Outlook

Hormonal manipulations in the luteal phase to coordinate subsequent antral follicle growth during ovarian stimulation

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Abstract

During the early follicular phase in the menstrual cycle, antral follicle sizes are often markedly heterogeneous. These follicular size discrepancies may, at least in part, result from the early exposure of FSH-sensitive follicles to gradient FSH concentrations during the preceding luteal phase. In addition, they potentially affect the results of ovarian stimulation. Indeed, pre-existing follicle size discrepancies may encumber coordinated follicular growth during ovarian stimulation, thereby reducing the number of follicles that reach maturation at once. To investigate this issue, three clinical studies were conducted to test the hypothesis that luteal FSH suppression could coordinate follicular growth. First, luteal FSH concentrations were artificially lowered by administering physiological oestradiol doses and measured follicular characteristics on the subsequent day 3. Second, it was verified whether luteal oestradiol administration could promote the coordination of follicular growth during ovarian stimulation and improve its results. Third, the effects of premenstrual gonadotrophin-releasing hormone (GnRH) antagonist administration on follicular characteristics were assessed during the early follicular phase. The results showed that luteal FSH suppression by either oestradiol or GnRH antagonist administration reduces the size and improves the homogeneity of early antral follicles during the early follicular phase, an effect that persists during ovarian stimulation. Coordination of follicular development may optimize ovarian response to short GnRH agonist and antagonist protocols, and constitutes an attractive approach to improving their outcome.

Keywords: early antral follicles, follicular synchronization, FSH, GnRH antagonist, oestradiol, ovarian stimulation

Introduction

During the first days of the follicular phase in the menstrual cycle, early antral follicles are differentially sensitive to FSH (McNatty *et al*., 1983; Fauser and Van Heusden, 1997) and exhibit dissimilar sizes, which range from 2 to 8 mm in diameter (Gougeon *et al*., 1983). Under physiological conditions, both of these interrelated phenomena are possibly implicated in the establishment of follicular dominance. Although it is conceivable that complex intrafollicular mechanisms combine to determine follicular sensitivity to FSH (Gougeon, 1996; De Felici *et al*., 2005), compelling

evidence indicates that larger follicles are more responsive to this hormone than are smaller follicles (Hillier *et al*., 1980; McNatty *et al*., 1983; Fauser and Van Heusden, 1997). The mechanisms underlying the heterogeneity of antral follicle sizes during the early follicular phase remain unclear. A possible explanation for this phenomenon involves the exposure of early antral follicles to gradient FSH concentrations during the late luteal phase. During the last days of the menstrual cycle, paralleling demise of the corpus luteum, FSH concentrations increase progressively (Mais *et al*., 1987; Roseff *et al*., 1989) to preserve antral follicles from atresia and ensure their subsequent growth (Chun *et al*., 1996;

Scott *et al*., 2004). 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 (Klein *et al*., 1996). The 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.

This physiological context is by no means without consequences on controlled ovarian stimulation outcome. In ovarian stimulation, most early antral follicles are required to grow coordinately in response to exogenous gonadotrophins to accomplish simultaneously functional and morphological maturation. Marked follicular size discrepancies at the end of ovarian stimulation may be counterproductive, since they imply that a substantial fraction of FSH-sensitive follicles fail to undergo satisfactory maturation, which reduces the number of viable oocytes and embryos and the probability of conception (Devreker *et al*., 1999). Hence, to optimize the results of controlled ovarian stimulation, it is plausible that the physiological size heterogeneity of early antral follicles should be primarily overcome.

Indeed, during the last 15 years, a number of attempts have been made to abridge the duration and complexity of ovarian stimulation and to improve its patient-friendliness as compared with the reference long gonadotrophin-releasing hormone (GnRH) agonist protocols for IVF–embryo transfer. Among the most relevant of these ranks the development of short GnRH agonist (Hazout *et al*., 1983; Macnamee *et al*., 1989) and GnRH antagonist (Diedrich *et al*., 1994; Olivennes *et al*., 1994) regimens. Some clinical trials have subsequently raised doubts concerning the effectiveness of these simplified approaches as compared with long GnRH agonist protocols (Tan *et al*., 1992; Cramer *et al*., 1999; Borm *et al*., 2000; Al-Inany *et al*., 2001). Nevertheless, the explanation for the poorer IVF–embryo transfer outcome achieved with these alternative protocols still remain unclear. In an effort to clarify this issue, it was noticed that both approaches to ovarian stimulation share common features that singularize them as compared with long GnRH agonist protocols. These include a shorter course of multiple follicles to maturation and smaller number of oocytes and embryos obtained (Tan *et al*., 1992; Cramer *et al*., 1999; Borm *et al*., 2000; Al-Inany *et al*., 2001). Based on this clinical background, it was postulated that the differential efficacy between long and short GnRH agonist and antagonist protocols could be, at least in part, related to pre-existing differences in the characteristics of early antral follicles before the start of gonadotrophin administration (Fanchin *et al*., 2003a,b).

To challenge the hypothesis that such size heterogeneities among antral follicles result from their early and progressive exposure to FSH during the luteal phase, it was decided to artificially lower endogenous luteal FSH secretion by administering either oestradiol (Fanchin *et al*., 2003a,b) or GnRH antagonist (Fanchin *et al*., 2004) and to measure follicular and hormonal profiles during the subsequent follicular phase. These hormonal pretreatments could be helpful in coordinating antral follicle growth and optimizing controlled ovarian stimulation for IVF–embryo transfer. The present article summarizes recent experience with both

approaches.

Luteal oestradiol administration

In a first study (Fanchin *et al*., 2003a), 66 female volunteers, 20–41 years of age, were prospectively investigated. All of them had regular, ovulatory menstrual cycles every 25–35 days, both ovaries present, no current or past diseases affecting ovaries or gonadotrophin or sex steroid secretion, clearance or excretion, body mass indexes (BMI) ranging from 18 to 27 kg/m^2, no current hormone therapy, and adequate visualization of ovaries in transvaginal ultrasound scans. Due to 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 were excluded from the analysis. Therefore, the population studied was limited to 60 participants undergoing 120 study cycles.

On day 3 of their menstrual cycles (baseline/day 3), all women underwent blood sampling for serum FSH, inhibin B, and oestradiol measurements and ultrasound scans of their ovaries. Subsequently, women were randomized to receive luteal oestradiol treatment or to serve as controls. Participants who were included in the oestradiol-treated group (*n* = 30) received micronized 17β-oestradiol oral tablets (4 mg/day; Provamès, Cassenne Laboratories, Puteaux, France), in the evening at 8 p.m., from day 20 of the same cycle until day 2 of their next cycle. Participants who were included in the control group (*n* = 30) remained untreated. On day 1 of oestradiol discontinuation in oestradiol-treated women (oestradiol/day 3) or on day 3 of the subsequent cycle in control women (control/day 3), similar hormonal and ultrasonographic measurements 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 oestradiol-treated women, compliance of treatment was monitored to detect any protocol violation.

Ultrasound scans were performed using a 4.5–7.2 MHz multi-frequency transvaginal probe (Siemens Elegra; Siemens S.A.S., Saint-Denis, France) by one single operator, who was not aware of the treatment schedule or the hormonal results. The objective of ultrasound examinations was to evaluate the number and sizes of early antral follicles and to calculate mean ovarian volume. All follicles that measured 2–12 mm in mean diameter (mean of two orthogonal diameters) were considered. In an attempt to optimize the reliability of ovarian follicular assessment, the ultrasound scanner used was equipped with a tissue harmonic imaging system (Thomas and Rubin, 1998), 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) (Sharara and McClamrock, 1999), were the mean volume for both ovaries. Intra-analysis coefficients of variation (CV) for follicular and ovarian measurements were <5% and their lower limit detection 0.1 mm respectively.

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

CV were respectively 5 and 7% and lower limit of detection was 0.1 mIU/ml for FSH. Serum inhibin B was determined by double antibody enzyme-linked immunosorbent assay (Serotec, Varilhes, France) as previously described (Groome *et al*., 1996). The lower limit for detection was 10 pg/ml, and intra-assay and interassay CV were <6 and <9% for inhibin B respectively. Serum oestradiol was determined by an immunometric technique using an oestradiol-60 Amerlite kit (Ortho Clinical Diagnostics, Strasbourg, France). The lower limit for detection was 14 pg/ml, and intra-assay and interassay CV were 8 and 9% for oestradiol respectively.

Measure of central tendency used was the mean and measure of variability was standard deviation (SD). Due to the pairwise design of this study, data from each participant on oestradiol/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 oestradiol/day 3 and from baseline/day 3 to control/day 3, the homogeneity of variances was tested using the Levene test for equal variances (Levene, 1960). This test is less sensitive than *F*-tests to departures from normality and allows the comparison of dispersion of data around the mean independently 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 0.05 significance concentration. A *P*-value <0.05 was considered statistically significant.

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 oestradiol-treated women and in controls. In contrast, a significant reduction of mean follicular sizes was observed in oestradiol-treated women, from baseline/day 3 to oestradiol/day 3 (*P* < 0.001), but not in controls. In agreement with this, mean ovarian volume decreased significantly in women treated with oestradiol and remained unchanged in controls (*P* < 0.02). In addition, a remarkable attenuation of follicular size discrepancies was observed on oestradiol/day 3 as compared with baseline/day 3 (*P* < 0.001). This phenomenon was not seen in controls, between baseline/day 3 and control/day 3. Consistently, SD/mean ratios for follicular sizes were significantly lower (*P* < 0.001) on oestradiol/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 oestradiol-treated women. Incidentally, it is noteworthy that ultrasonographic measurements made on baseline/day 3 were strictly similar in women included in the oestradiol-treated and control groups.

Hormonal results are also presented in **Table 1**. In women who were administered oestradiol during the luteal phase, serum inhibin B concentrations were significantly lower on oestradiol/day 3 as compared with baseline/day 3, whereas no significant longitudinal change in inhibin B concentrations was noted in controls. As expected, oestradiol administration raised serum oestradiol to concentrations comparable to those observed during the late follicular phase of the menstrual cycle (114 ± 57 pg/ml on oestradiol/day 3). Serum oestradiol concentrations did not vary significantly in controls, from baseline/day 3 to control/day 3. Administration of oestradiol lowered serum FSH concentrations on oestradiol/day 3 as compared with baseline/day 3. However, in women who did not receive oestradiol treatment, serum FSH concentrations remained steady from one cycle to the other. Coefficients of variation for FSH from the first to the second cycle were significantly higher in oestradiol-treated women than in controls (40 versus 17%, *P* < 0.01). As for ultrasonographic measurements, hormonal results obtained on baseline/day 3

Table 1. Ultrasonographic and hormonal results during two consecutive menstrual cycles in women receiving or not receiving oestradiol during the luteal phase.

| | Oestradiol-treated group | | | Control group | | |
	Baseline day 3	Oestradiol day 3	P-value	Baseline day 3	Control day 3	P-value
No. 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	<0.001	4.9 ± 0.8	5.0 ± 0.8	NS
SD/mean of follicular sizes	0.40	0.23	<0.001	0.40	0.39	NS
Mean ovarian volume (cm^3)	6.1 ± 3.0	5.1 ± 2.6	<0.02	6.1 ± 2.3	6.2 ± 2.1	NS
Serum inhibin B (pg/ml)	71 ± 32	34 ± 28	<0.001	77 ± 23	75 ± 21	NS
Serum oestradiol (pg/ml)	47 ± 29	114 ± 57	<0.001	43 ± 29	38 ± 22	NS
Serum FSH (mIU/ml)	7.3 ± 3.3	4.3 ± 1.9	<0.001	6.9 ± 2.6	7.5 ± 2.7	NS

NS: not significant; SD: standard deviation.

were closely similar in women receiving oestradiol as compared with those who served as controls.

As anticipated, the oestradiol-treated and the control groups were comparable in regard to ages of women (33.3 ± 0.6 versus 33.3 ± 0.5 years) and BMI (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 oestradiol (29.4 ± 1.3 days, $P < 000.1$) as compared with their baseline cycles (27.8 ± 1.2 days). This phenomenon was not observed in controls (28.0 ± 1.1 versus 27.9 ± 0.8 days respectively). Moreover, oestradiol treatment did not alter baseline cycle length in oestradiol-treated (27.8 ± 1.2 days) as compared with controls (27.9 ± 0.8 days). Participants did not refer any significant change on their menstrual bleeding characteristics in oestradiol-treated as compared with baseline cycles.

The second study on the effect of luteal of oestradiol administration on antral follicle growth (Fanchin *et al*., 2003b) prospectively analysed 100 female volunteers, 25–38 years of age, who met similar inclusion criteria as the preceding study (Fanchin *et al*., 2003a). Indications for IVF–embryo transfer were male factor (58%), tubal factor (27%), endometriosis (2%), or unexplained infertility (13%). Intracytoplasmic sperm injection was programmed in 46% of cases. Due to personal reasons ($n = 4$) or major protocol violation ($n = 6$), 10 women were excluded from the analysis. The population analysed was therefore limited to 90 participants.

Women randomly received luteal oestradiol treatment or served as controls. Those included in the luteal oestradiol group ($n = 47$) received micronized 17β-oestradiol oral tablets (4 mg/day; Provamès, Cassenne Laboratories, Puteaux, France), in the evening at 8 p.m., from day 20 of the same cycle until day 2 of their next cycle. Patients included in the control group ($n = 43$) remained untreated during the luteal phase.

On the first day of oestradiol discontinuation (cycle day 3) in oestradiol-treated group or on cycle day 3 in controls, r-FSH treatment (Gonal-F; Serono Laboratories, Saint Cloud, France) was started at a fixed dose set at 225 IU/day, SC, for 5 days. Further r-FSH administration was adjusted according to usual parameters of follicle growth determined by serum oestradiol concentrations and ultrasound monitoring. When at least one follicle exceeded 13 mm in diameter (de Jong *et al*., 2000), a potent, third-generation GnRH antagonist, cetrorelix acetate (Cetrotide, 3 mg; Serono Laboratories), was administered subcutaneously in a single dose at 8 p.m. An intramuscular injection of 10,000 IU of human chorionic gonadotrophin (HCG, Gonadotrophine Chorionique 'Endo'; Organon Laboratories, Saint-Denis, France) was performed when at least five follicles ≥16 mm in diameter were obtained. Follicular sizes (mean of two orthogonal diameters) exceeding 20 mm in diameter were avoided as far as possible. Cycle cancellation for inadequate ovarian response to ovarian stimulation was decided when <5 follicles ≥12 mm in diameter were observed after 10 days of r-FSH treatment. Oocytes were retrieved 36 h after HCG administration by transvaginal ultrasound-guided aspiration. Adequate embryo quality was defined as embryos having uniform sized and shaped blastomeres, ooplasm having no granularity and a maximum fragmentation of 10%. All embryo transfers were performed 2

days after oocyte retrieval using a Frydman catheter (CCD Laboratories, Paris, France). Luteal phase was supported with 400 mg of micronized progesterone (Estima Gé; Effik Laboratories, Bièvres, France) administered daily (200 mg in the morning, 200 mg in the evening) by vaginal route starting on the day of embryo transfer.

Ultrasound scans were performed using a 4.5–7.2 MHz multi-frequency transvaginal probe (Siemens Elegra; Siemens S.A.S., Saint-Denis, France) in the morning at approximately 8 a.m. by operators who were not aware of the treatment schedule. In addition to usual ultrasonographic monitoring of ovarian stimulation, by design, all women underwent a detailed ultrasound scan of their ovaries on day 8 of r-FSH treatment. During this examination, the number and sizes (mean of two orthogonal diameters) of antral follicles were evaluated. Among them, follicles ≥8 mm in diameter were considered as growing. Inter- and intra-analysis CV for follicular measurements were <5% and their lower limit detection 0.1 mm respectively.

Serum hormonal (oestradiol, progesterone, and LH) measurements that were performed on baseline (just before the start of r-FSH administration), on days 6 and 8 of r-FSH therapy, and on the day of HCG were included in the present analysis. Statistical methodology was conducted as in the previous study (Fanchin *et al*., 2003a).

The population included in the oestradiol-treated and control groups was similar with regard to women's ages (median, 33 years, range 26–38 versus 33 years, range 25–38 respectively), indications for IVF–embryo transfer (male factor, 62 versus 54%; tubal factor, 21 versus 32%; endometriosis, 2 versus 2%; unexplained infertility, 15 versus 12% respectively), duration of infertility (4.3 ± 0.2 versus 4.1 ± 0.2 years respectively), rank of the current IVF–embryo transfer attempts (2.6 ± 0.3 versus 2.1 ± 0.2 respectively), average menstrual cycle length (29.5 ± 0.4 versus 29.4 ± 0.4 days respectively), and ovarian status assessment by day 3 serum FSH (6.1 ± 0.2 versus 6.2 ± 0.2 mIU/ml respectively) and oestradiol (31.6 ± 2.4 versus 29.1 ± 2.6 pg/ml respectively) measurements performed within 3 months before inclusion in the protocol. Luteal oestradiol treatment lasted 11.3 ± 0.6 days. This treatment was well tolerated by patients, who did not experience any unwanted side effects. Luteal oestradiol administration did not alter the expected onset of menstrual bleeding.

Follicular development characteristics and embryologic data in both groups are presented in **Table 2**. As expected, the number of growing follicles was similar in the two groups on day 8. In contrast, a significant reduction was observed in the mean follicular sizes on day 8 of r-FSH treatment in the luteal oestradiol as compared with the control group ($P < 0.001$). In addition, calculation of homogeneity of variances indicated a noticeable attenuation of follicular size discrepancies in the luteal oestradiol as compared with the control group on the same observation day ($P < 0.01$). In line with this, CV of follicular sizes on day 8 were slightly, yet significantly, lower in the luteal oestradiol than in the control group ($P < 0.02$), which further confirms the attenuation in follicular size disparity after oestradiol pretreatment. No woman in both groups received GnRH antagonist before day

Table 2. Follicular development during ovarian stimulation and embryological results in women pretreated or not pretreated with oestradiol during the luteal phase.

	Luteal oestradiol group	Control group	P-value
No. follicles >10 mm on day 8	16.4 ± 1.0	16.8 ± 0.9	NS
Mean follicular size on day 8 (mm)	9.9 ± 0.2	11.1 ± 0.3	<0.001
CV of follicular sizes on day 8	0.22	0.26	<0.02
Day of GnRH antagonist administration	9.1 ± 0.2	8.5 ± 0.2	<0.01
Day of HCG administration	11.9 ± 0.2	10.8 ± 0.2	<0.001
No. follicles ≥16 mm on day of HCG	9.9 ± 0.5	7.9 ± 0.5	<0.01
No. mature oocytes	9.3 ± 0.7	7.3 ± 0.5	<0.03
No. available embryos	6.4 ± 0.6	4.6 ± 0.3	<0.01
No. embryos transferred	2.6 ± 0.1	2.7 ± 0.1	NS
Clinical pregnancy rates/cycle (%)	34	25	NS

NS: not significant; CV: coefficient of variation.

8. However, both cetrorelix acetate and HCG were administered later in the luteal oestradiol group as compared with the control group. Luteal oestradiol patients tended to require a higher r-FSH dose than controls, but not significantly (2674 ± 91 versus 2463 ± 100 IU respectively). Six ovarian stimulation cycles (three in each group) had to be cancelled due to unexpected, inadequate response to ovarian stimulation. On the day of HCG administration, more follicles had exceeded 15 mm in diameter in the oestradiol-treated as compared with the control group. Consistently, luteal oestradiol pretreatment was associated with more mature oocytes and available embryos than in the control group. Intracytoplasmic sperm injection was performed in 43 and 50% of cases included in luteal oestradiol and control groups respectively. Whereas the number of embryos transferred was similar in both groups, a higher prevalence of good quality embryos transferred was observed in women pretreated with oestradiol as compared with controls (63 versus 44% respectively, $P < 0.03$), which probably reflects the improved embryo selection from a larger embryo cohort. Although the present investigation was not powered to detect differences in IVF–embryo transfer outcome, a trend for increased pregnancy rates in luteal oestradiol group was incidentally observed.

As an expected result of luteal oestradiol administration, serum oestradiol concentrations remained higher in the oestradiol-treated as compared with the control group (139 ± 54 versus 34 ± 8 pg/ml) at baseline. Conversely, on day 6 and on day 8, serum oestradiol reached slightly, but significantly ($P < 0.05$), lower concentrations in patients pretreated with luteal oestradiol, probably reflecting a slower pace of follicle development. On the day of HCG, however, serum oestradiol concentrations in the luteal oestradiol group tended to exceed control values, which is in keeping with the larger number of mature follicles obtained in that group. Serum progesterone and LH concentrations remained similar in both groups throughout r-FSH treatment but, as for oestradiol,

progesterone showed a trend to higher concentrations in the luteal oestradiol group as compared with the control group.

Premenstrual GnRH antagonist administration

In the third study (Fanchin et al., 2004), another approach was tested that aimed at sparing early antral follicles from the putative uncoordinating effects of luteal FSH. It involved administrating a potent GnRH antagonist, cetrorelix acetate, during the late luteal phase. At a 3-mg dose, this molecule has been shown to bring down endogenous FSH secretion for at least 2 days (Erb et al., 2001), which opportunely corresponds to the timing during which luteal FSH reaches significant serum concentrations (Mais et al., 1987; Roseff et al., 1989). For this, follicular and hormonal profiles were assessed on day 2 in two consecutive menstrual cycles pretreated or not by GnRH antagonist during the late luteal phase.

Thirty female volunteers, 20–41 years of age, were prospectively studied. All participants had similar inclusion criteria to the preceding studies. An informed consent was obtained from all women and this investigation received the approval of the internal Institutional Review Board. Due to major protocol violation ($n = 4$) or personal reasons ($n = 1$), five women did not go through the two subsequent observation cycles required by the protocol and had to be excluded from the analysis. Therefore, the population studied was limited to 25 participants undergoing 50 study cycles.

On day 2 of their menstrual cycles (control/day 2), women underwent blood sampling for serum FSH, inhibin B, and oestradiol measurements at approximately 9 a.m. Later in the morning, ultrasound scans of their ovaries were performed. On day 25, participants received a single injection of GnRH antagonist (cetrorelix acetate, 3 mg; Cetrotide, Serono Laboratories, Boulogne, France), in the evening at approximately 8 p.m. On day 2 in the subsequent cycle (premenstrual GnRH antagonist/day 2), similar hormonal and ultrasonographic measurements as in the preceding cycle (control/day 2) were performed. It was decided to perform

follicular hormonal measurements on day 2 of the cycle because, in controlled ovarian stimulation protocols using GnRH antagonists, exogenous gonadotrophin administration usually starts on day 2. Furthermore, participants were asked to compute their control and subsequent menstrual cycle lengths and to report possible subjective changes in menstrual bleeding characteristics.

Ultrasonographic measurements in the present study followed similar methodological characteristics to those reported elsewhere (Fanchin *et al*., 2003a,b). The objective of ultrasound examinations was to evaluate the number and sizes of early antral follicles. All follicles that measured 2–12 mm in mean diameter (mean of two orthogonal diameters) were considered. Serum FSH, inhibin B, and oestradiol concentrations were determined and data were statistically analysed according to similar methodology as described previously (Fanchin *et al*., 2003a,b).

Mean ages of the women were 33.0 ± 4.5 years and mean BMI values were 21.5 ± 3.0 kg/m^2. Baseline menstrual cycle duration was 28.1 ± 0.6 days, which corresponded to a mean time elapsed from GnRH antagonist administration until the onset of menstrual bleeding of 3.1 ± 0.6 days (range, 2–4 days). Participants did not report any remarkable change in their menstruation characteristics, or in the onset of menstrual bleeding, in GnRH-antagonist-pretreated cycles as compared with control cycles. However, a slight yet significant lengthening of GnRH-antagonist-pretreated cycles as compared with the baseline cycle (28.8 ± 1.0 versus 28.1 ± 0.6 days, $P < 0.004$) was observed.

Follicular measurement results are summarized in **Table 3**. Mean time elapsed between GnRH antagonist administration and ultrasonographic assessment of early antral follicles was 4.1 ± 0.6 days (range, 3–5 days). A significant reduction was observed in the mean follicular sizes in GnRH-antagonist-pretreated cycles as compared with baseline cycles ($P < 0.001$). In addition, both the calculation of homogeneity of variances and CV of follicular size indicated a noticeable attenuation of follicular size discrepancies on premenstrual GnRH antagonist/day 2 ($P < 0.001$). Further, no significant differences in the magnitude of follicular size modifications were identified, irrespective of the time elapsed between

GnRH antagonist and ovarian measurements. Hormonal results are also presented in **Table 3**. As expected, cetrorelix acetate significantly decreased serum FSH concentrations on premenstrual GnRH antagonist/day 2 ($P < 0.001$). In line with this, and consistent with the significant reduction in follicular sizes, serum oestradiol and inhibin B concentrations were significantly lower on premenstrual GnRH antagonist/day 2 than on control/day 2 ($P < 0.001$ and $P < 0.01$ respectively).

Discussion

The present article summarizes three studies aimed at challenging the hypothesis that developmental asynchrony of early antral follicles is possibly due to the gradual FSH elevation occurring during the late luteal phase. Progressive FSH elevation may promote asynchronous growth of follicles because of their dissimilar intrinsic sensitivity to FSH (McNatty *et al*., 1983; Fauser *et al*., 1997). The first two trials showed that luteal oestradiol administration, through its putative suppressive effect on FSH secretion (le Nestour *et al*., 1993; de Ziegler *et al*., 1998), attenuates antral follicle size heterogeneity. In the third investigation (Fanchin *et al*., 2004), it was shown that a single administration of cetrorelix acetate, 3 mg, during the last days of the luteal phase in regularly menstruating volunteers effectively reduces both the size discrepancies and the mean diameter of early antral follicles during the subsequent follicular phase. The observed lengthening of the ensuing menstrual cycle after either luteal oestradiol (Fanchin *et al*., 2003a) or premenstrual GnRH antagonist (Fanchin *et al*., 2004) administration is in keeping with this effect, and presumably results from a longer growth course of smaller antral follicles to ovulation, which corroborates data by other investigators (Skarin *et al*., 1982; Mais *et al*., 1986; Hall *et al*., 1991).

When administered before controlled ovarian stimulation, luteal oestradiol administration effectively reduces the pace of multi-follicular growth in response to r-FSH (Fanchin *et al*., 2003b). Indeed, a slower increase in serum oestradiol concentrations was observed during ovarian stimulation and reduced antral follicle sizes on day 8 in oestradiol-pretreated ovarian stimulation. In addition, follicles took longer to achieve maturation and required later GnRH antagonist and HCG administration in oestradiol-pretreated patients. These

Table 3. Ultrasonographic and hormonal data on day 2 during two consecutive menstrual cycles pretreated or not pretreated with gonadotrophin-releasing hormone (GnRH) antagonist during the late luteal phase.

	Control/day 2	Premenstrual GnRH antagonist/day 2	P-value
No. follicles (range)	9.4 ± 2.9 (3–19)	9.3 ± 2.2 (2–20)	NS
Mean follicular size (mm)	5.5 ± 1.0	4.1 ± 0.9	<0.001
CV of follicular sizes (%)	38	20	<0.001
Serum inhibin B (pg/m)	76 ± 33	52 ± 30	<0.01
Serum oestradiol (pg/ml)	46 ± 26	23 ± 13	<0.001
Serum FSH (mIU/ml)	6.7 ± 2.4	4.5 ± 1.9	<0.001

NS: not significant; CV: coefficient of variation.

effects may be due to an overall reduction in early antral follicle sizes at the start of r-FSH treatment. Luteal oestradiol pretreatment also fostered follicular growth coordination during controlled ovarian stimulation, as corroborated by the attenuation of follicle size discrepancies on day 8 and the increased number of follicles reaching maturation at once. These follicular effects significantly increased the number of viable oocytes and available embryos ($P < 0.03$ and $P < 0.01$ respectively). Indeed, the number of embryos produced has been shown to influence positively IVF–embryo transfer outcome (Devreker et al., 1999), possibly through the optimization of embryo selection for embryo transfer. The observation that oestradiol-pretreated patients had more top-quality embryos transferred and showed a trend for improved pregnancy rates is consistent with this hypothesis.

The present data also provide alternative insights into the reported improvement of ovarian stimulation outcome with combined oral contraceptive pretreatment (Gonen et al., 1990; Schoolcraft et al., 1997). Indeed, due to their potent anti-FSH action, oral contraceptives may exert similar, or even stronger, coordinating effects on early follicular development as compared with luteal oestradiol or premenstrual GnRH antagonist administration. In line with this, Gonen et al. (1990) reported an increase in the number of mature follicles and oocytes in clomiphene citrate cycles preceded by oral contraceptives as compared with controls. Nevertheless, combined oral contraceptives have putative shortcomings, such as lengthy treatment course, menstrual bleeding postponement, and adverse effects that are not shared by the 10-day administration of physiological 17β-oestradiol doses used in the first two studies described in this paper (Fanchin et al., 2003a,b). Comparative studies between the effects of oral contraceptive and luteal oestradiol or GnRH antagonist pretreatments on follicular cohort characteristics and ovarian stimulation outcome are needed to clarify this issue.

The reduction in the pace of follicular development observed after premenstrual GnRH antagonist administration is likely to result from its FSH-suppressive effect. Indeed, cetrorelix acetate has been shown to induce a rapid, transient, and dose-dependent decrease in endogenous gonadotrophins that is faster and more pronounced for LH than FSH (Erb et al., 2001). Both the shorter half-life of LH (Erb et al., 2001) and the possible GnRH-independent regulation of FSH secretion (Hall et al., 1990, 1991) may combine to explain these differential effects on gonadotrophins. The deceleration in early follicular growth induced by cetrorelix acetate is in agreement with the reported arrest of antral follicle growth resulting from the action of another GnRH antagonist, Nal-Glu, administered during the mid-follicular phase in the menstrual cycle (Kettel et al., 1991). This suggests that a noticeable impairment of follicular development may occur even in presence of incomplete endogenous FSH suppression. Consistently, in the study by Kettel et al. (1991), 3-day Nal-Glu antagonist administration reduced mean immunoreactive FSH concentrations by approximately 45%, whereas 3-mg cetrorelix acetate administration reportedly reduces FSH concentrations by 42% (Fanchin et al., 2004). Therefore, the significant modification in early antral follicle development observed after luteal cetrorelix acetate administration leads to the inference that such treatment keeps transitorily serum FSH concentrations beneath the FSH threshold for early follicular

development.

Another pertinent yet controversial (Roseff et al., 1989; Hall et al., 1991) issue is the possible luteolytic action of GnRH antagonists. Corpus luteum sensitivity to LH suppression presumably shows interindividual variations (Roseff et al., 1989), and its degree of LH dependence has been the matter of debate (Roseff et al., 1989; Hall et al., 1991). As cetrorelix acetate was administered during the premenstrual phase in the present study, the occurrence of spontaneous or induced luteolysis remained indiscernible to the participants, who did not present any clinical evidence of menstrual advancement. Yet, from a practical standpoint, any acceleration in the onset of menses would not hamper the primary clinical application of the present approach, since exogenous gonadotrophin treatment for controlled ovarian stimulation can be started as early as complete corpus luteum demise occurs.

In conclusion, the possibility of reducing size differences among FSH-sensitive follicles during the early follicular phase may foster follicular growth coordination in response to exogenous gonadotrophin administration. This issue is particularly key with regard to ovarian stimulation protocols deprived of luteal FSH control, such as short GnRH agonist (Macnamee et al., 1989; Hazout et al., 1993) and GnRH antagonist (Diedrich et al., 1994; Olivennes et al., 1994) regimens. These approaches represent potential alternatives to GnRH agonist or oral contraceptive pretreatment to synchronize multi-follicular development and improve ovarian stimulation results. However, larger studies are needed to confirm whether follicular growth coordination induced by luteal oestradiol (Fanchin et al., 2003a,b) and premenstrual GnRH antagonist (Fanchin et al., 2004) administration improves IVF–embryo transfer pregnancy rates with GnRH antagonist or short GnRH agonist protocols.

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