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Coordination of early antral follicles by luteal estradiol administration provides a basis for alternative controlled ovarian hyperstimulation regimens

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Objective: To investigate whether luteal E_2 administration reduces size discrepancies of early antral follicles.

Design: Prospective, crossover study.

Setting: ART unit, Clamart, France.

Patient(s): Sixty women and 120 cycles.

Intervention(s): On cycle day 3 (baseline day 3), all women underwent measurements of early antral follicles by ultrasound and serum FSH and ovarian hormones. From day 20 until the next cycle day 2, 30 of them received oral 17β-E_2, whereas the remaining women served as controls. The day after E_2 discontinuation (E_2 day 3) or on subsequent cycle day 3 (control day 3), participants were reevaluated as on baseline day 3.

Main Outcome Measure(s): Magnitude of follicular size discrepancies.

Result(s): Follicular size discrepancies and follicular diameters were significantly attenuated on E_2 day 3 (3.7 $\pm$ 0.5 mm) as compared with baseline day 3 (4.9 $\pm$ 1.0 mm), but not in controls (5.0 $\pm$ 0.8 vs. 4.9 $\pm$ 0.8 mm). FSH (4.3 $\pm$ 1.9 vs. 7.3 $\pm$ 3.3 mIU/mL) and inhibin B (34 $\pm$ 28 vs. 71 $\pm$ 32 pg/mL) levels were consistently lower on E_2 day 3 than on baseline day 3 but remained unchanged in controls.

Conclusion(s): Luteal E_2 administration reduces the size and improves the homogeneity of early antral follicles on day 3. This approach may be instrumental in synchronizing follicular development during controlled ovarian hyperstimulation. (Fertil Steril® 2003;79:316–21. ©2003 by American Society for Reproductive Medicine.)

Key Words: FSH, estradiol, early antral follicles, controlled ovarian hyperstimulation

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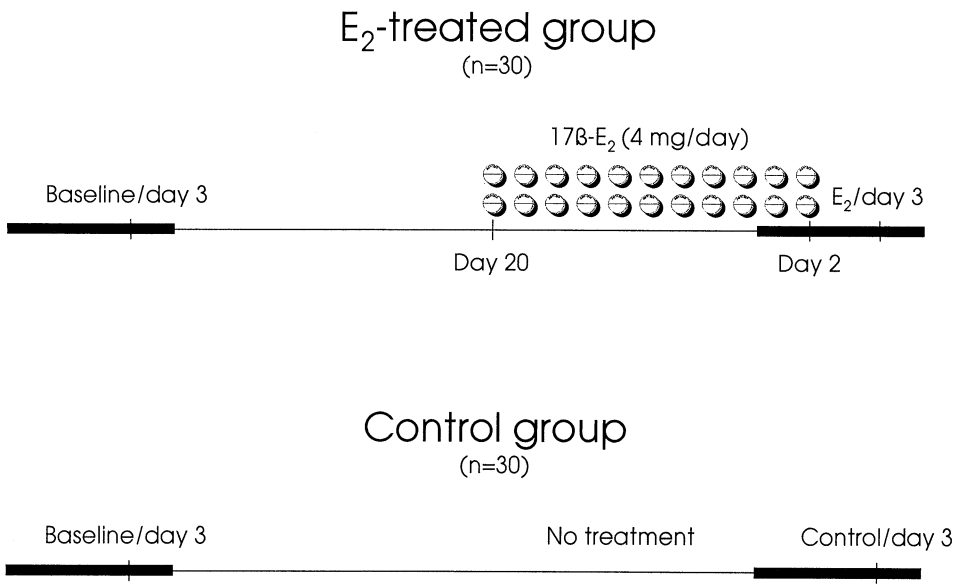
The foremost objective of controlled ovarian hyperstimulation (COH) is to ensure the adequate functional and morphologic maturation of early antral follicles to increase the number of viable oocytes and the probability of conception. One of the key points of such a procedure is the achievement of adequate synchronization of follicular growth so that ovulation can be triggered when most follicles have reached concomitant maturation. However, to attain satisfactory follicular coordination during COH, the physiological heterogeneity of selectable follicles observed during the early follicular phase should probably be overcome.

During the first days of the follicular phase in the menstrual cycle, early antral follicles are discrepantly sensitive to FSH (1, 2) and exhibit dissimilar sizes, which range from 2 to 8 mm in diameter (3). Under physiological conditions, both of these interrelated phenomena may be instrumental in the establishment of the follicular dominance. Indeed, although it is conceivable that complex intrafollicular mechanisms concur to determine follicular sensitivity to FSH (4), compelling evidence indicates that larger follicles are more responsive to this hormone than are smaller follicles (1, 2, 5). Yet the mechanisms underlying the heterogeneity of early antral follicles sizes during the early follicular phase remain unclear.

A possible explanation for this phenomenon involves the exposure of early antral follicles to gradient FSH levels during the late luteal phase. During the ultimate phase of the menstrual cycle, up to 5 days before the onset of

FIGURE 1

Study protocol. Horizontal *black bars* represent menstrual bleeding. On baseline/day 3, E_2/day 3, and control/day 3, hormonal and ultrasound measurements were performed.



Fanchin. Coordination of early antral follicles. Fertil Steril 2003.

menstrual bleeding (6, 7), paralleling the corpus luteum demise, FSH levels increase progressively to preserve antral follicles from atresia and ensure subsequent growth (8). According to their inherent sensitivity to FSH, it is possible that some early antral follicles are able to respond to lower amounts of FSH than others and, therefore, to start their development during the late luteal phase (9). This premature, gradual exposure of follicles to FSH may accelerate the development of more sensitive follicles and accentuate size discrepancies observed during the first days of the subsequent cycle.

Hence, we decided to challenge the hypothesis that size discrepancies among early antral follicles result, at least in part, from their early and progressive exposure to FSH. For this we elected to artificially lower endogenous FSH secretion during the luteal phase by administering E_2 (10, 11) and to measure follicular and hormonal profiles on day 3 in two consecutive menstrual cycles that were either preceded or not preceded by luteal E_2 administration.

MATERIALS AND METHODS

Subjects

We prospectively studied 66 female volunteers, 20–41 years of age. All participants met the following inclusion criteria: [1] regular, ovulatory menstrual cycles every 25–35 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 27 kg/m^2; [5] no current hormone therapy; [6] adequate visualization of ovaries in transvaginal ultrasound scans. Informed consent was obtained from all women, and this investigation received the approval of our internal Institutional Review Board. For personal reasons (n = 4) or major protocol violation (n = 2), six women did not complete the two subsequent observation cycles required by the protocol and had to be excluded from the analysis. Therefore, the population studied was limited to 60 participants undergoing 120 study cycles.

Study Protocol

The study protocol is summarized in Figure 1. On day 3 of their menstrual cycles (baseline/day 3), all 60 women underwent blood sampling for serum FSH, inhibin B, and E_2 measurements at approximately 9:00 A.M. Later in the morning, ultrasound scans of their ovaries were performed. Subsequently, women were randomized to receive luteal E_2 treatment or to serve as controls. As shown in Figure 1, participants who were included in the E_2-treated group (n = 30) received micronized 17β-E_2 oral tablets (4 mg/day; Provamès, Cassenne Laboratories, Puteaux, France) in the evening at 8:00 P.M. from day 20 of the same cycle until day 2 of their next cycle. We chose the 4 mg/day dose for E_2 p.o. administration because of its reported efficacy in reducing endogenous FSH and in preventing early follicular growth

(11). Participants who were included in the control group (n = 30) remained untreated.

On the first day of E_2 discontinuation in E_2-treated women (E_2/day 3) or on day 3 of the subsequent cycle in control women (control/day 3), hormonal and ultrasound measurements similar to the preceding cycle (baseline/day 3) were performed. In addition, participants were asked to compute their baseline and subsequent menstrual cycle lengths and to report possible subjective changes in menstrual bleeding characteristics. In E_2-treated women, compliance of treatment was monitored to detect any protocol violation.

Ultrasound Measurements

Ultrasound scans were performed using a 4.5–7.2 MHz multifrequency transvaginal probe (Siemens Elegra, Siemens S.A.S., Saint-Denis, France) by one single operator (J.S.C.F.), who was not aware of the treatment schedule or the hormonal results. The objective of the ultrasound examinations was to evaluate the number and sizes of early antral follicles and to calculate the mean ovarian volume. We considered all follicles that measured 2–12 mm in mean diameter (mean of 2 orthogonal diameters). The choice of not excluding follicles measuring 9–12 mm was based on the fact that, according to our own experience, some women display marked acceleration of follicular development.

In an attempt to optimize the reliability of ovarian follicular assessment, the ultrasound scanner that was used was equipped with a tissue harmonic imaging system (12), which allowed improved image resolution and adequate recognition of follicular borders. Ovarian volumes, calculated according to the formula for an ellipsoid (0.526 × length × height × width) (13), were the mean volume for both ovaries. Intra-analysis coefficients of variation (CVs) for follicular and ovarian measurements were <5%, and their lower limit of detection was 0.1 mm.

Hormonal Measurements

All blood samples were obtained by venipuncture, and serum was separated and frozen in aliquots at −20°C for subsequent centralized analysis. Serum FSH was measured by an immunometric technique using an Amerlite kit (Ortho Clinical Diagnostics, Strasbourg, France). Intra-assay and interassay CVs were, respectively, 5% and 7%, and lower limit detection was 0.1 mIU/mL for FSH. Serum inhibin B was determined by double antibody enzyme-linked immunosorbent assay (Serotec, Varilhes, France) as described elsewhere (14). Lower limit detection was 10 pg/mL, and intra-assay and interassay CVs were <6% and <9%, respectively, for inhibin B. Serum E_2 was determined by an immunoometric technique using an Estradiol-60 Amerlite kit (Ortho Clinical Diagnostics). Lower limit detection was 14 pg/mL, and intra-assay and interassay CVs were 8% and 9%, respectively, for E_2.

Statistics

The measure of central tendency used was the mean and the measure of the variability was the SD. Because of the pairwise design of this study, data from each participant on E_2 day 3 or on control day 3 were compared with corresponding data for the same participant on baseline day 3 by using the paired Student's *t*-test. To evaluate the magnitude of follicular size discrepancies from baseline day 3 to E_2 day 3 and from baseline day 3 to control day 3, we tested the homogeneity of variances by using the Levene test for equal variances (15). This test is less sensitive than F-tests to departures from normality and allows a comparison of the dispersion of data around the mean independent of mean values. In addition, SD:mean ratios for follicular sizes were also calculated. The present crossover study was powered to detect anticipated differences of 0.5 mm for follicular sizes and 2 mm for ovarian volume calculation at >80% power at a 0.5 significance level. $P<.05$ was considered statistically significant.

RESULTS

Ultrasound Results

Follicular and ovarian measurement results are summarized in Table 1. As expected, the number of antral follicles did not change significantly from one cycle to another in E_2-treated women and in controls. In contrast, we observed a significant reduction of mean follicular sizes in E_2-treated women from baseline/day 3 to E_2/day 3 but not in controls. In agreement with this, mean ovarian volume decreased significantly in women treated with E_2 and remained unchanged in controls. In addition, we observed a remarkable attenuation of follicular size discrepancies on E_2 day 3 as compared with baseline day 3 ($P<.0001$). This was not noticed between baseline day 3 and control day 3. Consistently, SD:mean ratios for follicular sizes were significantly lower on E_2/day 3 than on baseline/day 3 but not on control/day 3 as compared with baseline/day 3, which confirms the improvement in follicular size homogeneity observed in E_2-treated women. Incidentally, it is noteworthy that ultrasound measurements made on baseline/day 3 were strictly similar in women included in the E_2-treated and control groups.

Hormonal Results

Hormonal results are also presented in Table 1. In women who were administered E_2 during the luteal phase, serum inhibin B levels were significantly lower on E_2/day 3 as compared with baseline day 3, whereas no significant longitudinal change in inhibin B levels was noted in controls. As expected, E_2 administration raised serum E_2 to levels comparable to those observed during the late follicular phase of the menstrual cycle (114 ± 57 pg/mL on E_2 day 3). Serum E_2 levels did not vary significantly in controls from baseline/day 3 to control/day 3. Administration of E_2 lowered serum

TABLE 1

Ultrasound and hormonal results during two consecutive menstrual cycles in women receiving E_2 during the luteal phase and in controls.

	E_2 treated group			Control group		
	Baseline/day 3	E_2/day 3	*P*	Baseline/day 3	Control/day 3	*P*
No. of follicles (range)	10.4 ± 4.3 (3–19)	10.3 ± 4.9 (2–20)	NS	10.6 ± 4.1 (3–20)	10.5 ± 3.9 (3–20)	NS
Mean follicular size (mm)	4.9 ± 1.0	3.7 ± 0.5	<.001	4.9 ± 0.8	5.0 ± 0.8	NS
SD/mean of follicular sizes	0.40	0.23	<.001	0.40	0.39	NS
Mean ovarian volume (cm^3)	6.1 ± 3.0	5.1 ± 2.6	<.02	6.1 ± 2.3	6.2 ± 2.1	NS
Serum inhibin B (pg/mL)	71 ± 32	34 ± 28	<.001	77 ± 23	75 ± 21	NS
Serum E_2 (pg/mL)	47 ± 29	114 ± 57	<.001	43 ± 29	38 ± 22	NS
Serum FSH (mIU/mL)	7.3 ± 3.3	4.3 ± 1.9	<.001	6.9 ± 2.6	7.5 ± 2.7	NS

Fanchin. Coordination of early antral follicles. Fertil Steril 2003.

FSH levels on E_2/day 3 as compared with baseline/day 3. However, in women who did not receive E_2 treatment, serum FSH levels remained steady from one cycle to the other. CVs for FSH from the first to the second cycle were significantly higher in E_2-treated women than in controls (40% vs. 17%, $P<.01$). As for ultrasound measurements, hormonal results obtained on baseline/day 3 were closely similar in women receiving E_2 as compared with those who served as controls.

Clinical Results

As anticipated, the E_2-treated and the control groups were comparable with regard to ages of women (33.3 ± 0.6 vs. 33.3 ± 0.5 years) and body mass index (21.8 ± 0.4 and 21.8 ± 0.3 kg/m^2). A significant lengthening of mean menstrual cycle duration was observed in participants receiving E_2 (29.4 ± 1.3 days, $P<.0001$) as compared with their baseline cycles (27.8 ± 1.2 days). This phenomenon was not observed in controls (28.0 ± 1.1 vs. 27.9 ± 0.8 days, respectively). Moreover, E_2 treatment did not alter the baseline cycle length in E_2-treated patients (27.8 ± 1.2 days) as compared with controls (27.9 ± 0.8 days). Participants did not indicate any significant change in their menstrual bleeding characteristics in E_2-treated as compared with baseline cycles.

DISCUSSION

The present study was designed to investigate whether E_2 administration during the luteal phase could affect the degree of development of early antral follicles during the first days of the subsequent follicular phase. We wanted to challenge the hypothesis that developmental asynchrony of early antral follicles possibly results from the gradual FSH elevation that occurs during the late luteal phase. This phenomenon may promote asynchronous growth of follicles because of their intrinsic dissimilar sensitivity to FSH (1, 2, 16). Hence, through its putative suppressive effect on FSH secretion (10, 11, 17), E_2 administration might attenuate follicular size heterogeneity. To address this issue, our investigation set stringent methodological parameters such as population selection, randomized crossover design, accurate ultrasound technology, and independent data analysis.

Our results indicated that E_2 administration during the luteal phase effectively reduced both the size discrepancies and the mean diameter of early antral follicles, consistent with a decrease in mean ovarian volume. The observation of a subtle but significant lengthening of menstrual cycle duration in women pretreated with E_2 is in keeping with this effect. Indeed, although ovulation has not been monitored in the present study, menstrual cycle lengthening after E_2 treatment may be attributed to a longer growth course of smaller antral follicles to ovulation. Furthermore, these results are in accordance with data from previous experiments conducted in rhesus monkeys (18).

Earlier studies have shown that administration of physiological E_2 doses effectively prevents and postpones the intercycle FSH elevation (11, 17). In line with this, the present investigation showed lower FSH levels (<4.5 mIU/mL) on the day after E_2 discontinuation as compared with baseline (>7 mIU/mL). This phenomenon may not be attributable to physiological intercycle FSH fluctuations because it was not duplicated in the control group. In addition, the intercycle CV for FSH observed in E_2-treated patients (40%) significantly exceeded the intercycle CVs in controls (17%) and the CVs for FSH measured in the early follicular phase as reported by other investigators (<18%) (20).

Therefore, both the reduction of follicular size and heterogeneity observed by the present study lead us to hypothesize that, after E_2 administration, serum FSH levels might have remained beneath the putative FSH threshold for early antral follicular development (2, 19). Indeed, during the late luteal phase of the menstrual cycle, spontaneous demise of the corpus luteum leads to a progressive rise in FSH that starts approximately 5 days before menses and reaches serum levels >7 mIU/mL as early as 3 days before the onset of menstrual bleeding (6). It is possible that the obtained

reduction of FSH secretion might have altered the pace of early follicular development during the intercycle transition.

It is noteworthy that E_2 administration also led to a marked decrease in serum inhibin B levels as compared with baseline. Although the mechanisms implicated in this phenomenon are unclear, it is conceivable that the E_2-induced reduction of mean follicular size and the decreased FSH secretion acted together to lower serum inhibin B to levels similar to those described during the luteal phase of the menstrual cycle (14).

The coordination of early antral follicles achieved by luteal E_2 administration may provide a basis for new therapeutic perspectives. During COH, the variability in size of growing follicles may be counterproductive. In IVF-ET, because of the discordant maturation of follicular-oocyte complexes, the precise timing for hCG administration is hard to determine for all follicles at once. This leads to fewer mature oocytes and fewer available embryos, a factor that limits embryo selection for ET. Indeed, the large number of available embryos constitutes an important prognostic factor of IVF-ET outcome, possibly by increasing the probability that at least one good-quality embryo will be selected for ET (21, 22).

In addition, our present data provide a possible explanation for the larger number of follicles, oocytes, and embryos reported with COH protocols preceded by GnRH agonist or oral contraceptive administration as compared with COH protocols without luteal FSH control (23–26). These differences are possibly due to improved synchronization of follicular development achieved after luteal FSH suppression as a result of GnRH agonist or oral contraceptive pretreatment. Yet studies comparing the characteristics of early antral follicles after pituitary desensitization by GnRH agonists or oral contraceptive administration with those observed after luteal E_2 administration are needed to verify this assumption. Similarly, the faster follicular growth, the reduced number of oocytes and available embryos, and the remarkable trend to poorer IVF-ET outcome observed in GnRH antagonist, as compared with long GnRH agonist protocols (27), are consistent with this hypothesis. Hence, the possible improvement of GnRH antagonist protocol outcome by E_2 pretreatment should be addressed.

In conclusion, luteal E_2 administration reduces size discrepancies of early antral follicles during the early follicular phase. This approach may constitute a simple and promising method to foster follicular growth synchronization during COH. If these results are confirmed, luteal E_2 administration represents a potential, more physiological alternative to GnRH agonist or oral contraceptive pretreatment. Yet, further studies are needed to investigate whether the coordination of selectable follicles induced by E_2 administration during the luteal phase improves the results of short GnRH agonist or antagonist protocols and IVF-ET outcome.

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