The effect of delayed initiation of gonadotropin-releasing hormone antagonist in a flexible protocol on in vitro fertilization outcome

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Objective: To determine the proportion of patients stimulated on a flexible GnRH antagonist regimen who meet the criteria for antagonist administration after stimulation day 6 (S6) and to compare their clinical characteristics and cycle outcome with those patients who start the antagonist on S6 or earlier.
Design: Retrospective study.
Setting: Tertiary university hospital.
Patient(s): Patients undergoing IVF (n = 442) using a flexible GnRH antagonist protocol.
Intervention(s): Ovarian stimulation was performed using gonadotropins and GnRH antagonists. Group A (n = 323) patients met the criteria for antagonist administration (follicle size >12 mm, E_2 >300 pg/mL) on S6 or earlier. Group B patients (n = 119) started the antagonist later.
Main Outcome Measure(s): Implantation rate.
Result(s): Comparable implantation (30.4% vs. 33.7%), clinical (47.4% vs. 52.9%), and ongoing pregnancy rates (41.2% vs. 47.9%) were observed in groups A and B, respectively. Group B patients had a significantly higher body mass index, longer stimulation, increased gonadotropins dosage, fewer oocytes and two pronuclei oocytes, fewer frozen embryos, and fewer cycles with embryo freezing. Patients with polycystic ovary syndrome were more likely to be in group B.
Conclusion(s): A considerable proportion of patients on a flexible regimen begin GnRH antagonist administration later than S6. Despite different stimulation and laboratory characteristics, their reproductive outcome is not compromised as compared with patients with an earlier antagonist start. (Fertil Steril® 2013;99:725–30. ©2013 by American Society for Reproductive Medicine.)
Key Words: Gonadotropin-releasing hormone antagonists, in vitro fertilization, flexible protocol, implantation

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After a decade of doubts and hesitation, GnRH antagonist protocols have become extremely popular and are now widely used for prevention of premature LH surges during controlled ovarian stimulation (COS) before IVF-ET. A recent meta-analysis has indicated a similar therapeutic efficacy in terms of live-birth rates and improved safety in terms of ovarian hyperstimulation syndrome risk reduction as compared with long GnRH agonist protocols (1).

In early studies the most commonly used method for administration of the GnRH antagonist was on a fixed day, usually gonadotropin stimulation day 6 (S6). The rationale behind the fixed method was double: to avoid any risk of an early LH surge, as LH rise before S6 was thought to be unlikely, and to simplify and standardize the treatment protocol. Subsequently, it has been suggested that because of individual variations in patient response, commencement of antagonist

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administration could be flexible, according to the size of the lead follicle (2). In a meta-analysis of four randomized controlled trials (RCTs), the fixed and the flexible GnRH antagonist regimens have been found comparable in terms of clinical pregnancy rates, but a statistically significant reduction in both GnRH antagonist and exogenous gonadotropins requirements was observed when the flexible antagonist regimen was used (3).

The comparisons made in the RCTs between fixed and flexible GnRH antagonist regimens were of limited value in one aspect, as the majority of patients in the flexible arms received the antagonist at an early stage of the cycle (S6 or earlier), identical to the patients on the fixed regimen, thus decreasing the probability of detecting a potentially existing difference between the two protocols. It has been shown in two studies that the subset of patients in the flexible arm who did not reach the prespecified criteria for antagonist administration on S6 and had delayed administration of the antagonist achieved lower pregnancy rates, but the number of patients included was too small to reach firm conclusions (4, 5). Thus, no specific attention has so far been given to patients in whom antagonist administration is deferred, as there is no study in the literature that directly compares this group with the group of patients that start antagonist administration on S6 or earlier.

Our objective was to determine the proportion of patients who reach the criteria for GnRH antagonist administration later than S6 and to compare their clinical characteristics and cycle outcome with patients who began antagonist administration on S6 or earlier in a large retrospective cohort of patients treated with the flexible GnRH antagonist protocol.

MATERIALS AND METHODS

This was a retrospective study approved by our Institutional Review Board. Included in the study were women aged ≤ 40 years undergoing their first IVF cycle using a GnRH antagonist protocol at the IVF unit of the Edith Wolfson Medical Center. Excluded from the study were women aged >40 years, women who had more than three previous failed IVF cycles, or women who had precycle treatment with oral contraceptives.

The flexible GnRH antagonist protocol was used in all cycles. Recombinant human FSH (r-hFSH; Gonal-F, Merck-Serono, or Puregon, MSD) was started on day 2 or 3 of the menstrual cycle at an individualized dose according to patient's age, ovarian reserve testing, and previous response to gonadotropin therapy. The first monitoring visit was scheduled to stimulation day 5 (S5). The gonadotropins dose could then be adjusted according to the patient's response as assessed by serum E_2 and P levels and follicle number and diameter assessed by ultrasound. After 5 days of r-hFSH stimulation, the gonadotropin preparation was switched to human menopausal gonadotropin (hMG; Menogon or Menopur; Ferring), at the same dose as the r-hFSH dose, unless dose adjustment was necessary. A daily dose of 0.25 mg of GnRH antagonist (Cetrotide, Merck-Serono, or Orgalutran, MSD) was initiated when the leading follicle size was ≥ 13 mm or the serum E_2 level reached ≥ 300 pg/mL. HCG (250 μg, Ovitrelle, Merck-Serono) was administered when at least two follicles reached 18 mm in diameter and oocyte retrieval was undertaken 36 hours later. Standard IVF or intracytoplasmic sperm injection was applied as indicated. Embryos were transferred on day 3 or 5 of culture under ultrasound guidance. Two to three embryos were transferred. The luteal phase was supported by transvaginal micronized P 200 mg 3 times per day (Uterogestan, Besins Iscovesco) and oral E_2 2 mg 2 times per day (Estrofem, Novo Nordisk).

Patients were divided into two groups: group A consisted of patients who reached the criteria for GnRH antagonist administration on S6 or earlier, and group B of patients who started the GnRH antagonist later than S6. The demographic, clinical, and laboratory cycle outcome parameters were compared between the two groups. The primary outcome measure was the embryo implantation rate.

Serum E_2 and P levels were measured by means of the automated Elecsys Immunoanalyser (Roche Diagnostics). Intra-assay and interassay coefficients of variation were $<5\%$ and $<10\%$ for E_2 and $<3\%$ and $<5\%$ for P, respectively.

Implantation rate was calculated by dividing the number of gestational sacs by the number of embryos transferred. Clinical pregnancy was defined as intrauterine pregnancy with at least one fetus with positive heart beat at 6 weeks' gestation or later. Ongoing pregnancy was defined as intrauterine pregnancy with at least one fetus with positive heart beat at 12 weeks or later.

Analysis of data was carried out using SPSS 19.0 statistical analysis software (IBM Inc.). Normality of distribution of continuous variables was assessed using the Kolmogorov-Smirnov test (cutoff at $P=.01$). Descriptive statistics for continuous variables were reported as means $\pm$ SD. Categorical variables were described using frequency distributions and are presented as frequency (%). The *t* test for independent samples or the Mann-Whitney *U*-test were used as appropriate to compare continuous variables by group (the use of GnRH antagonist before vs. after S6). The χ^2-test (exact as needed) was used to compare categorical variables by group. A logistic regression model of group (the use of GnRH antagonist before vs. after S6) was developed, and odds ratios (OR) were estimated with the 95% confidence intervals (CI). All tests were two-sided and considered significant at $P<.05$.

RESULTS

From January 2006 through November 2011, 1,095 women had COS using the flexible GnRH antagonist protocol. Of them, 442 patients met the inclusion criteria. Their mean age was 31 ± 4.7 years, the mean duration of infertility was 2.8 ± 2.1 years, and the mean number of previous IVF cycles was 0.7 ± 0.9. Of the 442 women included, 323 women (73%) reached the criteria for GnRH antagonist administration on S6 or earlier (group A), while 119 women (27%) had delayed administration of the antagonist according to the prespecified criteria (group B). None of the patients in group A or B had a premature LH surge that led to cycle cancellation.

The following variables had distributions significantly deviating from normal using the Kolmogorov-Smirnov test: body mass index (BMI), days before GnRH antagonist administration, duration of gonadotropin stimulation, total dose of FSH used,

TABLE 1

Patient diagnosis in the early (group A) and late (group B) GnRH antagonist administration group.

Diagnosis	Group A, % (n = 323)	Group B, % (n = 119)	*P* value
Male factor	38.7	32.7	NS
Unexplained infertility	21.8	16.7	NS
Tubal factor	19.5	21	NS
PCOS[a]	4.3	21.7	<.0001
Endometriosis	3.1	0.4	NS
More than one factor	12.6	7.5	NS

Note: NS = not significant.
[a] Polycystic ovary syndrome.

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and the number of 2 pronuclei (PN) oocytes achieved. These variables were compared by treatment group using the Mann-Whitney *U*-test, while all other continuous variables were compared using the *t* test for independent samples.

Causes for infertility are shown in Table 1. Patients with polycystic ovarian syndrome (PCOS) made up 21.7% of group B patients and only 4.3% of group A patients ($P<.0001$). Patients' demographic and stimulation characteristics are shown in Table 2. Women in group B were found to have a significantly higher BMI, required a significantly longer ovarian stimulation period, and consumed significantly higher doses of gonadotropins but had a similar duration of GnRH antagonist administration. The starting day of the GnRH antagonist was 5.3 ± 0.6 vs. 7.8 ± 1.7 in groups A and group B, respectively ($P<.001$). There was no difference between the groups in the E_2 and P level at the initiation of GnRH antagonist administration and on the day of hCG administration.

Laboratory and reproductive outcome parameters are shown in Table 3. While women in group B had significantly fewer oocytes retrieved, fewer 2 pronuclei (PN) oocytes, fewer embryos to freeze, and overall fewer cycles with frozen embryos, no between-group differences were observed in fertilization rate, number of transferred embryos, implantation rate, or clinical, multiple, and ongoing pregnancy rates.

Further subanalysis revealed that the embryo implantation rate did not differ when comparing women receiving the GnRH antagonist on stimulation day 8 or later with women receiving antagonist on S6 or earlier (29% vs. 30%; $P=.97$).

Women with PCOS had an increased likelihood of being in group B (OR, 4.54; 95% CI, 1.95–10.56; $P<.001$). In logistic regression analysis, every 1 kg/m^2 increase in BMI was associated with a 7.3% increase in likelihood of being in group B (OR, 1.073; 95% CI, 1.007–1.143; $P=.029$). Subgroup analysis in group B revealed that patients with PCOS (26/119) had a significantly higher BMI (27.6 ± 3.2 vs. 25.4 ± 2.8; $P=.047$) and a prolonged duration of stimulation (13.61 ± 3.25 vs. 11.70 ± 2.0 days; $P=.002$) and required a lower total amount of FSH administered (2,127 ± 913 vs. 2,768 ± 1,439 IU; $P=.025$) compared with all other group B patients. There was no difference between PCOS patients in group B and all other group B patients in terms of duration of GnRH antagonist administration (8.5 ± 3.1 vs. 7.6 ± 0.96 days; NS), peak E_2 levels (2,480 ± 1,394 vs. 2,203 ± 1,200 pg/mL), number of oocytes retrieved (7.9 ± 5.7 vs. 8.0 ± 4.9; NS) and fertilized (4.5 ± 3.7 vs. 4.6 ± 2.8; NS), and implantation rates (38.5% vs. 31.6%; NS).

DISCUSSION

Our study indicates that a significant proportion of patients (27%) from the general IVF population who are treated with the flexible GnRH antagonist protocol reach the criteria for antagonist administration later than S6. Our study also shows that there is no difference in implantation, clinical, and ongoing pregnancy rates according to the timing of GnRH antagonist administration. The implantation and clinical pregnancy rates were also comparable when analyzing the subgroup of patients receiving the antagonist on stimulation day 8 or later.

Several studies have been conducted comparing the fixed and flexible protocols of GnRH administration, with very little attention paid to patients treated with a flexible regimen in which antagonist initiation was deferred beyond S6. Data

TABLE 2

Patient characteristics and response to ovarian stimulation in the early (group A) and late (group B) GnRH antagonist administration group.

	Group A (n = 323)	Group B (n = 119)	*P* value
Age	31.0 ± 4.6	31.2 ± 5.1	NS
Basal day 3 FSH IU/L	6.15 ± 2.16	6.15 ± 2.14	NS
BMI[a]	23.9 ± 4.67	26.0 ± 5.20	<.001
Starting day of the GnRH antagonist	5.3 ± 0.60	7.8 ± 1.7	<.001
Duration of GnRH antagonist administration, d	5.61 ± 1.46	5.95 ± 2.10	NS
Duration of stimulation, d[a]	9.3 ± 1.6	12.2 ± 2.45	<.001
Total amount of FSH used, IU[a]	1779 ± 724	2669 ± 1388	<.001
Serum E_2 on first day of GnRH antagonist administration, pg/mL	627 ± 519	645 ± 582	NS
Serum P on first day of GnRH antagonist administration, ng/mL	0.86 ± 0.69	0.73 ± 0.44	NS
Serum E_2 on day of hCG, pg/mL	2,196 ± 1,306	2,260 ± 2,095	NS
Serum P on day of hCG, ng/mL	1.12 ± 0.86	1.06 ± 0.84	NS

Note: Values expressed as mean ± SD. NS = not significant.
[a] Distribution of this variable differed significantly from normal and was compared by treatment group using the Mann-Whitney *U*-test; all other continuous variables were compared using the *t* test for independent samples.

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TABLE 3

Stimulation data and clinical outcome in the early (group A) and late (group B) GnRH antagonist administration group.

	Group A (n = 323)	Group B (n = 119)	*P* value
Recovered oocytes	8.95 ± 4.70	7.81 ± 4.99	.02
Fertilization rate, %	65	62.5	NS
No. of 2PN oocytes[a]	5.64 ± 3.39	4.52 ± 2.98	.002
No. of transferred embryos	1.96 ± 0.63	1.99 ± 0.75	NS
No. of frozen embryos	1.94 ± 2.89	1.31 ± 2.42	.035
Cycles with frozen embryos, %	43.7	31.1	.023
Implantation rate, %	30.4	33.7	NS
Clinical pregnancy rate, %	47.4	52.9	NS
Ongoing pregnancy rate, %	41.2	47.9	NS
Multiple pregnancy rate, %[a]	27.3	29.5	NS

Note: All multiple pregnancies were twins. NS = not significant.
[a] Distribution of this variable differed significantly from normal and was compared by treatment group using the Mann-Whitney *U*-test; all other continuous variables were compared using the *t* test for independent samples.

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from two RCTs (4, 5) relating to a small number of patients suggested that delaying antagonist administration beyond S6 adversely affects cycle outcome. The ultimate study to assess the effects of delayed antagonist administration, as previously proposed by Kolibianakis et al. (6), should include patients that have not reached the prespecified criteria for antagonist administration by S6. At this point patients should be randomized either to start the antagonist or to continue stimulation until the criteria are met. To the best of our knowledge, such a study has not been published yet. We therefore conducted this retrospective study to better characterize and describe the outcome of cycles with delayed GnRH antagonist administration.

Implantation rate, the primary outcome measure of our study, was comparable for the two groups, as were the clinical and ongoing pregnancy rates. Implantation rate remained comparable even if antagonist administration was initiated on stimulation day 8 or later. These results were obtained despite the fact that the two study groups were considerably different from a clinical point of view. Patients in group B were more likely to have PCOS, had a higher BMI, required prolonged stimulation and increased amounts of gonadotropins, and produced fewer oocytes (Tables 1–3). Moreover, in the laboratory, group B patients had fewer 2PN oocytes, fewer frozen embryos and fewer cycles with embryo freezing. Despite significant differences in clinical and laboratory characteristics, transfer of a similar number of embryos resulted in comparable implantation and pregnancy rates. It can be therefore concluded that neither embryo quality nor endometrial receptivity were adversely affected by delayed administration of the GnRH antagonist.

Lack of between-group differences in clinical outcomes must be considered in the framework of study design limitations. Implantation and clinical pregnancy rates were more than 10% higher, and ongoing pregnancy rate was more than 16% higher in group B than in group A. The study was not powered to detect these differences. With a sample size of approximately 5,000 subjects, these differences would have been statistically significant. The question remains as to their clinical significance. Further, the study population was drawn from a convenience sample of patients treated at a single center and is not a probability sample from a target population. This creates some difficulty in generalizing to all patients undergoing IVF. However, we included a relatively large cohort of patients from the general IVF population that represents common daily practice, rather than a uniform group of the good-prognosis patients who are usually included in RCTs.

The fact that group B patients reached the criteria for GnRH antagonist administration later than group A is somewhat surprising, as this group is overrepresented by patients with PCOS who are generally high responders. Subgroup analysis in group B reveals that patients with PCOS had a higher BMI, but a similar number of oocytes was retrieved while using a significantly lower total dose of FSH administered compared with other group B patients. This is probably because of the extreme caution we take while treating patients with PCOS who always receive a soft GnRH antagonist protocol when stimulated for the first time. A favorable implantation rate of 38.5% was observed in group B patients with PCOS.

Our study did not aim to compare a fixed protocol with an individualized flexible protocol. We focused on and compared patients who met the criteria for GnRH antagonist administration up to S6 or later. The results of our study do not lead to the conclusion that delaying antagonist administration is appropriate but rather that patients who meet follicle and E_2 criteria later do as well as those who meet criteria earlier. This is a new finding that contrasts with what has been suggested in the past (4, 5).

Our study included patients from the general IVF population, without using diagnosis-specific inclusion or exclusion criteria. It therefore represents a very realistic spectrum of patients who are routinely encountered in IVF clinics. Patients who have a higher BMI, patients with PCOS treated with soft protocols, and perhaps (although not specifically addressed in our study) patients with a lower ovarian reserve all tend to have a prolonged pattern of ovarian response to COS and reach the criteria for GnRH antagonist administration at a later stage. Again, if good-quality embryos are available for transfer, the reproductive outcome of cycles with delayed

GnRH antagonist initiation is favorable and not compromised compared with cycles with earlier initiation of GnRH antagonist.

In contrast to early RCTs (2, 4, 5, 7), we used an individualized starting dose of r-hFSH that is probably more conducive to an optimal ovarian response. In accordance with changing trends in antagonist administration, as discussed below, we started monitoring on S5 rather than on S6 and initiated antagonist administration at a follicle size $\geq$13 mm, taking into account serum E_2 levels as well, rather than only the size of 14–16 mm previously used in the RCTs (2, 4, 5, 7). We routinely supplement the late follicular phase of all our COS cycles with LH activity (in the form of hMG) and include exogenous E_2 in our regimen for luteal phase support. We believe that the favorable reproductive outcome (Table 3) that was achieved for both groups in our study is related to the incorporation of the above interventions in our practice.

Since the introduction of GnRH antagonists to clinical practice there has been an ongoing attempt to optimize existing stimulation protocols. The first protocol developed was the fixed protocol in which the antagonist is administered on S6 (8). To reduce the number of antagonist injections and duration of stimulation, a flexible protocol was developed in which the GnRH antagonist is started when the follicles reach a size of 14–16 mm. Five RCTs have been performed so far comparing the fixed and flexible protocols (2, 4, 5, 7, 9). In a meta-analysis summarizing four of the early studies, there was a trend toward lower pregnancy rates in the flexible protocol, but this trend did not reach statistical significance (OR, 0.70; 95% CI, 0.47–1.05) (3).

In two of the above studies that provided the relevant data, >50% of patients in the flexible arm started the antagonist beyond S6 (4, 5), and the authors have suggested that applying the antagonist later than S6 could have negative effects on cycle outcome (4, 5). In the study performed by Mochtar (4), only one pregnancy was achieved in 21 women in whom GnRH antagonist was administered on stimulation day 8 or later. Mochtar (4) performed a subanalysis on cycle outcome according to the presence or absence of a $\geq$15 mm follicle on S6. In the fixed arm, ongoing pregnancy rates with and without a $\geq$15 mm follicle present were 47% and 15.6%, respectively. Patients in the fixed arm received the GnRH antagonist on S6 irrespective of the presence of the $\geq$15 mm follicle, suggesting that it is the pattern of ovarian response rather than the actual timing of GnRH antagonist administration that could affect cycle outcome.

In contrast, Kolibianakis et al. (5) performed a similar subanalysis in their RCT but achieved somewhat conflicting results. In the fixed arm, ongoing pregnancy rate without a $\geq$15 mm follicle present on S6 was 38.1%, as compared with 25% in patients with a $\geq$15 mm follicle present. Ongoing pregnancy rate in the flexible group without a $\geq$15 mm follicle present was as low as 16.1%. Similarly, implantation rates decreased significantly when no follicle $\geq$15 mm was present on S6 and the flexible protocol was applied as compared with the fixed protocol (8.8% vs. 23.9%) (5). It appears from the results of the above study that antagonist administration on S6 allows for a favorable outcome irrespective of the presence of a large follicle. Kolibianakis et al. attributed an adverse outcome of delayed GnRH antagonist administration to higher exposure of the genital tract to LH and E_2 present in the flexible group that is believed to affect negatively endometrial receptivity. Delayed antagonist administration beyond S6 has been associated with increased endometrial advancement at the time of oocyte retrieval, which in extreme cases could result in a reduced probability of pregnancy (10). The reasons for the discrepancy in results between the two studies despite a similar study design remains unclear.

In the recent years there has been a shift in the practice of GnRH antagonist administration. In the early introductory RCTs of GnRH antagonists, all done using a fixed protocol, the majority of LH rises occurred before the initiation of the antagonist on S6 (11–14). These observations and the accumulation of wide clinical experience from a decade of GnRH antagonists use have led to the assumption that an earlier initiation of antagonist administration could be associated with a lower incidence of LH rises and an improved outcome. Since there is no clear understanding and agreement regarding the time during COS at which a premature LH surge is possible and likely to occur, "strict criteria" for antagonist initiation based on ultrasound and hormonal parameters have been developed (9, 15).

While in the classical fixed protocol the first monitoring visit is scheduled on S6, with strict criteria the monitoring begins on S4 (15) or even S3 (9). Lainas et al. (15), showed that when using criteria that combine follicle diameter >14 mm, E_2 level >600 pg/mL, and LH >10 IU/L, 66% of patients received GnRH antagonist before S6 (15). Surprisingly, patients who started antagonist administration on S6 had significantly lower ongoing pregnancy rates. A very small proportion of patients started the antagonist after S6, and they were not included in the analysis. Recently, Kolibianakis et al. (9) have reported the results of an RCT comparing fixed and flexible antagonist administration regimens, where early monitoring and strict criteria were applied in the flexible arm. In the fixed group, antagonist administration was started on S6, and in the flexible arm the antagonist was started when lead follicle diameter was >12 mm and/or serum E_2 level was >150 pg/mL. In this trial the GnRH antagonist was started after 3.1 $\pm$ 0.9 days of stimulation in the flexible arm and after 5.0 $\pm$ 0.0 days in the fixed arm (P=.01). No difference in the incidence of LH rises or in reproductive outcome was observed, but the consumption of GnRH antagonist was significantly increased in the flexible group (9). This is a true shift, almost a paradox, as in early studies one of the potential advantages suggested for the flexible protocol was a significant reduction in GnRH antagonist consumption (2, 4, 7).

In conclusion, a considerable proportion of patients on a flexible GnRH antagonist regimen begin antagonist administration later than S6. Despite different stimulation and laboratory characteristics, their reproductive outcome is not compromised as compared with patients with an earlier start of GnRH antagonist. While the world is still divided regarding the use of fixed and flexible protocols, there is

certainly a need of further studies to optimize GnRH antagonist administration.

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