Initiation of Gonadotropin-Releasing Hormone Antagonist on Day 1 as Compared to Day 6 of Stimulation: Effect on Hormonal Levels and Follicular Development in *in Vitro* Fertilization Cycles

EFSTRATIOS M. KOLIBIANAKIS, CAROLA ALBANO, MICHEL CAMUS, HERMAN TOURNAYE, ANDRE C. VAN STEIRTEGHEM, AND PAUL DEVROEY

Centre for Reproductive Medicine, Dutch-Speaking Brussels Free University, 1090 Brussels, Belgium

The objective of the present study was to assess the effect of altering the timing of GnRH antagonist initiation on the hormonal environment and follicular development in *in vitro* fertilization cycles. Sixty women undergoing *in vitro* fertilization participated in a prospective randomized controlled trial. Patients were stimulated with a fixed dose of 200 IU recombinant FSH, starting on d 2 of the cycle, and with GnRH-antagonist, starting either on d 1 (n = 30) or on d 6 of stimulation (n = 30). A significantly lower exposure to LH ($P < 0.001$) and estradiol ($P < 0.001$) during the follicular phase was observed in the d-1 group, compared with the d-6 group of an-

tagonist administration. No differences in follicular development were seen between the two groups on either d 6 of stimulation or on the day of human chorionic gonadotropin administration. Similar fertilization rates, implantation rates, and ongoing pregnancy rates per transfer were, in addition, present between the two groups compared. In conclusion, administration of GnRH antagonist on d 1 (compared with d 6) of stimulation is associated with a lower exposure to LH and estradiol, which does not seem to affect follicular development. (*J Clin Endocrinol Metab* 88: 5632–5637, 2003)

G ONADOTROPIN-RELEASING HORMONE antagonists have been introduced in *in vitro* fertilization (IVF) to prevent premature LH surge. GnRH antagonists are administered either as a daily dose of 0.25 mg (1) or as a single 3-mg dose, supported by additional daily doses of antagonist if necessary (2). Initiation of GnRH antagonist is carried out either as a fixed scheme on d 6 of stimulation (3–7) or as a flexible scheme when follicular growth is present in ultrasound (US), after at least 5 d of stimulation (8).

The decision to start GnRH antagonist after at least 5 d of stimulation with gonadotropins is based on the reduced possibility of observing a premature LH rise in the early follicular phase, and the quick suppression of endogenous gonadotropins after initiation of the antagonist (9). In phase-3 comparative studies between GnRH antagonists and GnRH agonists (10), a premature LH rise has indeed been observed in a low proportion of patients in the antagonist group, not significantly different from that present in the agonist group.

An earlier administration of GnRH antagonist could probably eliminate the problem of premature LH rise. However, it might be important for additional reasons. Recent data suggest that histology of endometrium at oocyte retrieval is positively related to LH level at initiation of stimulation and to the duration of FSH stimulation before antagonist administration (11). Moreover, exposure to LH and estradiol (E2) levels before antagonist initiation is negatively associated with the chance of achieving an ongoing pregnancy after

embryo transfer (12). Administration of GnRH antagonist on d 1 of stimulation has been shown to be effective for polycystic ovary syndrome patients undergoing ovulation induction for intrauterine insemination (13). Currently, however, no information is available describing the way the follicular phase of IVF cycles is modified by earlier administration of GnRH antagonist.

The purpose of this randomized controlled trial was to compare the hormonal environment and follicular development of IVF cycles in which GnRH antagonist is started either on d 1 or on d 6 of stimulation.

Subjects and Methods

Patient population

Sixty women undergoing IVF treatment at the Centre for Reproductive Medicine of the Dutch-Speaking Free University of Brussels, from May 2002 to January 2003, were included in the study. Patients could participate in the study only once.

Inclusion criteria were: age less than 39 yr, no more than three previous ART attempts, body-mass index between 18–29 kg/m^2, regular menstrual cycles, no polycystic ovaries, no endometriosis or previous poor response to ovarian stimulation, and basal hormonal levels at initiation of stimulation (FSH < 10 IU/liter, LH < 10 IU/liter, E2 < 80 pg/ml, and progesterone (P) < 1.6 ng/ml).

Patients were randomized by a computer-generated list at initiation of stimulation, to receive GnRH antagonist starting either from d 1 (n = 30) or from d 6 (n = 30) of stimulation. The research project was approved by our Institutional Review Board, and an informed consent was obtained from all patients.

Ovarian stimulation

Recombinant FSH (rec-FSH) (Puregon, NV Organon, Oss, The Netherlands) and GnRH antagonist Ganirelix (Orgalutran; NV Organon) were used for ovarian stimulation. Started on d 2 of the menstrual cycle

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Abbreviations: AUC, Area under the curve; COC, cumulus-oocyte complex; E2, estradiol; hCG, human chorionic gonadotropin; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; P, progesterone; rec-FSH, recombinant FSH; US, ultrasound.

at 200 IU per day, the rec-FSH dose remained the same in all patients during stimulation. Ovulation triggering was performed using 10,000 IU of human chorionic gonadotropin (hCG) (Pregnyl, NV Organon) as soon as at least three follicles of at least 17 mm were present on US scan. Conventional IVF was performed in 26 couples, and intracytoplasmic sperm injection (ICSI) in 34 couples.

Hormonal measurements and US assessment of follicular development

Hormonal assessment was performed at initiation of stimulation, on d 3, 6, 8, and 10 of rec-FSH stimulation and on the day of hCG administration. Serum LH, FSH, E2, and P levels were measured by means of the automated Elecsys immunoanalyzer (Roche Diagnostics, Mannheim, Germany). Intraassay and interassay coefficients of variation were less than 3% and less than 4% for LH, less than 3% and less than 6% for FSH, less than 5% and less than 10% for E2, and less than 3% and less than 5% for P, respectively.

US was performed concomitantly with hormonal assessment at each visit, or more frequently to ensure that all patients received hCG for ovulation triggering as soon as they satisfied the criteria for follicular development mentioned above. In addition, US was performed in all patients before initiation of stimulation. No follicles more than 10 mm were present on d 1 of stimulation in the patients analyzed.

Statistical analysis

It was calculated that 26 patients in each group would be required to achieve 81% power to detect a difference of 300 pg/ml in E2 levels between the two groups of antagonist administration on d 6 of stimulation, assuming an E2 level of 600 pg/ml in the d-6 group and that the actual distribution of E2 is uniform, using a two-sided Mann-Whitney U test with a significance level (α) of 0.05.

Normally distributed metric variables were analyzed by the independent sample t test while not normally distributed by the Mann-Whitney U test. Nominal variables were analyzed in the form of a frequency table by the use of the χ^2 test or Fisher exact test. The exposure of the genital tract to LH, E2, and P and FSH was assessed by the area under the curve (AUC), which was calculated by using the trapezoidal rule (NCSS statistical software, Kaysville, UT). All tests were two-tailed, with a confidence level of 95% ($P < 0.05$). Values are expressed as mean ± sem, unless stated otherwise.

Results

No differences were observed between the d-1 and d-6 groups, with regard to the age of the patients analyzed (31.9 ± 0.7 yr *vs.* 32.0 ± 0.6 yr, respectively), the number of previous IVF/ICSI trials performed (0.5 ± 0.2 *vs.* 0.7 ± 0.2, respectively), and serum FSH levels at initiation of stimulation (Table 1).

Similar proportions ($P = 0.9$) of couples in the d-1 and d-6 groups presented with andrological infertility (63.3% *vs.* 66.7%, respectively), tubal infertility (20% *vs.* 16.7%, respectively), and idiopathic infertility (16.7% *vs.* 16.6%, respectively).

A similar duration of stimulation was required in the d-1 and d-6 groups to reach the criteria for hCG administration (8.7 ± 0.3 d *vs.* 9.0 ± 0.3 d, respectively; $P = 0.38$), and no significant difference was present in the number of rec-FSH units used (1740 ± 65 *vs.* 1800 ± 76, respectively; $P = 0.38$).

Hormonal levels on d 1, 3, 6, and 8 of stimulation for patients with at least 8 d of stimulation, as well as hormonal levels on the day of hCG, are presented in Table 1. Significantly lower levels of LH and E2 were present in the d-1 group, compared with the d-6 group, of antagonist administration on both d 3 and 6 of stimulation and on the day of hCG administration for E2 (Table 1 and Fig. 1).

A significantly lower exposure to LH and E2 during the

TABLE 1. LH, E2, P, and FSH levels during the follicular phase of IVF cycles stimulated with GnRH antagonists and rec-FSH

	Median (interquartile range)		*P*
	d 1	d 6	
LH IU/liter			
d 1	4.4 (2.5)	4.5 (2.0)	0.8
d 3	1.0 (0.6)	2.4 (2.2)	0.001
d 6	0.5 (0.6)	1.9 (2.0)	0.001
d 8	0.8 (1.2)	1.1 (1.4)	0.1
d of hCG	1.0 (1.3)	0.7 (1.4)	0.4
E2 pg/ml			
d 1	39 (21)	41 (24)	0.7
d 3	96 (60)	122 (86)	0.01
d 6	280 (176)	528 (548)	0.001
d 8	679 (459)	1075 (836)	0.2
d of hCG	1385 (1095)	1815 (1040)	0.04
P ng/ml			
d 1	0.7 (0.2)	0.7 (0.6)	0.5
d 3	0.6 (0.2)	0.6 (0.5)	0.8
d 6	0.6 (0.3)	0.6 (0.6)	0.7
d 8	0.7 (0.3)	0.8 (0.6)	0.3
d of hCG	0.9 (0.6)	1.2 (0.6)	0.2
FSH IU/liter			
d 1	7.2 (3.5)	7.3 (3.2)	0.8
d 3	12.0 (3.5)	11.6 (4.6)	0.9
d 6	13.5 (3.5)	13.0 (5.2)	0.7
d 8	14.0 (4.2)	12.9 (4.6)	0.2
d of hCG	13.5 (5.0)	12.5 (4.0)	0.1

follicular phase, as expressed by AUC, was observed in the d-1 group, compared with the d-6 group of antagonist administration, overall as well as from d 1 to d 6 and from d 6 to the day of hCG administration (Table 2). No significant differences were observed with regard to exposure to P and FSH during the follicular phase between the two groups of antagonist administration (Table 2).

Similar numbers of follicles with diameters less than 11 mm, 11–<15 mm, 15–<17 mm, 17 mm or more, and 11 mm or more were present between d-1 and d-6 group of antagonist administration, both on d 6 of stimulation and on the day of hCG (Table 3 and Fig. 2). Similar numbers of cumulus-oocyte complexes (COCs) ($P = 0.5$) were retrieved in the d-1 group (9.5 COCs; interquartile range, 10.5) and d-6 group of antagonist administration (11.0 COCs; interquartile range, 7.7). A similar proportion of metaphase-2 oocytes was present in the two groups of GnRH antagonist administration in cycles where ICSI was performed (81.6% *vs.* 80.0%, respectively).

Similar fertilization rates were observed in the d-1 group (60.5%) and d-6 group (58.9%). In one patient in the d-6 group, no embryo transfer was performed (because of high risk of ovarian hyperstimulation syndrome); and in three patients in the d-1 group, because of fertilization failure (n = 2) or because of poor embryo morphology (n = 1). Similar implantation rates (d 1, 33.9%; d 6, 30.5%) and ongoing pregnancy rates per transfer (d 1, 51.9%; d 6, 51.7%) were present in the d-1 group and d-6 group, respectively.

Discussion

This study has shown that initiation of GnRH antagonist on d 1 of stimulation for IVF, compared with d 6 of stimulation, is associated with a significantly lower exposure to LH
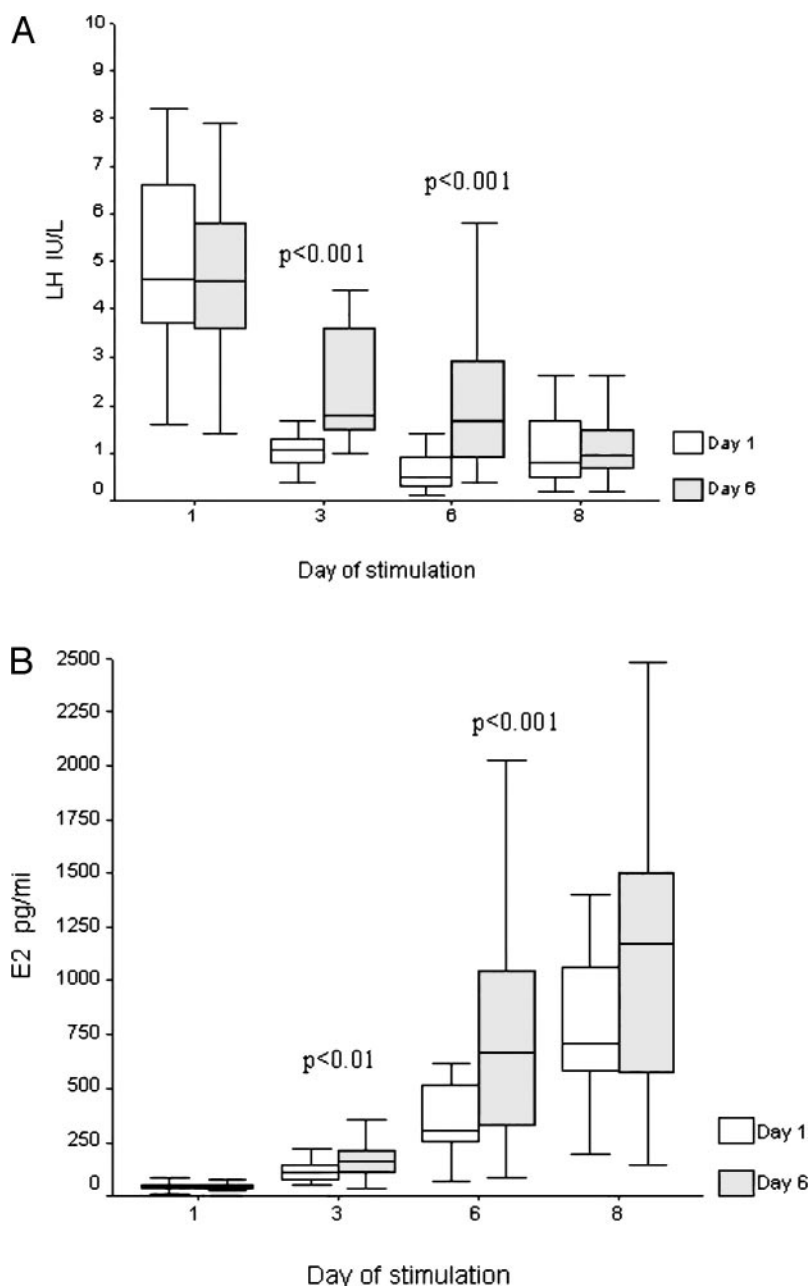
FIG. 1. Box plots of LH and E2 levels during the follicular phase, according to timing of GnRH antagonist initiation in patients with at least 8 d of stimulation. Differences between groups of GnRH antagonist administration are significant on d 3 and on d 6 of stimulation.

and E2 in the follicular phase and a similar pattern of follicular development in the two groups.

This is the first study that describes hormonal parameters and follicular development in IVF cycles where GnRH antagonist is initiated concomitantly with rec-FSH stimulation. Using a standard fixed dose of rec-FSH and applying strict criteria for hCG administration, a comparison with a fixed scheme of GnRH antagonist administration starting on d 6 of stimulation was carried out in a selected population of women undergoing IVF.

The differences observed between the hormonal levels and the follicular development of the two groups compared are therefore not biased by different doses of gonadotropin used during stimulation. In addition, E2 levels were not considered in deciding on hCG administration.

As a result, duration of stimulation reflects only follicular development.

A lower exposure to E2 in the d-1 group, compared with the d-6 group, is probably associated with the lower exposure to LH in the former group. It is well known that, under the influence of LH, theca cells produce androgens, which are then converted to estrogen in the granulosa cells by FSH (14). In addition, it has been shown previously that FSH is able to induce LH receptors in granulosa cells (15), a process enhanced by estrogen (16). The stimulation of these receptors by LH results in an increase in steroidogenesis (17); as in the developing follicle, the aromatase system becomes directly responsive to FSH as well as to LH (18). Thus, LH stimulates both androgen synthesis (in theca cells) and aromatization (in granulosa cells), regulating estrogen secretion (18, 19).

TABLE 2. Exposure to LH, E2, P, and FSH during the follicular phase, according to timing of GnRH antagonist administration

	AUC Median (interquartile range)		P
	d 1	d 2	
Overall exposure			
LH	10.9 (9.6)	18.6 (14.3)	0.000
E2	3898 (2572)	5316 (3222)	0.001
P	5.6 (2.0)	6.8 (5.2)	0.07
FSH	103.8 (31.9)	99.1 (30.5)	0.82
From d 1 to d 6 of stimulation			
LH	8.6 (6.3)	14.4 (8.5)	0.000
E2	790 (512)	1392 (1365)	0.005
P	3.3 (1.1)	3.3 (2.4)	0.5
FSH	56.7 (14.5)	56.1 (18.1)	0.9
From d 6 of stimulation to the d of hCG			
LH	3.1 (3.1)	5.3 (5.3)	0.005
E2	2941 (536)	3914 (1355)	0.004
P	2.4 (2.6)	3.2 (2.4)	0.07
FSH	45.5 (28.4)	45.5 (21.2)	0.8

LH AUC is measured in LH IU/liter·days; E2 AUC is measured in E2 pg/ml·days; P AUC is measured in P ng/ml·days; FSH AUC is measured in FSH IU/liter·d.

It should be noted that the two-cell, two-gonadotropin theory has been challenged by the demonstration of follicular development and an increase of E2 in the absence of detectable biological activity of LH (20). However, these data are derived from patients down-regulated with GnRH agonists, who do not respond to gonadotropins in the same way as do patients with hypogonadotropic hypogonadism. In the latter group, LH has been shown to be obligatory for E2 synthesis (21–23).

Interestingly, during the period from d 6 to hCG administration, LH exposure in the d-6 group remained significantly higher than in the d-1 group, despite suppression of endogenous LH by GnRH antagonist (Table 2). Although no clear explanation is available for this higher LH exposure after GnRH antagonist initiation, it probably leads to the higher exposure to E2 in the d-6 group, compared with the d-1 group, during the same period.

A similar pattern of hormonal differences in the early follicular phase has been observed in phase-3 comparative studies between GnRH agonists and GnRH antagonists (3, 4). Values of E2 on d 6 of stimulation in the antagonist group (before antagonist initiation) were reported to be more than twice as high as those in the agonist group (3, 4). Furthermore, LH values were much higher in the antagonist group on the same day. The similarity between phase-3 comparative studies (long agonist protocol *vs.* d-6 antagonist) and the current study (initiation of GnRH antagonist, either on d 1 or on d 6 of stimulation) is obviously associated with the similar pattern of gonadotropin suppression present in the two groups compared.

In contrast to the present study, however, phase-3 comparative studies showed that more follicles were present in the antagonist group on d 6 than in the agonist group. This has been attributed to the earlier recruitment of follicles, which starts in the luteal phase in the antagonist but not in the agonist group.

Such a difference in the number of follicles present between the two groups on d 6 of stimulation was not observed

TABLE 3. Follicular development on d 6 of stimulation and on the day of hCG, according to timing of GnRH antagonist administration

	Median (interquartile range)		P
	d 1	d 6	
Day 6			
Total follicles ≥ 11	5.0 (5.0)	4.0 (5.5)	0.4
Follicles < 11 mm	7.0 (7.0)	6.0 (5.7)	0.7
Follicles 11–<15	4.0 (4.0)	4.0 (4.2)	0.3
Follicles 15–<17	0.0 (1.0)	0.0 (1.0)	0.8
Follicles ≥ 17	0.0 (0.2)	0.0 (0.0)	0.3
Day of hCG			
Total follicles ≥ 11	11.0 (9.2)	12.0 (8.0)	0.4
Follicles < 11	2.5 (6.0)	2.0 (4.0)	0.2
Follicles 11–<15	5.0 (8.2)	5.5 (4.5)	0.6
Follicles 15–<17	2.0 (4.0)	2 (4.0)	0.2
Follicles ≥ 17	4.0 (2.0)	4.0 (2.0)	0.9

in the current study. This is probably attributable to the fact that developing follicles in both groups of antagonist administration were under the influence of a similar hormonal environment, the environment of the luteal phase of the cycle before stimulation.

Similar serum FSH levels seem to be present in both the d-1 and d-6 groups, despite suppression of endogenous FSH by GnRH antagonist in the d-1 group. This probably means that exogenous stimulation outweighs endogenous FSH suppression in the d-1 group, resulting in similar serum FSH levels, which lead to similar follicular development in the two groups compared. It can also be observed that a significantly different exposure to LH levels in the follicular phase, between the two groups of antagonist administration compared, did not seem to influence follicular development.

Furthermore, it seems that significantly lower exposure to LH and E2 in the d-1 group is not associated with the maturation of the oocytes retrieved, because similar proportions of metaphase-2 oocytes were observed in the two groups. Moreover, the endocrine differences, present between the two groups, seem to have no effect on fertilization and implantation rates achieved. It should be noted that a definitive proof of an obligatory role of estrogen in folliculogenesis and the elucidation of the mechanisms subserving their different actions in follicular cells remains elusive (24). Although knock-out and mutant animal models are a valuable source of information in this respect, it is still difficult to establish the exact role of estrogen independently from that of gonadotropins or other ovarian hormones or factors (25).

On the other hand, the impact of the endocrinological differences observed between the two groups on endometrial receptivity remains to be assessed. Endometrium in IVF cycles has been shown to be histologically advanced, even before hCG administration (26). It has been hypothesized that a lower exposure to estrogen during the early follicular phase could be beneficial by preventing the premature induction of P receptors in endometrium and thus avoid a P action on endometrium before the luteal phase starts (11).

In conclusion, this study shows that administration of GnRH antagonist on d 1 of stimulation results in a lower exposure to LH and E2 in the follicular phase, compared with initiation of GnRH antagonist on d 6 of stimulation. However, this does not seem to affect follicular development or

FIG. 2. Proportion of follicles with diameters less than 11 mm, 11 to <15 mm, 15 to <17 mm, and 17 mm or more, on the day of hCG administration, according to GnRH antagonist initiation.
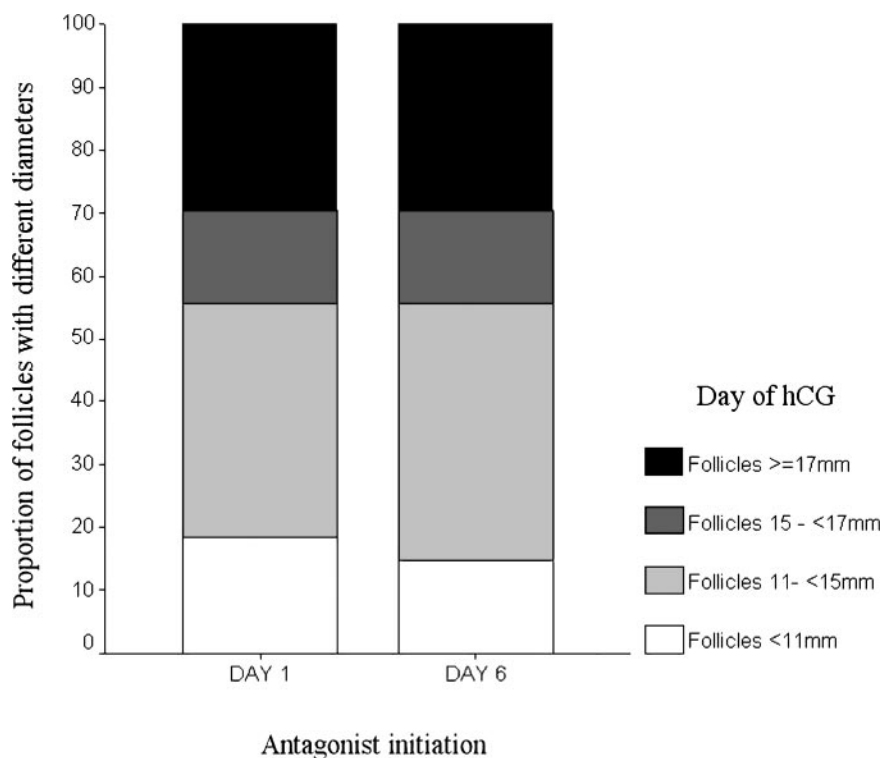
the proportion of mature oocytes retrieved. How these hormonal differences affect endometrial maturation at oocyte retrieval and implantation potential remains to be determined in larger trials.

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