adequate levels of P that are high and steady during the luteal phase to support implantation and early pregnancy. Those practices include administering exogenous P and/or stimulating endogenous P production by hCG injections (21, 22). A recent Cochrane systematic review that included 59 studies concluded that in IVF-ET cycles involving down-regulation with a long-acting GnRH agonist, P supplementation (administered vaginally or by IM injections) was associated with significantly higher ongoing pregnancy rates per ET when compared with placebo or no P supplementation (odds ratio [OR], 2.38; 95% confidence interval [CI], 1.32–4.29) (23). Various doses, durations, and types of luteal phase support by P administration have been implemented without agreement on the optimal supplementation scheme (11). Different routes of exogenous P administration for luteal phase support have been reported, including oral, vaginal, and parenteral routes, for example, IM (12). Despite the several disadvantages of IM-P_4 injections including side effects and painful injections (11), as well as inflammatory reactions and abscesses (24), this is still the most commonly used route of P administration for luteal support in IVF centers in North America.

When studied in the absence of endogenous P production (absence of corpus luteum), vaginal administration of P was found to result in significantly higher local tissue levels in the endometrium when compared with IM-P_4 (25). On the other hand, vaginal administration of P has been found to be associated with minimal systemic absorption and negligible circulating serum levels of P (26), while IM-P_4 has been associated with steady significantly higher levels of circulating serum P (25). This could explain the universal practice of luteal support by IM-P_4 injection in women undergoing IVF treatment, that is, IM-P_4 injection is associated with reliable P absorption that will lead to steady levels of serum P. Such steady levels of serum P absorbed after IM-P_4 would supply adequate amounts of P support to the endometrium and the uterus. Such a belief could also explain the empirical practice of measuring serum P levels during the luteal phase to monitor the adequacy of P administration for luteal support. Furthermore, some clinicians even adopt the empirical practice of increasing IM-P_4 doses when P serum levels are found to be below acceptable levels, as empirically determined by different centers. Also, such practices seem to be based on the unsupported thought that most of the circulating serum P levels in IVF women receiving IM-P_4 come from P absorbed after its exogenous administration (IM-P_4 injections) rather than from the endogenously produced P by the corpora lutea.

The hypothesis of this study was that vaginal administration of P for luteal support in women undergoing IVF-ET treatment would be associated with no differences in the treatment outcomes (ongoing pregnancy rates and rates of pregnancy loss) when compared with IM-P_4 luteal support, that is, vaginal P administration provides luteal support that is no different from the IM-P_4 support.

The objectives of this study were to determine the outcomes of IVF-ET in women who used vaginal micronized P versus those who used IM-P_4 for luteal support and to investigate serum P levels during the luteal phase and early pregnancy in those women.

MATERIALS AND METHODS

This is a retrospective cohort study that included a group of 544 consecutive women who underwent IVF-ET treatment at Michigan IVF from July 2005 through January 2006. Each woman completed one fresh treatment cycle of IVF-ET, using her own oocytes. In 145 women (the vaginal P group), the micronized vaginal P Endometrin (a vaginal insert containing 100 mg of micronized P that is used at a dose of 2 or 3 times every day; Ferring Pharmaceuticals, Parsippany, NJ) was used for luteal support. The IM-P_4 group included 399 women who received IM-P_4 in oil (100 mg injection every day) for luteal support. In both groups, P luteal support started on the day after oocyte retrieval and continued until a negative pregnancy test was confirmed, pregnancy loss occurred, or up to 12 weeks' gestation was achieved (ongoing pregnancy). The vaginal P group included women who were enrolled in an Institutional Review Board–approved phase III multicenter randomized controlled trial who received Endometrin as 100-mg vaginal inserts either 2 or 3 times a day as reported elsewhere (27). The IM-P_4 group included women who met the eligibility criteria for enrollment into the same randomized trial (27) but declined enrollment and preferred receiving P IM for luteal support. In the Endometrin randomized clinical trial (27), there was no significant difference in the outcomes of IVF-ET treatment (clinical and ongoing pregnancy rates and rates of pregnancy loss) between the two Endometrin subgroups who used Endometrin 100 mg 2 or 3 times a day (data not shown). In the current study, women in the two Endometrin subgroups were included together in the analysis of the results as one combined vaginal P group. Institutional Review Board approval was obtained for the analysis of the data collected from the charts of the vaginal and IM-P_4 groups.

Included Women

The inclusion/exclusion criteria and the details of the IVF-ET protocol were the same as those followed in the Endometrin randomized clinical trial (27). Women were included in the vaginal and IM groups if they met the following inclusion criteria: healthy women between 18 and 42 years of age, with a body mass index $\leq$34 kg/m^2, a baseline FSH level $\leq$15 mIU/mL on day 3 of menstrual period, a history of infertility requiring IVF, a normal uterine cavity and adnexae, and a male partner or donor sperm with semen analysis results adequate for IVF. Exclusion criteria included a history of recurrent pregnancy loss (defined as three or more spontaneous miscarriages), abnormal uterine bleeding of undetermined origin, or a history of either poor response to gonadotropins (defined as two or fewer mature follicles) or two previous cancelled IVF cycles. Women who had clinically relevant systemic disease or male partners with obvious leukospermia or signs of infection in a recent semen sample were also excluded from the study. Any woman who failed to produce at least three oocytes for retrieval in the study cycle was not included.

IVF-ET and P Luteal Support Protocol

The P administration for luteal support was part of the standard IVF treatment regimen in which the IVF cycle was divided into two phases: the first phase included a pretreatment phase, and the second phase included a randomization/treatment phase. The first phase (pretreatment phase) included screening, down-regulation using an injectable GnRH-agonist, ovarian stimulation with gonadotropins, and oocyte retrieval. The second phase (randomization/treatment phase) began on the day after oocyte retrieval and lasted up to approximately 10 weeks if the patient became pregnant (12 weeks' gestation). In this phase, women in the vaginal P group were randomized to receive the micronized vaginal P 2 or 3 times a day, while women in the IM-P_4 group received IM-P_4 100 mg in oil once a day for luteal support. Women who succeeded in becoming pregnant remained on their P treatment regimen for the entire 10 weeks of pregnancy. All women with a negative pregnancy test discontinued P supplementation immediately after a second confirmatory pregnancy test (βhCG serum level $<$5 mIU/ml) that was done 2 days later.

Treatment Protocol

Screening for eligibility included medical and fertility history, physical and gynecological examination, transvaginal ultrasound examination, hormone evaluations, and semen analysis. Upon successful completion of the screening procedures, pituitary down-regulation was performed with an injectable GnRH agonist, using a long protocol. GnRH agonist injections started during the luteal phase with daily injections. Documentation of down-regulation of the pituitary and ovaries was confirmed by a serum E_2 level $<$50 pg/mL, endometrial lining $<$7 mm, and no evidence of ovarian activity on transvaginal ultrasound. Absence of ovarian activity was defined as absence of ovarian follicles with a mean diameter $>$12 mm). After documentation of adequate down-regulation of the pituitary/ovarian axis, gonadotropin treatment was started. Gonadotropin therapy was performed with the combined use of hMG (Menopur; Ferring Pharmaceuticals, Parsippany, NJ) and highly purified FSH (Bravelle;, Ferring Pharmaceuticals). All women received at least one vial of hMG daily during the period of COH. Gonadotropin stimulation was stopped when the lead follicle mean diameter was $\geq$18 mm, and a single IM injection of 10,000 IU hCG (Novarel;, Ferring Pharmaceuticals) was administered to trigger final follicular maturation. Oocytes were retrieved within approximately 36 hours after hCG administration. ET was done on day 3 or day 5 with no more than three cleaving embryos transferred on day 3 after retrieval or no more than two blastocysts transferred on day 5 after retrieval. About 2 weeks after ET, a serum pregnancy test was performed to document biochemical pregnancy. If positive, a repeat serum pregnancy test was performed about 2 days later. At about 2 weeks after a positive serum pregnancy test, clinical pregnancy was confirmed by transvaginal ultrasound. The women

who were pregnant continued taking P support for 10 weeks, until 12 weeks' gestational age.

Outcome Measures

The primary outcome was ongoing pregnancy rate defined as clinical pregnancy beyond 12 weeks of gestation. Secondary outcomes included other IVF-ET outcomes: clinical pregnancy rate and rates of pregnancy loss (chemical and miscarriage), as well as serum P levels during the luteal phase and early pregnancy. Clinical pregnancy was defined as the presence of an intrauterine gestational sac. Biochemical pregnancy loss was defined as dropping βhCG serum levels before detection of a gestational sac, and miscarriage was defined as loss of a clinical or ongoing pregnancy before 20 weeks of gestation.

Blood Draws for Serum P Assays

All women in this study underwent serial measurements of the serum P levels during the luteal phase, and during the early weeks of pregnancy in pregnant women for up to 12 weeks of gestation or until pregnancy loss occurred, whichever occurred earlier. The analysis included only those women who had at least one blood draw for serum P assay on the following days (after oocyte retrieval): day 3 or 4, day 5 or 6, day 7 or 8, day 9 or 10, day 11 or 12, day 13 or 14, and between days 15 and 18. In pregnant women, a total of at least three blood draws for P assays were collected during early pregnancy (between day 19 after oocyte retrieval and 12 weeks of gestation) as follows: at least one blood draw during each of the following four intervals: days 19–22 interval, days 23–26 interval, day 27 or beyond until 12 weeks of gestation. Blood was drawn for P assays between 8:00 and 10:00 A.M. P (vaginal or IM-P_4 injection) was administered 1–2 hours before blood draws. Table 1 summarizes the windows of blood draws for obtaining serum P levels. A cohort of 373 women out of the total cohort of women included in this study (544 women) completed the blood draws (98 women from the vaginal P group and 275 women from the IM-P_4 group).

P Assay

Venous blood samples were drawn in standard gel tubes and were allowed to clot before centrifugation at 3000 *g* for 10 minutes to separate the serum. Samples were assayed on the same day. P assay was performed using an Immulite-2000 (Diagnostic Products Corporation, DPC, Los Angeles), solid-phase, competitive chemiluminescent enzyme immunoassay. The assay had a calibration range of 0.2–40 ng/mL (0.6–127 nmol/L) and analytical sensitivity of 0.1 ng/mL (0.3 nmol/L). The analytical performance characteristics had a coefficient of variation that was 8% and 10% for intra- and interassay precision, respectively.

Statistical Analysis: Power Calculation for Adequacy of Included Sample Size

To test the null hypothesis that there is no statistically significant difference in the primary outcome (ongoing pregnancy rate) between the vaginal P and the IM-P_4 groups, the following assumptions were made:

1. Type I error or alpha error at 0.05
2. Type II error or beta error at 0.20
3. A change of ongoing pregnancy rate of 25% or more to be clinically significant, 48% (the average ongoing pregnancy rate in the center) down to 36%

A sample size of at least 526 women in both groups was found to be enough to achieve a power of 0.80 to show no difference (noninferiority) in the vaginal P group compared with the IM-P_4 P group. Given that 145 women were included in the vaginal P group and 339 women in the IM-P_4 group, according to the above assumptions, the power for the sample

TABLE 1

Windows of blood draws for obtaining serum P levels.

Window of blood draw (no. of days after the day of oocyte retrieval)	Women who underwent at least one blood draw
Day 3 or 4	
Day 5 or 6	
Day 7 or 8	
Day 9 or 10	All women
Day 11 or 12	
Day 13 or 14	
Between days 15 and 18	
Between days 19 and 22	Pregnant women (with ongoing pregnancies)
Between days 23 and 26	
Between days 27 and 12 weeks' gestation (day 72)	

Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

size (544 women) included in this study was found to be about 0.83.

The sample size of 544 women included in this study was powered to test some of the secondary outcomes including the implantation rates and clinical pregnancy rates (power calculations for the included sample size were found to be about 93 and 87, respectively). However, the sample size was not powered to test the rates of pregnancy loss (biochemical and miscarriage). The calculated power was about 65.

Regarding the power calculation for the other secondary outcome, mean P levels, the number of women who completed blood draws (373 women) provided a large enough sample size to draw powered calculations. To test the null hypothesis that there is no statistically significant difference in the mean values of P levels between the vaginal P and the IM-P_4 groups the following assumptions were made:

1. Alpha error at 0.05
2. Delta (difference in population means) of 0.01
3. Sigma (SD) of 0.05
4. M (the ratio between the control and experimental patients) of 3

A sample size of at least 262 women in both groups was found to be enough to achieve a power of 0.80. Given that 98 women were included in the vaginal P group and 275 women in the IM-P_4 group, according to the above assumptions, the power for the sample size included in this study was found to be 0.92.

Outcome measurements were analyzed by the appropriate statistical tests including Student's *t*-test and χ^2-test (for continuous and dichotomous variables, respectively). $P<.05$ was considered statistically significant. Analysis of mean P levels during the luteal phase and early pregnancy was done between the two patient groups (the vaginal group and the IM-P_4 group) using Student's *t*-test and analysis of variance (ANOVA). Student's *t*-test was used to compare each two corresponding mean P levels (samples obtained on the same corresponding luteal or early pregnancy blood draw interval window). ANOVA was done to compare between all the samples together in the two groups. Correlation analysis between luteal P levels and clinical pregnancy rates was applied for each group separately. Women were subgrouped according to mean luteal P levels into five subgroups. The ranges of mean P levels for each of the five groups were increments of SD (the range between the highest and lowest mean P level when divided by the SD the outcome of the division was 5, ie the number of the subgroups). The trend test was used to calculate the *P*-value to determine statistical significance among the subgroups of P mean values. Statistical analysis was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago).

RESULTS

Table 2 shows patients and cycle characteristics in the vaginal and IM-P_4 groups. The two groups were matching without significant differences in age (women and male partner) or cycle characteristics (number of stimulation days, E_2, and P levels on day of hCG administration or number of retrieved mature oocytes or transferred embryos). The type and duration of infertility as well as the number of prior IVF-ET cycles and semen characteristics were not different between the two groups (data not shown). Table 3 shows that the number and stage of ET were not different between the two groups either.

There was no statistically significant difference between the vaginal and IM-P_4 groups with regard to treatment outcomes including rates of positive pregnancy tests, clinical and ongoing pregnancy (OR, 1.0675; 95% CI, 0.7587–1.5020; $P=.71$, for ongoing pregnancy rates), or rates of total pregnancy loss, chemical and miscarriage (OR, 1.0775; 95% CI, 0.7383–1.5727; $P=.70$). Also, the implantation rates were not significantly different between the two groups. The absence of statistical difference between the two groups was

TABLE 2

Characteristics of the vaginal P and the IM-P_4 luteal support groups.

	Vaginal P_4	IM-P_4
No. of women	145	399
Age of woman, years	33.1 ± 4.5 (22–41)	33.6 ± 3.4 (24–41)
Age of male partner, years	36.3 ± 5.7 (24–55)	36.4 ± 5.8 (24–76)
No. of stimulation days	11.5 ± 1.9 (8–16)	10.8 ± 1.9 (8–14)
E_2 level on day of hCG administration, pg/mL	2174 ± 1008 (248–6118)	3710 ± 938 (865–9650)
P level on day of hCG administration, ng/mL	0.8 ± 0.4 (0.2–2.6)	1.0 ± 0.25 (0.4–1.0)
No. of retrieved oocytes	13.3 ± 6.8 (3–37)	13.7 ± 7.1
No. of transferred embryos	2.4 ± 0.6 (1–3)	2.6 ± 0.8 (1–3)

Note: Data are presented as mean ± SD (range). All comparisons were statistically not significant ($P>.05$).

Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

TABLE 3

Number and stage of transferred embryos in the vaginal P and IM-P_4 groups.

Stage of transferred embryo	Vaginal P_4 (n = 145)	IM-P_4 (n = 399)
Cleavage Stage (day 3):		
No. of cycles	82	205
No. of transferred embryos	2.7 ± 0.6 (1–3)	2.9 ± 0.7(1–3)
Blastocyst stage (day 5):		
No. of cycles	63	194
No. of transferred embryos	1.8 ± 0.2 (1–2)	1.9 ± 0.2 (1–2)

Note: Data are presented as mean ± SD (range). All comparisons were statistically not significant.

Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

true when all women were included in the analysis (Table 4) and when the analysis was done according to the woman's age (Table 5) and stage of ET (Table 6). When the diagnosis of infertility was considered, women with a diagnosis of endometriosis (surgical diagnosis by laparoscopy) had statistically significantly higher implantation ($P<.04$) and pregnancy rates (positive βhCG, clinical and ongoing pregnancy; $P<.04$) in the vaginal P luteal support group compared with those with endometriosis who received IM-P_4 luteal support (Table 7).

There was a positive correlation between mean luteal P levels and clinical pregnancy rates in the vaginal P group and IM-P_4 group (Table 8). The correlation coefficients were 0.8543 and 0.7970 for the vaginal P and IM-P_4 groups, respectively. The test for trend showed a statistically significant trend, with $P<.0001$ and $<.003$ for the vaginal and IM-P_4 groups, respectively (Table 8).

When all women were included in the analysis, during the luteal phase (from day 3 to day 18 after oocyte retrieval), mean serum P levels were not significantly different between the vaginal P group and the IM-P_4 group. However, during the early pregnancy phase (after day 18 after oocyte retrieval until 12 weeks of gestation), mean serum P levels were significantly higher in the vaginal P group when compared with the IM-P_4, group as shown in Figures 1 and 2.

When the analysis was restricted to women with clinical pregnancy (Figs. 3 and 4), statistically significantly higher mean P levels were found in women who used vaginal P during early pregnancy but not during the "early" part of the luteal phase. Serum P levels started to be statistically significantly higher in the vaginal P group as early as days 9 and 10 after oocyte retrieval.

In the nonpregnant women (Figs. 5 and 6), there was insignificant difference in the mean P levels along the luteal phase between the vaginal P and the IM-P_4 groups, except that the mean P level on days 9 and 10 was statistically significantly "lower" in the vaginal P group. However, there was a maintained pattern of consistently higher (but not statistically significant) mean P levels associated with IM-P_4 compared with the vaginal P throughout the whole luteal phase.

In all women who received IM-P_4, mean serum P levels were found to be highest during the first few days after oocyte retrieval (peak mean level of 111 ng/mL achieved on days 5 and 6 after oocyte retrieval). Mean P levels dropped to statistically significantly lower mean levels on days 7 and 8 and on days 9 and 10 ($P<.01$), reaching a nadir (40 ng/mL) around the midluteal phase (9–10 days after oocyte retrieval). At that time, levels started to rise again, only in pregnant women, to reach statistically significantly higher levels by the end of

TABLE 4

Treatment outcomes between the vaginal P luteal support and the IM-P luteal support group: All women.

	Vaginal P_4 (n = 145)	IM-P_4 (n = 399)
Pregnancies (positive βhCG)	84 (58) [50–66]	229 (57) [53–62]
Clinical pregnancies	71 (49) [41–57]	210 (53) [48–58]
Ongoing pregnancies	64 (44) [36–52]	188 (47) [42–52]
Chemical pregnancies	13 (16) [8–23]	19 (8) [5–12]
Miscarriage	7 (8) [2–14]	22 (10) [6–13]
Total pregnancy loss	20 (24) [15–33]	41 (18) [13–23]
Implantation rate, %	30 [23–37]	29 [25–33]

Note: Data are presented as number (%) [95% CI]. *P* is not statistically significant for all comparisons.

Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

TABLE 5

Women <35 years and ≥35 years old.

	Vaginal P_4 (n = 145)	IM-P_4 (n = 399)
Women <35 years old (n =313):		
No. of women	87	226
Pregnancies (positive βhCG)	51 (59) [48–69]	132 (58) [52–65]
Clinical pregnancies	47 (54) [44–65]	123 (54) [48–61]
Ongoing pregnancies	41 (47) [37–58]	111 (49) [43–56]
Chemical pregnancies	4 (8) [1–15]	9 (7) [33–11]
Miscarriage	6 (12) [3–21]	13 (10) [5–15]
Total pregnancy loss	10 (20) [8–31]	22 (17) [10–23]
Implantation rate, %	34% [26–42]	30% [26–34]
Women ≥35 years old (n = 231):		
No. of women	58	173
Pregnancies (positive β-hCG)	33 (57) [44–70]	97 (56) [49–64]
Clinical pregnancies	24 (41) [29–52]	87 (50) [43–58]
Ongoing pregnancies	23 (40) [27–52]	78 (45) [38–53]
Chemical pregnancies	9 (27) [12–43]	10 (10) [4–16]
Miscarriage	1 (3) [0–9]	9 (9) [4–15]
Total pregnancy loss	10 (30) [15–46]	19 (20) [12–28]
Implantation rate	26 [17–35]	27 [23–32]

Note: Data are presented as number (%) [95% CI]. *P* is not statistically significant for all comparisons.

Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

TABLE 6

Cleavage and blastocyst stage ET.

	Vaginal P_4 (n = 145)	IM-P_4 (n =399)
Cleavage stage ET (n = 287):		
No. of women	82	205
Pregnancies (positive βhCG)	47 (57) [47–68]	117 (57) [50–64]
Clinical pregnancies	41 (50) [39–61]	107 (52) [45–59]
Ongoing pregnancies	37 (45) [34–56]	98 (48) [41–55]
Chemical pregnancies	6 (13) [3–22]	10 (9) [4–14]
Miscarriage	4 (9) [1–17]	9 (8) [3–13]
Total pregnancy loss	10 (21) [10–33]	19 (16) [10–23]
Implantation rate, %	26 [21–31]	25 [21–29]
Blastocyst stage ET (n = 257):		
No. of women	63	194
Pregnancies (positive βhCG)	37 (59) [47–71]	112 (58) [51–65]
Clinical pregnancies	30 (48) [35–60]	103 (53) [46–60]
Ongoing pregnancies	27 (43) [31–55]	90 (46) [39–53]
Chemical pregnancies	7 (19) [6–32]	9 (8) [3–13]
Miscarriage	3 (8) [0–17]	13 (12) [6–18]
Total pregnancy loss	10 (27) [13–41]	22 (20) [12–27]
Implantation rate, %	39 [31–47]	33 [29–37]

Note: Data are presented as number (%) [95% CI]. *P* is not statistically significant for all comparisons.

Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

TABLE 7

Women with endometriosis.

	Vaginal P_4 (n = 33)	IM-P_4 (n = 49)	*P*
Pregnancies (positive βhCG)	24 (73) [58–88][a]	23 (47) [33–61][a]	.04
Clinical pregnancies	23 (70) [54–86][a]	23 (47) [33–61][a]	.04
Ongoing pregnancy rate	18 (55) [30–72][a]	18 (37) [24–52][a]	.04
Chemical pregnancies	1 (4) [0–11]	0	NS
Miscarriage	5 (21) [7–35]	5 (22) [10–34]	NS
Total pregnancy loss	6 (25) [10–40]	5 (22) [10–34]	NS
Implantation rate	42 [31–53][a]	24 [17–31][a]	.03

[a] Statistically significant ($P<.05$).

Note: Data are presented as number (rate) [95% CI]. NS = not statistically significant.

Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

the luteal phase (82 ng/mL) on days 15–18 days after oocyte retrieval and throughout early pregnancy weeks (90 ng/mL). The *P*-values were $<.01$ and $<.001$, respectively.

In all women who received vaginal P luteal support, mean serum P levels throughout the luteal phase and early pregnancy followed the same pattern, with an almost identical curve to that produced by P levels achieved in women who received IM-P_4 as explained above. The peak mean serum P level during the luteal phase (105 ng/mL) was achieved on days 5–6 after oocyte retrieval. The peak of the mean P level dropped to statistically significantly lower mean levels on days 7–8 and on days 9–10 ($P<.01$), reaching a nadir (29 ng/mL) around the midluteal phase (9–10 days after oocyte retrieval). In pregnant women only, nadir levels started to rise again, to reach statistically significantly higher levels by the end of the luteal phase (83 ng/mL) on days 15–18 days after oocyte retrieval and continued to rise further to much higher levels throughout early pregnancy weeks (118 ng/mL). The *P*-values were $<.01$ and $<.001$, respectively.

In nonpregnant women, mean P levels remained around the nadir level attained on days 9 and 10 throughout the rest of the luteal phase until a negative pregnancy test was confirmed in both groups (vaginal and IM-P_4 groups). The nadir achieved around days 9 and 10 after oocyte retrieval was statistically significantly lower in nonpregnant women who used the vaginal P when compared with the nonpregnant women who used IM-P_4. Such a nadir was not statistically significantly different in the pregnant women who used IM-P_4 when compared with the pregnant women in the vaginal P group.

In the vaginal P group, there was no statistically significant difference in mean serum P levels between women who used the vaginal P twice versus those used it 3 times a day. Mean P

TABLE 8

Correlation between clinical pregnancy rate and mean serum P levels during the luteal phase.

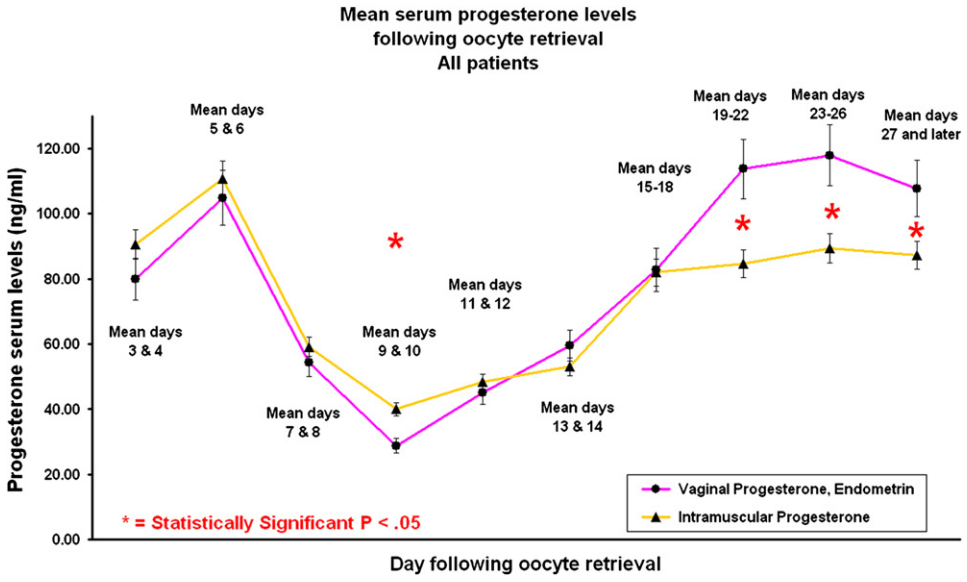
Luteal support group and range of mean P levels (ng/mL)	No. of women	No. of clinical pregnancies	Clinical pregnancy rate	*P* (test for trend)
Vaginal P:				<.0001
16–48	37	11	30	
49–80	27	15	56	
81–112	22	17	77	
113–144	8	8	100	
145–173	4	3	75	
IM-P_4:				<.003
11 to 46	47	27	57	
47–81	142	59	42	
82–116	54	33	61	
117–151	20	16	80	
152–193	12	10	83	

Note: The ranges for each of the five groups were increments of SD (the range between the highest and lowest mean P level divided by the SD).

Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

FIGURE 1

Mean serum P levels during the luteal phase (all women) and early weeks of gestation (pregnant women) in the vaginal P group and in the IM-P_4 group. Serum P levels are presented as mean ± SEM. During the luteal phase, the mean serum P level on days 9 and 10 after oocyte retrieval was statistically significantly higher in the IM-P_4 group. However, during early pregnancy, serum P levels were statistically significantly higher in the vaginal P group on mean days (after oocyte retrieval) of 19–22, 23–26, and 27days to 12 weeks of gestation.



Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

levels in the two groups (100 mg twice and 100 mg 3 times a day) followed identical parallel curves throughout the luteal phase and early pregnancy. This was true for all women and for the subgroup of women who achieved pregnancy (data not shown).

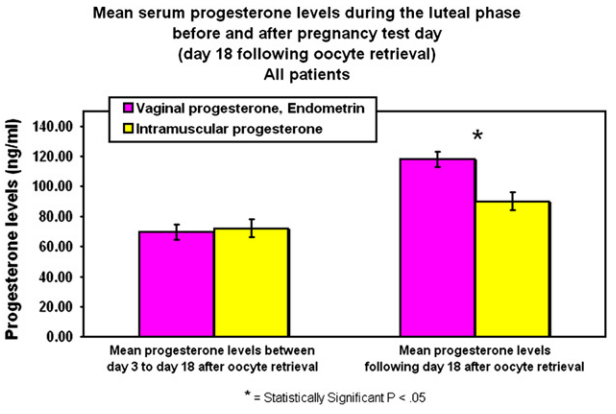
There were no serious adverse reactions or significant side effects reported in any of the patients in either group, vaginal P or IM-P_4, that necessitated discontinuation of treatment. All patients tolerated the treatment with administered P for the planned period of time.

DISCUSSION

This study shows that in women who underwent IVF-ET treatment according to the long GnRH agonist protocol, vaginal P luteal support was associated with treatment outcomes that were not different from those achieved in women who received IM-P_4 luteal support. Serum P levels during the luteal phase were not different between the two groups (vaginal and IM-P_4 luteal support) during the early part of the luteal phase, but significantly higher levels were found in pregnant women who used vaginal P starting after days 9–10 after oocyte retrieval. Interestingly, women with endometriosis who received vaginal P luteal support had statistically significantly better outcomes (higher implantation and pregnancy rates) when compared with those with endometriosis who received IM-P_4 luteal support.

FIGURE 2

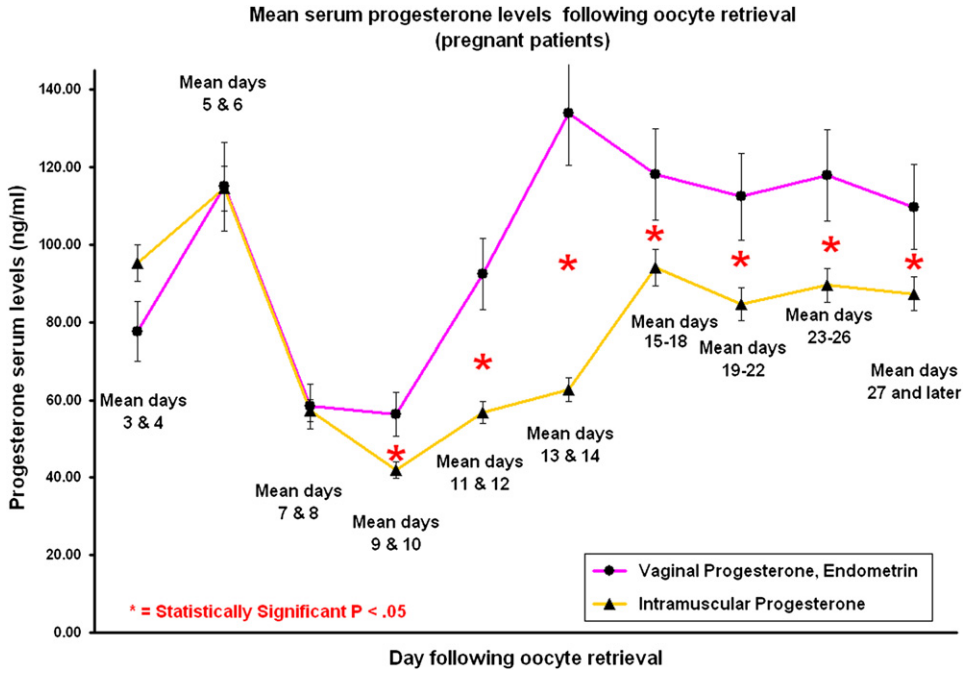
Mean P levels throughout the luteal phase (first 18 days after oocyte retrieval) in all women and throughout early pregnancy phase (18 days after oocyte retrieval until 12 weeks of gestation) in pregnant women. Data are presented as the mean ± SEM. While overall the mean serum P level was not different during the luteal phase, it was statistically higher in the vaginal P group during the early pregnancy phase.



Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

FIGURE 3

Mean serum P levels during the luteal phase (only pregnant women) and early weeks of gestation in the vaginal P group and in the IM-P_4 group. Serum P levels are presented as mean ± SEM. During the luteal phase, mean serum P levels were not statistically different between the vaginal P group and the IM-P_4 group. However, during early pregnancy, serum P levels were statistically significantly higher in the vaginal P group on mean days (after oocyte retrieval) of 19–22, 23–26, and 27 days to 12 weeks of gestation.



Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

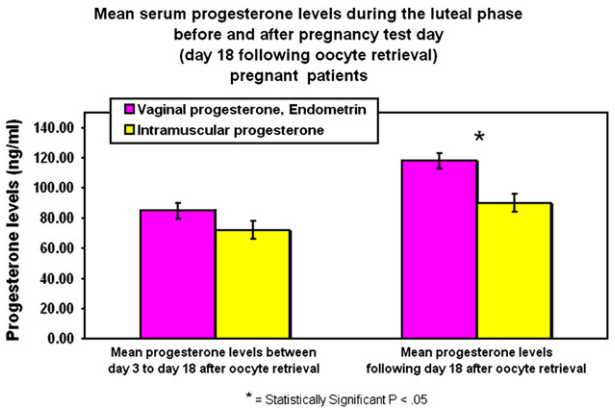
Different regimens of IM-P_4 have been empirically used for luteal phase support in women undergoing IVF-ET. This included different doses that varied between 25 and 100 mg per day and different starting dates after oocyte retrieval and for variable lengths of time through the early weeks of pregnancy. Those diverse regimens were not persistently found to be associated with clinically significant differences concerning the outcome of IVF-ET treatment, that is, achievement of pregnancy (28). Interestingly, despite several adverse effects that have been reported with IM-P_4 administration, including painful injections, rash (29), inflammatory reactions, and abscesses (24), as well as several case reports of the rare, although serious, complication of acute eosinophilic pneumonia (30), such a route is still the preferred routine practice in most of the IVF centers in North America.

Clearly, the vaginal route is associated with several advantages when compared with IM injection, including reduced local adverse effects and better compliance, as well as the anticipated high P levels in the uterine environment (24, 25, 31). P administered vaginally is expected to be associated with high uterine P concentrations and lower systemic absorption. This means higher P concentrations where they are desired (endometrium) but lower concentrations where they might not be desired (systemic). Lower systemic absorption is obviously advantageous, as less frequent systemic P side effects should be expected. The lower serum P levels and higher uterine endometrial tissue concentrations observed with vaginal P administration compared with IM-P_4 are believed to be due to two important reasons. The first reason is the countercurrent exchange in P transport between the anatomically close blood vessels (32), and the second is due to the uterine first pass effect, where liver metabolization is absent (33).

Part of the hypothesis in this study was that circulating serum P is mainly driven from endogenous production from corpora lutea rather than from what is absorbed from exogenously administered P. The current results found administration of vaginal P for luteal support to be associated with serum P levels that were not different from those levels achieved after IM-P_4 injections. Such levels of serum P might be achieved from endogenous corpora luteal production of P rather than systemic absorption from the vaginally administered exogenous P, which is known to be minimal. Interestingly, in pregnant women, significantly higher serum P levels were found to be associated with vaginal P when compared with IM-P_4 throughout the later part of the luteal phase (after the time of implantation) and early weeks of pregnancy. The proposed mechanism is that lower amounts of P absorbed into the systemic circulation after vaginal P administration would be associated with a more physiologically functioning corpora lutea. This could be due to a less suppressive effect of lesser

FIGURE 4

Mean P levels throughout the luteal phase (first 18 days after oocyte retrieval) in pregnant women and throughout early pregnancy phase (18 days after oocyte retrieval until 12 weeks of gestation) in pregnant women. Data are presented as the mean ± SEM. While overall the mean serum P level was not different during the luteal phase, it was statistically higher in the vaginal P group during the early pregnancy phase.



Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

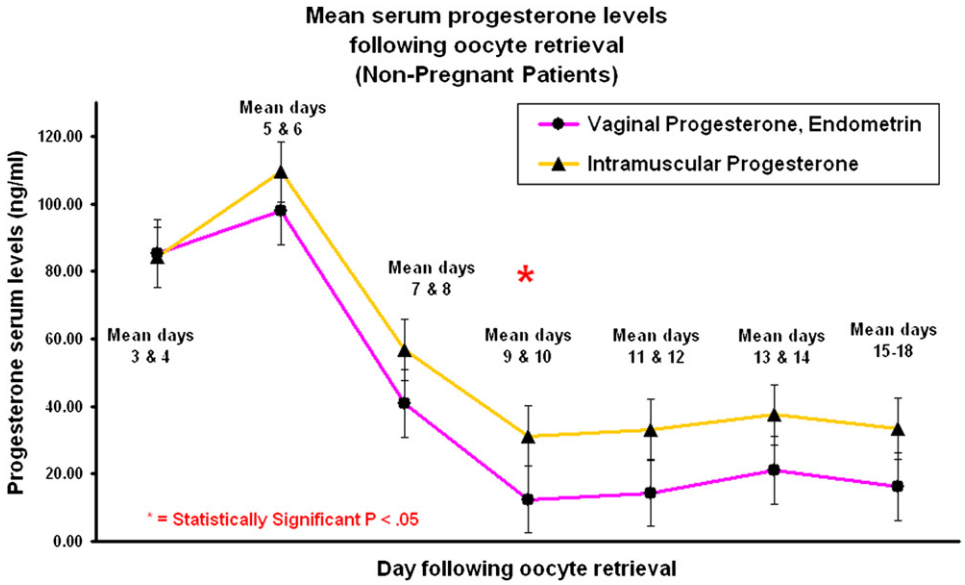
amounts of systemically absorbed P on the hypothalamus-pituitary-ovarian axis. Exogenously administered P is known to suppress endogenous gonadotropin production (34–36), including LH, which is particularly important for a properly functioning corpus luteum (34–36). Another viable mechanism could be that higher local uterine levels of P absorbed after vaginal administration (25) lead to a more favorable local effect on the corpora lutea through the connection between the ovarian and uterine circulation. Such a positive effect of P locally at the level of the corpus luteum has been suggested by several reports that found evidence for an intracrine favorable effect of P on the corpus luteum function, including prevention of its luteolysis, as has been reviewed before (37, 38).

The results of the current study provide indirect evidence in support of the hypothesis of a better physiologically responsive corpora lutea in association with vaginal P administration. In support of that notion, significantly higher P levels were found in pregnant women starting around the time of implantation that continued throughout the studied early pregnancy weeks. This seems to be a response to rising levels of βhCG in those pregnant women.

When studying serum P levels during the luteal phase in women undergoing IVF-ET while receiving exogenous P luteal support, an important question is how much is contributed by the absorbed P and how much comes from endogenous production by corpora lutea. The ideal method for studying such a question would be to measure serum P levels of exogenously administered labeled P. Such an experiment

FIGURE 5

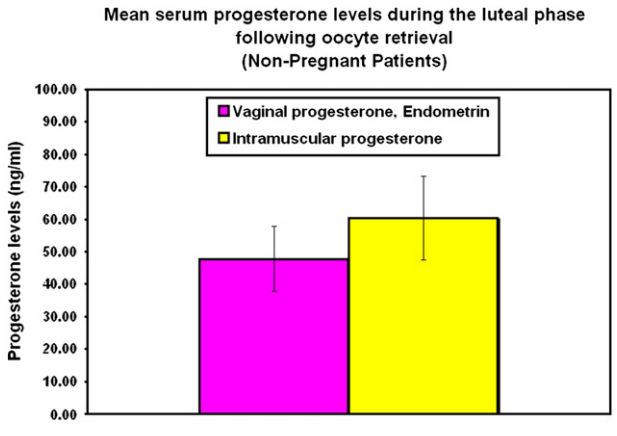
Mean serum P levels during the luteal phase (nonpregnant women) in the vaginal P group and in the IM-P_4 group. Serum P levels are presented as mean ± SEM. Mean serum P levels were consistently higher in the IM-P_4 group. However, the difference was not statistically insignificant except the mean level on day 9–10 that was significantly lower in the vaginal P group.



Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

FIGURE 6

Mean P levels throughout the luteal phase (first 18 days after oocyte retrieval) in nonpregnant women. Data are presented as the mean ± SEM. Overall the mean serum P level was not statistically different between the vaginal P group and the IM-P_4 group during the luteal phase.



Mitwally. Vaginal P_4 vs. IM-P_4 for luteal support. Fertil Steril 2010.

would not be possible in those women attempting to achieve pregnancy but might still be feasible in oocyte donors. In the current study, one answer to the question of how much of a contribution to circulating P comes from endogenous P production could be extrapolated indirectly by looking at the details of the curve for mean P levels throughout the luteal phase in the two study groups. Clearly, with a steady continuous absorption of P as expected after IM-P_4 injection, a more or less steady mean serum P level should be anticipated throughout the luteal phase. This would be true if the major fraction of the serum P level was contributed by absorbed P rather than by what was endogenously produced from the corpora lutea. Interestingly, this was not the case.

The two parallel curves representing mean serum P levels throughout the luteal phase and early pregnancy weeks in the two groups (vaginal and IM-P_4 luteal support groups) followed the expected pattern for hCG levels during the same period of time. Highest hCG levels would be expected immediately after oocyte retrieval (as a result of exogenously administered hCG for triggering ovulation). Those levels are expected to decline to a nadir in about 9–10 days (the half-life of the exogenously administered hCG is about 24 hours). The hCG levels are expected to rise again after the nadir only in pregnant women (endogenous hCG production by implanting embryo and early pregnancy). In a subgroup of women, serum levels of βhCG were assayed concurrently on the same days of P assays. Those βhCG levels were found to follow the expected pattern, with the highest levels found on days 3–4 after oocyte retrieval and declining to a nadir around days 9–10. In women who achieved pregnancy, βhCG levels were found to climb again after that nadir, around days 9–10, after oocyte retrieval (data not shown). The only discordance between the curve for βhCG levels and P levels was encountered on days 3–4 compared with days 5–6 after oocyte retrievals. Beta-hCG declined between the two windows of measurements, while P levels increased (followed an opposite direction). There are two explanations for such discordance. First, the maximal response of luteal granulosa cells to hCG stimulations takes a few days to achieve after ovulation when a switch from mainly estrogen production to mainly P production occurs. This is known as the maximal luteal P production and is physiologically achieved around the midluteal phase (37, 38). Second, the trauma induced by the follicular puncture, bleeding, and granulosa cell aspiration during oocyte retrieval might require few days before the corpora lutea can recover the full ability to produce P. However, such a mechanism has been debated as explained in the introduction.

In this study, the specific pattern of the curve for P levels achieved throughout the luteal phase and early pregnancy, paralleling that curve for hCG levels (exogenously administered to trigger ovulation and endogenously produced by the implanting embryo and early pregnancy), would support the hypothesis of a significant contribution by the endogenous P production from the corpora lutea to serum P levels. Furthermore, there is another support for this notion. The differences in the mean P levels between the nadir day (20 and 40 ng/mL, in the vaginal and IM-P_4 groups, respectively) and the maximal P levels (105 and 111 ng/mL) achieved during the first few days after oocyte retrieval were 85 and 71 ng/mL for the vaginal and IM groups, respectively. Such differences were almost twice the mean P levels on the nadir day, which is a very significant amount, particularly when considering what has been reported in the literature as adequate luteal P levels in normal cycles, levels around 10–20 ng/mL (37, 38). On the nadir day, the corpora lutea would be expected to produce the least amount of P, while P absorption after exogenous P administration should not be different. The findings in this study of P levels during early pregnancy that correlate with the pattern of hCG rise and the preovulatory dominant follicles are supported by what has been previously reported in the classic study by Cowan et al., who found P production in early gestations to be regulated by prior follicular events, as was the rate of hCG production (39).

Again, despite all the advantages of the vaginal route of P administration, many clinicians are still adherent to the practice of IM administration of P for luteal support in women undergoing IVF. This could be due to earlier evidence for a superiority of IM-P_4 compared with vaginal P as regards clinical pregnancy rates. In 2002, a meta-analysis (29) included five randomized trials comparing IM-P_4 with vaginal P application (40–43) for luteal support in women undergoing IVF pooled together for a total of 891 cycles for analysis from those studies. The clinical pregnancy rate and delivery rate were significantly higher when IM-P_4 was used (the relative risk clinical pregnancy rate/ET was 1.33 [95% CI, 1.02–1.75], and the relative risk for the delivery rate was 2.06 [95% CI, 1.48–2.88).

Interestingly, despite the conclusion of such a meta-analysis, the vaginal route of P supplementation in IVF patients has gained wide application as a first choice luteal support regimen worldwide (except in North America). This has been mainly due to patient compliance and comfort as well as to increased trust in effectiveness (43). In more recent studies, there has been accumulating evidence that compared with IM-P_4 luteal support, vaginal P is at least equally effective (44). Such evidence has initially been shown in studies involving two different forms of vaginal P that are available on the market in Europe (at the time of those studies): natural micronized P (Utrogestan Laboratories Besins International, Paris) and Crinone 8% (Fleet Laboratories Ltd., Watford, UK), a controlled and sustained-release vaginal gel. The recommended doses used in the studies were as follows: for Utrogestan, 100 mg capsules were administered vaginally by two capsules, 3 times daily (a total of 600 mg/day), whereas Crinone 8% was administered vaginally once a day, that is, 90 mg (45, 46). In more recent studies, the micronized vaginal P Endometrin has been reported to be equally effective as Cyclogest vaginal suppositories (46) and Crinone vaginal P gel for luteal support in women undergoing IVF (27). In a recent interim analysis of their original data that suggested a superiority of IM-P_4 injection over Crinone P gel (24), Yanushpolsky et al. found similar pregnancy rates, implantation rates, and early spontaneous abortion rates in IVF patients who received either Crinone or IM P injection for luteal support. The investigators reported fewer side effects and greater overall satisfaction by women receiving Crinone. This conclusion was achieved after an interim analysis that included women younger than 40 years old. It is important to mention that in this study (47), the day of P initiation was delayed for 1 day compared with in the current study, in which P supplementation was initiated on the day after oocyte retrieval.

In the current study, there was no statistically significant difference in any of the various outcomes of IVF-ET treatment (pregnancy rates and rates of pregnancy loss) between women who received vaginal P luteal support and those who used IM-P_4 support. However, in the subgroup of women with endometriosis, interestingly, both implantation and pregnancy rates (positive βhCG, clinical and ongoing) were statistically significantly higher in women who used vaginal P luteal support than in those with endometriosis who used IM-P_4 for luteal support. Serum P levels in this subgroup of women with endometriosis consistently followed the same pattern seen with all patients, that is, higher P levels during the later part of the luteal phase and early pregnancy in pregnant women. However, it is important to mention here that the sample size of the subgroups of women with endometriosis was not large enough to complete an adequately powered analysis while controlling for other factors that could affect the tested outcomes.

Endocrine dysfunction due to luteal phase defect and lower implantation potential has already been suggested in women with endometriosis (48). Several mechanisms are believed to contribute to the probable luteal phase defect in those women including P receptor defects and dysfunction in the hypothalamus-pituitary-ovarian axis. Furthermore, P resistance has been suggested due to the presence of the inhibitory P receptor (PR) isoform PR-A and the absence of the stimulatory isoform PR-B (49). Pituitary-ovarian dysfunction has also been suggested as a cause for the subfertility in women with endometriosis with the evidence reviewed by Cahill and Hull (50). The investigators presented evidence for impaired follicular growth leading to a reduction in circulating E_2 concentrations during the preovulatory phase as well as to a reduction in both E_2 and P during the early luteal phase. Such lower E_2 levels were associated with disturbed LH surge patterns and reduction in LH concentrations in preovulatory follicular fluid. Moreover, this was associated with impaired in vitro steroidogenic capacity of the granulosa cells (51). The investigators suggested that those mechanisms explain at least in part the reported reduced oocyte fertilization and implantation rates in women with endometriosis (49). In addition, luteal dysfunction has been suggested to lead to decreased uterine receptivity due to associated abnormal expression of endometrial biomarkers of implantation including integrins and other adhesion molecules (52, 53).

It is important to mention here that the association of a luteal phase defect with lower implantation in women with endometriosis is highly controversial. Moreover, the evidence presented involves studies of P production during unstimulated cycles in women with endometriosis. In addition, the outcome of IVF-ET in women with endometriosis-associated infertility is still a controversial issue, with conflicting conclusions among different studies as reviewed by Sallam et al. (54).

Commonly used vaginal P includes pharmacy-compounded P suppositories that are known to deliver variable and unreliable levels of P (55, 56). Interestingly, only one out of 10 compounding pharmacies that provided P vaginal suppositories provided a compound that was within the potency range required of similar Federal Drug Administration (FDA-) approved products (57). The scientific uncertainties associated with compounded hormones make their use less preferable (55), at least until supplementary clinical trials are available to determine the efficacy and safety of those compounded hormones (56). This led the FDA to issue a warning against the use of compounded and bioidentical hormones (58). It is relevant to reiterate here that, as mentioned earlier in a recent study, the micronized vaginal P inserts used in this study have been shown to provide reproducible levels with less variability than vaginal P gel in pharmacokinetic studies measuring serum concentrations over time (26). The formulation of the vaginal P compounds that have been tried in the clinical practice of luteal support could explain some of the early controversy regarding the effectiveness of vaginal P for luteal support in women undergoing IVF-ET.

In a recent Cochrane review (in 2008), the investigators withdrew their previous one (published in 2004) (23). To

determine the answer to three important questions—first, whether luteal phase support after assisted reproduction increases the pregnancy rate; second, what is the optimal hormone for luteal phase support, that is, hCG, P, or a combination of both; and third, what is the optimal route of P administration, that is, vaginal or IM—the investigators searched the Cochrane Menstrual Disorders and Subfertility Group trials register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (1971 to Deccember 2003), and EMBASE (1985 to December 2003). In addition, they hand-searched reference lists of relevant articles and abstract books from scientific meetings up to December 2003. Such a search resulted in the inclusion of 59 studies in the review and reached the following conclusions: luteal phase support with hCG provided significant benefit, compared with placebo or no treatment, in terms of increased ongoing pregnancy rates (OR, 2.38; 95% CI, 1.32–4.29) and decreased miscarriage rates (OR, 0.12; 95% CI, 0.03–0.50). However, such significant benefit was observed only in cycles in which GnRH agonists were used. Furthermore, such a significant benefit was associated with a major drawback that was the significant increase in the risk of severe ovarian hyperstimulation syndrome (OHSS). The odds of OHSS increased 20-fold when hCG was used in cycles with GnRH agonists. On the other hand, P luteal support was associated with a small but significant increase in pregnancy rates (OR, 1.34; 95% CI, 1.01–1.79) when trials with and without GnRH agonists were grouped together. Interestingly, P supplementation had no favorable effect in reducing the miscarriage rate as was observed with hCG supplementation. There was no significant difference between P and hCG or between P and P plus hCG or estrogen in terms of pregnancy or miscarriage rates. However, the odds of OHSS were more than twofold higher with treatments that included hCG than with those that included P alone (OR, 3.06; 95% CI, 1.59–5.86). Comparing routes of P administration, there was a lower clinical pregnancy rate with the oral route compared with the IM or vaginal routes. However, the difference did not reach statistical significance. On the other hand, there was evidence of a benefit of the IM route over the vaginal route as regards the outcomes of ongoing pregnancy and live birth. The vaginal P gel and other types of vaginal P resulted in comparable pregnancy rates without significant differences. The investigators concluded that luteal phase support with hCG or P after assisted reproduction resulted in an increased pregnancy rate with no superiority of hCG over P; rather, hCG was associated with a greater risk of OHSS when used with GnRH agonists. The available data did not support a superiority of one route over the other for P administration, and it is to be established with further studies (23).

In the current study, there are important limitations including the retrospective collection of the data and the sample size that was not large enough to allow an adequately powered analysis of important confounders (e.g., infertility diagnosis and duration) that could have affected the tested primary outcome (ongoing pregnancy). Also, the study was completed before obtaining data on all live births of ongoing pregnancies. At the time of the analysis of the data for the current study, all available records on birth outcomes showed that all ongoing pregnancies included in the study had already ended in delivery of live births or were ongoing beyond 28 weeks of gestation.

Another very important limitation in the current study is that P levels were determined after single blood draws during the specified time course of the study. Single blood draws for determining P levels would obviously not account for the pulsatile nature of P production by the corpus luteum. Such pulsatile production results in a wide range of P concentrations, as has been already reported during the mid- and late luteal phases. Levels as low as 2.3 ng/mL and as high as 40.1 ng/mL have been found within the relatively short interval of time spanning a single secretory pulse (60–90 minutes) (59). This has made the clinical utility of single or even serial serum P measurements of limited clinical utility in providing an accurate gauge of the quality of luteal function (10). Moreover, P levels during the early pregnancy weeks are known to range widely, particularly when conception follows treatment with ovulation-inducing drugs. This is due to the formation of multiple corpora luteal with possibly variable function as well as to a different potential for implantation by multiple embryos. However, in the current study, a subgroup of 30 women had multiple blood draws (hourly for 12 hours on the fifth day after oocyte retrieval and four hourly for 12 hours on four occasions during the luteal phase) for assays of serum P levels and other hormones. Interestingly, the means of P levels followed a comparable pattern of what has been found in the rest of the women in the current study, who had single blood draws. The curve for mean serum P levels followed a curve comparable to that achieved after the single P assays, including peak P in the first few days after oocyte retrieval, significantly lower nadir around the midluteal phase, and significantly higher rising levels thereafter in pregnant women (data not shown). A recent study looked at clinical intrauterine pregnancy rates, pregnancy loss, and live-birth rates between two matched groups of women who underwent IVF-ET treatment. One group received IM-P_4 for luteal support (200 women), and the second group received vaginal P for luteal support in the form of Endometrin 100 mg twice a day (12 women), Endometrin 100 mg 3 times a day (11 women), or Crinone 8% gel 90 mg every day (17 women). The investigators did not find significant differences in treatment outcomes between vaginal and IM P supplementation (60).

Summary

In conclusion, in women undergoing IVF-ET according to the long GnRH agonist protocol, luteal support with vaginal P was associated with treatment outcomes including pregnancy rates and rates of pregnancy loss that were not statistically different from those associated with IM-P_4 luteal support. Mean serum P levels during the luteal phase were not different in women who received vaginal P when compared with those who received IM-P_4. However, in pregnant women,

significantly higher mean P levels were found in women who received vaginal P luteal support. This could reflect better physiologically functioning corpora lutea due to less suppression than that associated with nonphysiological systemic levels of P achieved after IM-P_4 injections.

Acknowledgements: The authors thank Ferring Pharmaceuticals for supporting the conduction and writing of this study. The authors also thank M. Joseph Scobey, Ph.D., Joanna Gonsalves, Ph.D., and Paul Weathersbee, Ph.D., of Ferring Pharmaceuticals, Parsippany, New Jersey, for editorial assistance.

REFERENCES

1. Muasher SJ, Abdallah RT, Hubayter ZR. Optimal stimulation protocols for in vitro fertilization. Fertil Steril 2006;86:267–73.
2. Karande VC, Korn A, Morris R, Rao R, Balin M, Rinehart J, et al. Prospective randomized trial comparing the outcome and cost of in vitro fertilization with that of a traditional treatment algorithm as first-line therapy for couples with infertility. Fertil Steril 1999;71:468–75.
3. Soliman S, Daya S, Collins J, Jarrell J. A randomized trial of in vitro fertilization versus conventional treatment for infertility. Fertil Steril 1993;59:1239–44.
4. Hughes EG, Federkow DM, Daya S, Sagle MA, Van De Koppel P, Collins JA. The routine use of gonadotropin-releasing hormone agonists prior to in vitro fertilization and gamete intrafallopian transfer: a meta-analysis of randomized controlled trials. Fertil Steril 1992;58:888–96.
5. Gomel V, Cheung PCK. Vitro Fertilization and Assisted Reproduction. Bologna: Monsuzzi Editor, 1997:405–15.
6. de Mouzon J, Lancaster PI. World Collaborative Report on In Vitro Fertilization—1993. XVth World Congress on Fertility and Sterility 1995; Montpellier, September, 17–22.
7. Jones HW Jr, Jones GS, Andrews MC, Acosta A, Bundren C, Garcia J, et al. The program for in vitro fertilization at Norfolk. Fertil Steril 1982;38:14–21.
8. Csapo AI, Pulkkinen MO, Ruttner B, Sauvage JP, Wiest WG. The significance of the human corpus luteum in pregnancy maintenance. I. Preliminary studies. Am J Obstet Gynecol 1972;112:1061–7.
9. Csapo AI, Pulkkinen MO, Wiest WG. Effects of luteectomy and progesterone replacement therapy in early pregnant patients. Am J Obstet Gynecol 1973;115:759–65.
10. The Practice Committee of the American Society for Reproductive Medicine in collaboration with the Society for Reproductive Endocrinology and Infertility. Progesterone supplementation during the luteal phase and in early pregnancy in the treatment of infertility: an educational bulletin. Fertil Steril 2008;5:S150–3.
11. Fatemi HM, Popovic-Todorovic B, Papanikolaou E, Donoso P, Devroey P. An update of luteal phase support in stimulated IVF cycles. Hum Reprod Update 2007;13:581–90.
12. Penzias A. Luteal phase support. Fertil Steril 2002;77:318–23.
13. Carson DD, Baqchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, et al. Embryo implantation. Dev Biol 2000;223:217–37.
14. Edwards RG, Steptoe PC, Purdy JM. Establishing full-term human pregnancies using cleaving embryos grown in vitro. Br J Obstet Gynaecol 1980;87:737–56.
15. Jones GS. Luteal phase defect: a review of pathophysiology. Curr Opin Obstet Gynecol 1991;3:641–8.
16. Casper RF, Yen SS. Induction of luteolysis in the human with a long-acting analog of luteinizing hormone-releasing factor. Science 1979;205: 408–10.
17. Smitz J, Erard P, Camus M, Devroey P, Tournaye H, Wisanto A, et al. Pituitary gonadotrophin secretory capacity during the luteal phase in superovulation using GnRH-agonists and HMG in a desensitization or flare-up protocol. Hum Reprod 1992;7:1225–9.
18. Smitz J, Bourgain C, Van Waesberghe L, Camus M, Devroey P, Van Steirteghem AC. A prospective randomized study on oestradiol valerate supplementation in addition to intravaginal micronized progesterone in buserelin and HMG-induced superovulation. Hum Reprod 1993;8: 40–5.
19. Fauser BC, Devroey P. Reproductive biology and IVF: ovarian stimulation and luteal phase consequences. Trends Endocrinol Metab 2003;14: 236–42.
20. Kerin JF, Broom TJ, Ralph MM, et al. Human luteal phase function following oocyte aspiration from the immediately preovular graafian follicle of spontaneous ovular cycles. Br J Obstet Gynaecol 1981;88: 1021–8.
21. Mochtar MH, Hogerzeil HV, Mol BW. Progesterone alone versus progesterone combined with hCG as luteal support in GnRHa/HMG induced IVF cycles: a randomized clinical trial. Hum Reprod 1996;11:1602–5.
22. Hutchins-Williams KA, Decherney AH, Lavy G, Diamond MP, Naftolin F, Lunenfeld B. Luteal rescue in in vitro fertilization–embryo transfer. Fertil Steril 1990;53:495–501.
23. Daya S, Gunby J. Luteal phase support in assisted reproduction cycles [Review]. Cochrane Database Syst Rev 2004;(3). CD004830.
24. Propst AM, Hill JA, Ginsburg ES, Hurwitz S, Politch J, Yanushpolsky EH. A randomized study comparing Crinone 8% and intramuscular progesterone supplementation in in vitro fertilization-embryo transfer cycles. Fertil Steril 2001;76:1144–9.
25. Miles RA, 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.
26. Preston RA, Norris PM, Blake EJ, Yankov VI. Single day and multiple day pharmacokinetics of a novel vaginal micronized progesterone tablet (Endometrin) compared to Crinone vaginal gel in healthy female subjects. Reprod Sci 2007;14:198A.
27. Doody KJ, Schnell VL, Foulk RA, Miller CE, Kolb BA, Blake EJ, et al. Endometrin for luteal phase support in a randomized, controlled, open-label, prospective in-vitro fertilization trial using a combination of Menopur and Bravelle for controlled ovarian hyperstimulation. Fertil Steril. Published online April 18, 2008 [Epub ahead of print].
28. Pritts EA, Atwood AK. Luteal phase support in infertility treatment: a meta-analysis of the randomized trials. Hum Reprod 2002;17: 2287–99.
29. Lightman A, Kol S, Itskovitz-Eldor J. A prospective randomized study comparing intramuscular with intravaginal natural progesterone in programmed thaw cycles. Hum Reprod 1999;14:2596–9.
30. Bouckaert Y, Robert F, Englert Y, De Backer D, De Vuyst P, Delbaere A. Acute eosinophilic pneumonia associated with intramuscular administration of progesterone as luteal phase support after IVF: case report. Hum Reprod 2004;19:1806–10.
31. Kimzey LM, Gumowski J, Merriam GR, Grimes GJ Jr, Nelson LM. Absorption of micronized progesterone from a nonliquifying vaginal cream. Fertil Steril 1999;56:995–6.
32. Cicinelli E, Schonauer LM, Galantino P, Matteo MG, Cassetta R, Pinto V. Mechanisms of uterine specificity of vaginal progesterone. Hum Reprod 2000;15:159–65.
33. de Ziegler D, Seidler L, Scharer E, Bouchard P. Non-oral administration of progesterone: experiences and possibilities of the transvaginal route. Schweiz Rundsch Med Prax 1995;84:127–33.
34. Channing CP, Schaerf FW, Anderson LD, Tsafriri A. Ovarian follicular and luteal physiology. Int Rev Physiol 1980;22:117–201.
35. Leung PC, Armstrong DT. Interactions of steroids and gonadotropins in the control of steroidogenesis in the ovarian follicle. Ann Rev Physiol 1980;42:71–82.
36. Mahesh VB. The dynamic interaction between steroids and gonadotropins in the mammalian ovulatory cycle. Neurosci Biobehav Rev 1985;9:245–60.
37. Rothchild I. Regulation of the mammalian corpus luteum. Recent Prog Horm Res 1981;37:183–298.
38. Stocco C, Telleria C, Gibori G. The molecular control of corpus luteum formation, function, and regression. Endocr Rev 2007;28:117–49.
39. Cowan BD, Whitworth NS, Sopelak VM, Isaacs JD, Long CA. Regulation of early gestational corpus luteum function in spontaneous and follicular stimulated conceptions. Fertil Steril 1994;62:263–8.

40. Artini PG, Volpe A, Angioni S, Galassi MC, Battaglia C, Genazzani AR. A comparative, randomized study of three different progesterone support of the luteal phase following IVF/ET program. J Endocrinol Invest 1995;18:51–6.
41. Abate A, Perino M, Abate FG, Brigandi A, Costabile L, Manti F. Intramuscular versus vaginal administration of progesterone for luteal phase support after in vitro fertilization and embryo transfer: a comparative randomized study. Clin Exp Obstet Gynecol 1999;26:203–6.
42. Anserini P, Costa M, Remorgida V, Sarli R, Guglielminetti E, Ragni N. Luteal phase support in assisted reproductive cycles using either vaginal (Crinone 8%) or intramuscular (Prontogest) progesterone: results of a prospective randomized study. Minerva Ginecol 2001;53: 297–301.
43. Levine H. Luteal support in IVF using the novel vaginal progesterone gel Crinone 8%: results of an open-label trial in 1184 women from 16 US centers. Fertil Steril 2000;74:836–7.
44. Simunic V, Tomic V, Tomic J, Nizic D. Comparative study of the efficacy and tolerability of two vaginal progesterone formulations, Crinone 8% gel and Utrogestan capsules, used for luteal phase support. Fertil Steril 2007;87:83–7.
45. Ludwig M, Schwartz P, Babahan B, Katalinic A, Weiss JM, Felberbaum R, et al. Luteal phase support using either Crinone 8% or Utrogest: results of a prospective, randomized study. Eur J Obstet Gynecol Reprod Biol 2002;103:48–52.
46. Ng EH, Chan CC, Tang OS, Ho PC. A randomized comparison of side effects and patient convenience between Cyclogest suppositories and Endometrin tablets used for luteal phase support in IVF treatment. Eur J Obstet Gynecol Reprod Biol 2007;131:182–8.
47. Yanushpolsky E, Hurwitz S, Greenberg L, Racowsky C, Hornstein MD. Comparison of Crinone 8% intravaginal gel and intramuscular progesterone supplementation for in vitro fertilization/embryo transfer in women under age 40: interim analysis of a prospective randomized trial. Fertil Steril 2008;89:485–7.
48. Witz CA, Burns WN. Endometriosis and infertility: is there a cause and effect relationship? Gynecol Obstet Invest 2002;53(Suppl 1):2–11.
49. Attia GR, Zeitoun K, Edwards D, Johns A, Carr BR, Bulun SE. Progesterone receptor isoform A but not B is expressed in endometriosis. J Clin Endocrinol Metab 2000;85:2897–902.
50. Cahill DJ, Hull MG. Pituitary-ovarian dysfunction and endometriosis. Hum Reprod Update 2000;6:56–66.
51. Cunha-Filho JS, Gross JL, Bastos de Souza CA, Lemos NA, Giugliani C, Freitas F, et al. Physiopathological aspects of corpus luteum defect in infertile patients with mild/minimal endometriosis. J Assist Reprod Genet 2003;20: 117–21.
52. Donaghay M, Lessey BA. Uterine receptivity: alterations associated with benign gynecological disease. Semin Reprod Med 2007;25:461–75.
53. Kyama CM, Overbergh L, Mihalyi A, Meuleman C, Mwenda JM, Mathieu C, et al. Endometrial and peritoneal expression of aromatase, cytokines, and adhesion factors in women with endometriosis. Fertil Steril 2008;89:301–10.
54. Sallam HN, Garcia-Velasco JA, Dias S, Arici A. Long-term pituitary down-regulation before in vitro fertilization (IVF) for women with endometriosis. Cochrane Database Syst Rev 2006;(25). CD004635.
55. Cirigliano M. Bioidentical hormone therapy: a review of the evidence. J Womens Health (Larchmt) 2007;16:600–31.
56. Boothby LA, Doering PL. Bioidentical hormone therapy: a panacea that lacks supportive evidence. Curr Opin Obstet Gynecol 2008;20:400–7.
57. Mahaguna V, McDermott JM, Zhang F, Ochoa F. Investigation of product quality between extemporaneously compounded progesterone vaginal suppositories and an approved progesterone vaginal gel. Drug Dev Ind Pharm 2004;30:1069–78.
58. A response to the Wyeth petition. Available at http://www.iacprx.org/site/DocServer/APhA_Comments_on_2005P-0411.pdf?docID=1601.
59. Filicori M, Butler JP, Crowley WF Jr. Neuroendocrine regulation of the corpus luteum in the human. Evidence for pulsatile progesterone secretion. J Clin Invest 1984;73:1638–47.
60. Khan N, Richter KS, Newsome TL, Blake EJ, Yankov VI. Matched-samples comparison of intramuscular versus vaginal progesterone for luteal phase support after in vitro fertilization and embryo transfer. Fertil Steril. Published online June 13, 2008 [Epub ahead of print].