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Luteal estradiol administration strengthens the relationship between day 3 follicle-stimulating hormone and inhibin B levels and ovarian follicular status

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Objective: To investigate whether the prevention of early follicular growth by luteal E_2 administration improves the relationship between day 3 hormone measurements and the ovarian follicular status.

Design: Prospective, cohort study.

Setting: Assisted reproductive technology unit in Clamart, France.

Patient(s): One hundred sixty-two infertile women.

Intervention(s): Participants received oral 17β-E_2, 4 mg/day, from day 20 to the next cycle day 1 (n = 81) or served as controls (n = 81). Serum E_2, inhibin B, and FSH were measured during the 3 days after E_2 discontinuation (FD1, FD2, and FD3) in E_2-treated women and on cycle day 3 (CD3) in controls. Early antral follicles were counted at ultrasound scans on FD3 and CD3.

Main Outcome Measure(s): Hormonal-follicular correlations on FD3 and CD3.

Result(s): As expected, after E_2 withdrawal, inhibin B and FSH increased from FD1 to FD3 whereas E_2 decreased. Correlations between FSH and inhibin B and follicular counts were stronger on FD3 than on CD3.

Conclusion(s): Luteal E_2 administration notably strengthens the relationship between serum FSH and inhibin B levels and the number of antral follicles on day 3. This approach may represent an alternative test of ovarian follicular status. (Fertil Steril® 2003;79:585–9. ©2003 by American Society for Reproductive Medicine.)

Key Words: FSH, estradiol, ovarian reserve, controlled ovarian hyperstimulation

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During the luteal-follicular transition, inhibin B (1) and estradiol (E_2) (2) secretions by early antral follicles modulate their own stimulation by FSH. Given that reproductive aging is characterized by the quantitative depletion of the early antral follicular cohort (3, 4), the consequent reduction in peripheral inhibin B and E_2 concentrations amplifies intercycle FSH secretion, which culminates 2 to 3 days after the onset of menstrual bleeding (5). Hence, circulating FSH (6) and inhibin B (7) levels on day 3 of the menstrual cycle have become widely used as markers of ovarian status and responsiveness to controlled ovarian hyperstimulation (COH). However, growing evidence indicates that these hormone measurements, arbitrarily timed to the onset of menses, offer only a pale reflection of women's fecundity potential (8–11).

During the ultimate phase of the menstrual cycle, there is a progressive increase in FSH levels that parallels the corpus luteum demise and starts up to 5 days before the onset of menstrual bleeding (2, 5). In some women, premenstrual exposure of early antral follicles to FSH may accelerate their development during the first days of the follicular phase (12). Because larger follicles are likely to produce more inhibin B (13, 14) and E_2 (15), it is possible that such a phenomenon interferes with FSH secretion, thereby altering the predictability of peripheral day 3 FSH and inhibin B measurements on the ovarian follicular status.

Based on this contention, it is conceivable that the suppression of FSH secretion during the late luteal phase could avoid premature development of early antral follicles and im-

prove the reliability of hormonal measurements. Indeed, the administration of physiological E_2 doses during the luteal-follicular transition has been shown to effectively prevent intercycle FSH rise and, consequently, untimely follicular growth (16, 17). In addition, after E_2 discontinuation, FSH levels increase rapidly and peak 3 days later (16, 17). Hence, we hypothesized that the control of early antral follicular development by exogenous E_2 would be instrumental in strengthening correlations between FSH and inhibin B levels measured on day 3 and the ovarian follicular status.

MATERIALS AND METHODS

Patients

We prospectively studied 162 infertile women, 27 to 44 years of age who met the following inclusion criteria: 1) regular, ovulatory menstrual cycles every 25 to 35 days; 2) both ovaries present; 3) no current or past diseases affecting ovaries or gonadotropin or sex steroid secretion, clearance, or excretion; 4) body mass indexes ranging from 18 to 25 kg/m^2; 5) no current hormone therapy; and 6) adequate visualization of ovaries in transvaginal ultrasound scans. Infertility was unexplained (69%), or due to sperm (21%) or mild tubal (9%) abnormalities. An informed consent was obtained from all women and this investigation received the approval of our internal institutional review board.

Study Protocol

Participants either received luteal E_2 treatment or served as controls. Women included in the E_2-treated group (n = 81) were given oral micronized 17β-E_2 (4 mg/day; Provamès, Cassenne Laboratories, Puteaux, France) in the evening at 8:00 PM, from day 20 until day 1 of the next cycle. To document the hormonal profile following E_2 discontinuation, serum FSH, inhibin B, and E_2 levels were measured on the 3 subsequent days referred to as functional days (FD): FD1, FD2, and FD3, respectively. On FD3, early antral follicles were counted at ultrasound scans. Women included in the control group (n = 81) did not receive any treatment during the luteal phase and had similar hormonal and ultrasonographic measurements on the third day of the subsequent cycle, referred to as cycle day (CD) 3. Women in both groups were asked to compute the length of the menstrual cycle studied.

Hormonal Measurements

All blood samples were obtained by venipuncture, performed at approximately 8:00 AM, and serum was separated and frozen in aliquots at −20°C for subsequent centralized analysis. Serum FSH levels were determined by an automated multianalysis system using a chemiluminescence technique (ACS-180, Bayer Diagnostics, Puteaux, France). Intraassay and interassay coefficients of variation (CV) were, respectively, 3% and 5%, and the lower limit of detection was 0.1 mIU/mL for FSH. Serum inhibin B was determined by double antibody ELISA (Serotec, Varilhes, France) as previously described (13). For inhibin B, the lower limit of detection was 10 pg/mL, and the intraassay and interassay CV were <6% and <9%, respectively. Serum E_2 was determined by an automated multianalysis system using a chemiluminescence technique (ACS-180, Bayer Diagnostics, Puteaux, France). For E_2, the lower limit of detection was 15 pg/mL, and intraassay and interassay CV were 8% and 9%, respectively. The specificity of the FSH, inhibin B, and E_2 assays was 100%, 99.5%, and 100%, respectively.

Ultrasound Monitoring

Both on FD3 and on CD3, ultrasounds scans of the ovaries were performed with a 4.5–7.2 MHz multifrequency transvaginal probe (Siemens Elegra, Siemens S.A.S., Saint-Denis, France) by one single operator (C.R.) at approximately 9:00 AM. The objective of these examinations was to count the total number of early antral follicles (2 to 8 mm in diameter) in both ovaries. The operator was unaware of both treatment schedule and hormonal results. In an attempt to optimize recognition of ovarian follicles, the ultrasound scanner used was equipped with a tissue harmonic imaging system (18). Tissue harmonic imaging is based on ultrasound signal frequency changes during propagation through the target media. This change is called a harmonic and is essentially a doubling of the original frequency. Hence, the returning high frequency signal has to only travel one direction (return to the probe), which enhances signal penetration, reduces near field noise and image haze, and provides increased contrast resolution.

Statistics

The measure of central tendency used for continuous variables was the mean and the measure of variability was the standard error of the mean. Longitudinal hormonal changes were assessed by analysis of variance with repeated measures or paired Student's *t*-test when appropriate. Relationship between two different continuous variables were assessed by correlation. The Fisher *r* to *z* test was used to determine if correlation coefficients (*r*) were different from zero. Comparison of strength of correlations was performed using the Hotelling's *t*-test (19), which assesses the difference between coefficients of correlation for correlated samples. $P<.05$ was considered statistically significant.

RESULTS

Overall Data

As expected by design, E_2-treated and control groups were strictly comparable in regard to mean women's ages (33.9 ± 0.4 vs. 33.6 ± 0.5 years, respectively), frequency distributions of causes of infertility (unexplained, 68% and 70%; sperm abnormalities, 22% and 21%; mild tubal alteration, 9% and 9%, respectively), and early antral follicular counts (10.1 ± 0.7 follicles on FD3 and 10.6 ± 0.6 follicles on CD3). In addition, the number of early antral follicles and women's ages showed similar negative correlations in E_2-

treated as in control groups ($r = -0.41$, $P<.0001$ and $r = -0.39$, $P<.0001$, respectively). The mean length of the preceding cycle was not altered by luteal E_2 administration and remained similar in both groups (28.1 ± 0.2 versus 28.2 ± 0.2 days, respectively). However, the subsequent menstrual cycles lasted slightly, but significantly, longer in the E_2-treated women (29.1 ± 0.2 days) than in the controls (28.3 ± 0.2 days, $P<.02$).

The hormonal dynamics after exogenous E_2 discontinuation in the E_2-treated group as well as the comparison of hormone levels between FD3 and CD3 are depicted in Figure 1. Serum FSH levels were low at 3.8 ± 0.2 mIU/mL on the first day of E_2 withdrawal (FD1) and increased progressively on FD2 (7.2 ± 0.4 mIU/mL) and FD3 (10.5 ± 0.6 mIU/mL) ($P<.0001$). It is noticeable that FSH levels were significantly higher on FD3 than on CD3 (8.3 ± 0.4 mIU/mL, $P<.0006$). Similarly to FSH, serum inhibin B levels were low on FD1 (18.6 ± 1.1 pg/mL) and increased progressively from FD2 to FD3 (33.2 ± 2.9 and 71.2 ± 5.6 pg/mL, respectively) ($P<.0001$). Yet serum inhibin B levels on FD3 were lower than inhibin B levels on CD3 (90.0 ± 3.6 pg/mL, $P<.006$). Further, E_2 administration maintained serum E_2 at levels comparable to those observed during the late follicular phase of the menstrual cycle as measured on FD1 (152.7 ± 5.9 pg/mL). After oral treatment was discontinued, serum E_2 levels decreased progressively (73.6 ± 4.2 and 52.2 ± 3.2 pg/mL, on FD2 and FD3, respectively) ($P<.0001$). Serum E_2 levels were higher on FD3 than on CD3 (34.3 ± 2.3 pg/mL, $P<.0001$), presumably as a result of exogenous and endogenous contributions.

Follicular and Hormonal Relationships

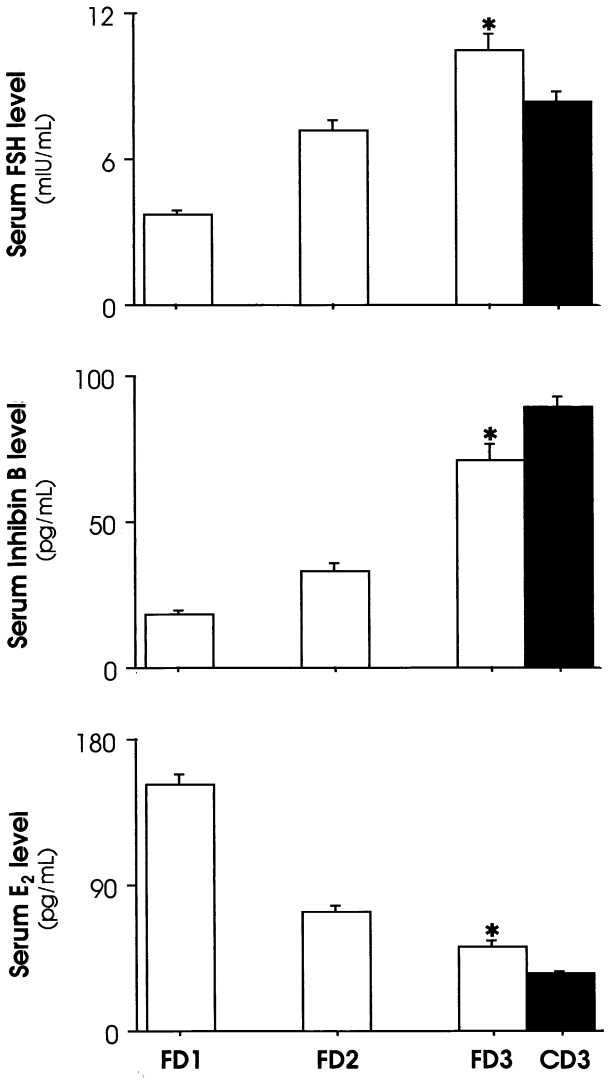
The strength of the correlations between serum hormone levels on FD3 (E_2-treated group) and on CD3 (control group) and early antral follicular counts are summarized in Table 1. Serum FSH levels showed a stronger negative correlation with the number of early antral follicles on FD3 ($r = -0.44$, $P<.0001$) than on CD3 ($r = -0.23$, $P<0.04$). Comparison of strength of correlations indicated that this difference was statistically significant ($P<.02$). Similarly, the correlation between serum inhibin B levels and early antral follicular counts was stronger ($P<.001$) on FD3 ($r = 0.59$, $P<.0001$) than on CD3 ($r = 0.24$, $P<.03$). In contrast, serum E_2 levels failed to correlate with early antral follicular counts on either FD3 or CD3. Furthermore, serum FSH and inhibin B were correlated to each other only in patients who were pretreated with E_2 ($r = -0.30$, $P<.0007$ vs. $r = -0.07$, $P>.05$). This difference was statistically significant ($P<.02$).

DISCUSSION

The results of the present investigation indicate that the magnitude of FSH and inhibin B elevation timed by luteal E_2 administration is better correlated to the ovarian follicular status than conventional, uncontrolled FSH and inhibin B

FIGURE 1

Serum FSH (*top panel*), inhibin B (*middle panel*), and E_2 (*bottom panel*) profiles during the first 3 days after E_2 discontinuation (FD1, FD2, and FD3) in the E_2-treated group. Both the progressive elevation in FSH and inhibin B levels, as well as the decrease in E_2 levels, from FD1 to FD3 were statistically significant (*open bars*, $P<.0001$). Hormone levels on FD3 were significantly different as compared to controls (CD3, *closed bars*, $^*P<.0001$).



Fanchin. FSH and inhibin B elevation timed by E_2. Fertil Steril 2003.

measurements performed on cycle day 3. The objective of E_2 administration was to control the timing of FSH elevation during the luteal-follicular transition to prevent premature follicular development and, therefore, optimize hormonal-follicular correlations.

Indeed, previous data have demonstrated that, during the late luteal phase of the menstrual cycle, spontaneous demise of the corpus luteum leads to a progressive FSH rise that

starts approximately 5 days before menses (5) and reach serum levels >7 mIU/mL 3 days before the onset of menstrual bleeding (5). On account of the enhanced sensitivity of early antral follicles to FSH during the late luteal phase (3, 20), it is presumable that luteal FSH secretion hastens the pace of follicular development (12). In addition, this phenomenon has been reported as being more striking in women who display some degree of follicular exhaustion and declined fecundity (12). Our data confirmed that administration of physiological E_2 doses during the late luteal phase effectively maintained FSH secretion at low levels (3.8 ± 0.2 mIU/mL on FD1), which is in keeping with results reported previously in both humans (17, 21) and animals (16).

As expected, a dramatic FSH elevation occurred during the 3 subsequent days after E_2 withdrawal. Interestingly, serum FSH levels were significantly higher on FD3 than on CD3. Some elements led us to infer that these differences were neither phenomenological nor due to patient heterogeneities. Indeed, our experimental design required population selection, randomization, and blinded measurements; also, clinical and ultrasonographic attributes of patients were remarkably similar in both groups. Taken together, these methodological characteristics minimized as far as possible the likelihood that individual discrepancies accounted for the observed hormonal differences. Moreover, these results corroborate previous experiments conducted in rhesus monkeys, in which removal of E_2 administration during the follicular phase led to larger FSH release as compared to untreated controls (16).

Hence, it is conceivable that FSH level differences between FD3 and CD3 result from luteal E_2 administration through a number of mechanisms. First, the overall reduced inhibin B and E_2 secretion by small early antral follicles is likely to insufficiently suppress FSH secretion. Indeed, our results indicated that inhibin B levels were very low on the day after E_2 withdrawal (FD1) and reached lower levels on FD3 than on CD3. Second, whereas FD3 corresponded precisely to the third day of FSH elevation in E_2-treated patients, the timing of FSH elevation could not be directed in controls. Hence, on CD3, serum FSH levels may have been influenced by the stage of follicular growth in untreated women. Finally, the putative negative feedback effect of prolonged E_2 treatment on GnRH pulse characteristics (22) and/or on pituitary gonadotropin secretion (23) could have elicited a select favoritism of FSH beta expression in E_2-treated patients. However, this issue deserves further clarification in additional studies.

Serum E_2 and inhibin B profiles from FD1 to FD3 in E_2-treated group markedly differed from each other. Whereas inhibin B levels dramatically increased in response to FSH stimulation, E_2 levels fell progressively from FD1 to FD3 as a result of the interrupted exogenous contribution. Incidentally, the pace of inhibin B increase after E_2 withdrawal observed in the present study is remarkably similar to that observed after profound FSH suppression by GnRH agonist administration and subsequent FSH treatment as reported previously (24). This strengthens the hypothesis that similar follicular status may be obtained after FSH suppression by exogenous E_2 as compared to pituitary desensitization by GnRH agonists.

In addition, it is noteworthy that serum inhibin B levels were lower on FD3 as compared to CD3. Given that inhibin secretion also depends on follicular sizes (13, 14), it is possible that early antral follicles were smaller in E_2-treated patients as compared with controls. In line with this, menstrual cycles lasted longer in women who were pretreated with E_2, possibly due to a longer growth course of smaller antral follicles to ovulation. An analogous phenomenon has been observed in E_2-treated monkeys (16). Unfortunately, the design of the present study did not include antral follicular size measurements, which prevents us from making any conclusion on this point. This issue is currently being investigated.

Furthermore, no evidence of increasing E_2 production as a result of endogenous FSH stimulus was observed in the E_2-treated group. In response to FSH, E_2 secretion by the early antral follicles is scarce and variable, probably due to the incipient aromatase activity in early antral follicles (3, 25, 26). In agreement, serum E_2 levels correlated with the number of early antral follicles on neither FD3 nor CD3, which challenges the reliability of day 3 E_2 measurements for prognosticating antral follicular reserve. Yet endogenous E_2 secretion by antral follicles participates in the regulation of FSH levels. Hence, the lack of mutual FSH and inhibin B correlation in the untreated group is consistent with the varying E_2 production by antral follicles displaying heterogeneous degrees of development.

The central observation of the present study was the more strict correlation between the magnitude of FSH and inhibin B rise timed by E_2 administration and the number of early antral follicles as compared to controls. This indicates that the control of follicular exposure to FSH by E_2 administration may refine the analysis of hormonal-follicular relationships, commonly timed to the onset of menses, and improve their clinical usefulness. Indeed, it is conceivable that the putative prognostic value of early antral follicular counts (5, 27, 28) on women's fertility and responsiveness to controlled ovarian hyperstimulation may be further improved by concomitant hormonal measurements.

In conclusion, the intercycle FSH and inhibin B rise timed by E_2 administration represents an interesting, alternative strategy to assess ovarian follicular status. The observed improvement of hormonal-follicular correlations may be explained by a possible modification of early follicular growth and steroidogenesis due to decreased premenstrual FSH secretion. Additional investigation is needed to assess possible consequences of luteal E_2 administration on the early antral follicle characteristics during the early follicular phase. More

studies including a large number of cases are also required to confirm the hypothesis that the magnitude of FSH and inhibin B increases after E_2 withdrawal can improve the predictive value of hormonal measurements performed during the early follicular phase for assessing women's fertility potential.

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