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A Randomized Comparison of Two Ovarian Stimulation Protocols with Gonadotropin-Releasing Hormone (GnRH) Antagonist Cotreatment for *in Vitro* Fertilization Commencing Recombinant Follicle-Stimulating Hormone on Cycle Day 2 or 5 with the Standard Long GnRH Agonist Protocol

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Extending the FSH window for multifollicular development by administering FSH from the midfollicular phase onward constitutes a novel mild protocol for ovarian stimulation for *in vitro* fertilization (IVF) based on the physiology of single dominant follicle selection in normo-ovulatory women. We compared outcomes from this protocol with two other stimulation protocols. One hundred and forty-two normo-ovulatory patients with an indication for IVF (or IVF/ICSI) were randomized to a GnRH agonist long protocol (group A; n = 45) or one of two GnRH antagonist protocols commencing recombinant FSH on cycle d 2 (group B; n = 48) or cycle d 5 (group C; n = 49). A fixed dose (150 IU/d) of rFSH was used for ovarian stimulation, and GnRH antagonist cotreatment was initiated on the day when the leading follicle reached 14 mm diameter.

Group C showed a shorter duration of stimulation (median duration, 11, 9, and 8 d for groups A, B, and C, respectively; $P < 0.001$), reflected in a significantly lower total dose of rFSH used (median amount of rFSH, 1650, 1350, and 1200 IU for groups A, B, and C, respectively; $P < 0.001$). In group C more cycles were cancelled during the stimulation phase due to insufficient response, resulting in a lower percentage of oocyte retrievals (84%, 73%, and 63% for groups A, B, and C; $P = 0.02$). However, women in group C obtained better quality embryos (percentage of embryo score of 1 for best embryo, 29%, 37%, and 61% for groups A, B, and C, respectively; $P = 0.008$), resulting in more transfers per oocyte retrieval (68%, 71%, and 90% for groups A, B, and C, respectively; $P = 0.04$). After profound ovarian stimulation (groups A and B) only 7% of the patients who retrieved four oocytes or less conceived, whereas after mild stimulation (group C) 67% of these patients conceived ($P < 0.01$). Overall, no differences were found among the three groups comparing pregnancy rate per started IVF cycle.

In conclusion, application of the described mild ovarian stimulation protocol resulted in pregnancy rates per started IVF cycle similar to those observed after profound stimulation with GnRH agonist cotreatment despite shorter stimulation and a 27% reduction in exogenous FSH. A higher cancellation rate before oocyte retrieval was compensated by improved embryo quality concomitant with a higher chance of undergoing embryo transfer. A relatively low number of oocytes retrieved after mild ovarian stimulation distinctly differs from the pathological reduction in the number of oocytes retrieved after profound ovarian stimulation (poor response) associated with poor IVF outcome. The relatively small number of oocytes obtained after mild ovarian stimulation may represent the best of the cohort in a given cycle. (*J Clin Endocrinol Metab* 88: 166–173, 2003)

CONVENTIONAL OVARIAN STIMULATION protocols aim to stimulate the growth of many follicles to obtain multiple oocytes for *in vitro* fertilization (IVF) and thus multiple embryos, allowing selection for transfer (1). Currently applied standard IVF protocols take many weeks, are complex and expensive, and are not without risk. Problems related to ovarian stimulation include emotional stress, abdominal discomfort, short-term complications such as ovarian hyperstimulation syndrome and multiple gestation, and uncertainties regarding long-term health consequences (2). Many of the problems associated with current IVF stimulation regimens relate to the unphysiological approach to ovarian (hyper)stimulation (3). Preceding the administration of high doses of gonadotropins, pituitary down-regulation is normally achieved by prolonged administration of GnRH agonists (the so-called long protocol). To date, IVF practice has focused on optimizing success in terms of pregnancy rate per started IVF cycle. Profound ovarian stimulation is therefore applied, despite the above-mentioned side-effects, risks, and costs. If the balance between the risks and benefits of IVF treatment is to improve, a paradigm shift is required in the approach to treatment and in the way success from IVF is defined. Increasing knowledge regarding the physiology of ovarian follicle development and dominant follicle selection (4) together with the clinical availability of new compounds, such as GnRH antagonists (5), have presented the opportunity to develop novel, milder approaches for ovarian stimulation for IVF (6).

During the luteo-follicular transition in the normal menstrual cycle, FSH concentrations rise and surpass the thresh-

Abbreviations: E2, Estradiol; ET, embryo transfer; hCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization; P, progesterone; rFSH, recombinant FSH; TVS, transvaginal sonography.

old, stimulating a cohort of small antral follicles to grow (4). Around the midfollicular phase, the most mature follicle gains dominance over other cohort follicles (7). This dominant follicle continues its growth despite decremental FSH concentrations (8), whereas the remaining follicles from the recruited cohort enter atresia due to insufficient stimulation by FSH. This decreasing FSH level and subsequent closure of the FSH gate (9) or window (10) appears essential for single dominant follicle selection.

Multifollicular growth is established in current IVF protocols by generating FSH serum concentrations far above the threshold from the early follicular phase for an extended period. However, single dominant follicle selection may be disrupted by the administration of low doses of exogenous FSH during the mid to late follicular phase, effectively preventing the physiological decrease in FSH concentrations (11, 12). The clinical introduction of GnRH antagonists in IVF (13–17) allows ovarian stimulation to be commenced in the undisturbed menstrual cycle. Extending the FSH window by administering low dose exogenous FSH from the mid to late follicular phase may indeed be effective in IVF (18), resulting in a shorter and more patient-friendly treatment cycle. However, it remains unclear whether the reduced number of oocytes obtained after mild stimulation may impair outcome (2). To address these issues, we carried out a prospective randomized study comparing stimulation characteristics and IVF outcomes of the standard long GnRH agonist protocol for ovarian stimulation with two GnRH antagonist protocols commencing FSH in the early or midfollicular phase.

Subjects and Methods

Subjects and study design

This study was approved by the local ethics review committee. Written informed consent was obtained from each participant. Between November 1999 and May 2001, 169 patients with an indication for IVF with or without intracytoplasmic sperm injection (ICSI) were recruited. After assignment to IVF or IVF/ICSI (those patients with a total motile sperm count $<1.0 \times 10^6$), randomization was performed to 1 of the 3 treatment groups using a computer-generated randomization schedule assigned via numbered sealed envelopes.

Inclusion criteria were 1) age between 20–38 yr; 2) body mass index (body weight divided by the square of body height) between 19–29 kg/m^2; 3) history of regular menstrual cycles, ranging from 25–35 d; 4) no relevant systemic disease, severe endometriosis, or uterine and ovarian abnormalities; 5) no more than three previous IVF cycles; and 6) no previous IVF cycle with a poor response or ovarian hyperstimulation syndrome.

Group A was treated with the GnRH agonist triptoreline (Decapeptyl, Ferring Pharmaceuticals Ltd., Hoofddorp, The Netherlands; 1 mg/d, sc) starting 1 wk before the expected menses (usually cycle d 21). After down-regulation was achieved [serum estradiol (E2), <150 pmol/liter], ovarian stimulation was commenced with a fixed daily dose of 150 IU recombinant FSH (rFSH), sc (Gonal-F, Serono Benelux BV, The Hague, The Netherlands). Groups B and C were treated with the GnRH antagonist cetrorelix (Cetrotide, ASTA Medica, Amsterdam, The Netherlands; 0.25 mg/d, sc) commencing when the largest follicle had reached a diameter of 14 mm, as previously described (18). rFSH was initiated on cycle d 2 (group B) or 5 (group C). Triptoreline and cetrorelix were continued up to and including the day of human chorionic gonadotropin (hCG) administration. When the leading follicle had reached a diameter of 18 mm or more and at least three follicles had reached a diameter of 15 mm or more, rFSH was stopped, and a single sc bolus of 10,000 IU hCG (Pregnyl, NV Organon, Oss, The Netherlands) was administered 35 h before the planned time of oocyte retrieval. All follicles 12 mm or larger were aspirated. Subsequently, IVF with or without ICSI was performed, and a maximum of two embryos were transferred 3–5 d thereafter, as described previously (19). All embryos were scored on the day of embryo transfer (ET) between 0800–1000 h. Luteal support in the form of intravaginal progesterone (P; Progestan, Organon; 200 mg, three times daily) was given from the day of oocyte retrieval until a urine pregnancy test was performed 17 d later. A schematic description of the applied treatment regimens is given in Fig. 1.

Assessments

Baseline blood sampling and transvaginal sonography (TVS) were performed on cycle d 2 or 3 of the pretreatment cycle in group A and on cycle d 2 of the treatment cycle in groups B and C. Monitoring of response during the treatment cycle consisted of TVS and blood sam-

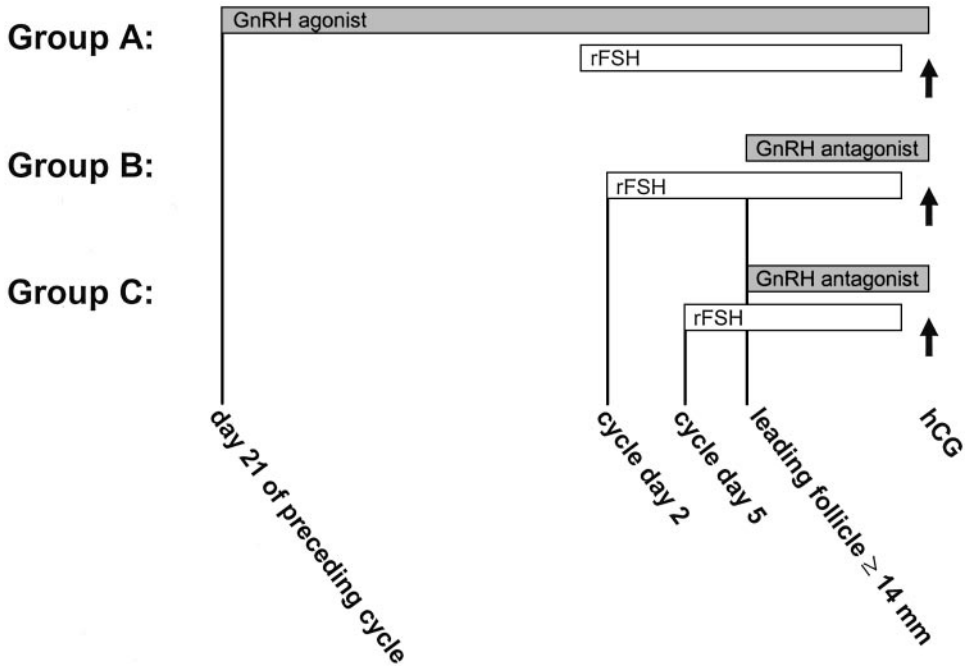

FIG. 1. Schematic representation of the three studied stimulation regimens: a GnRH agonist long protocol and two late follicular phase GnRH antagonist protocols with start of a fixed dose of rFSH on cycle d 2 or cycle d 5.

pling (cycle d 2, 5, and 8; day of hCG administration; day of oocyte retrieval, and 7 d thereafter) for hormonal analysis (E2, FSH, LH, and P). Additional TVS monitoring was performed as clinically indicated.

Embryo scoring was carried out on the day of ET (3, 4, or 5 d after oocyte retrieval) blinded to the stimulation protocol. Scoring was based on developmental stage and morphology, using previously described criteria (19, 20). Scoring criteria for d 3 embryos included cell number, regularity of blastomeres, fragmentation, and morphological aspects such as granulation. Criteria for d 4 embryos included the degree of embryo compaction and the presence of separated cells or fragments. Day 5 embryos were scored using criteria including embryonic stage, cavitation, inner cell mass, and cell morphology. When no fragmentation was evident and the developmental stage was appropriate for their age, embryos were described as high grade and given an embryo score of 1. Embryos showing developmental delay and more than 50% fragmentation were described as low grade and scored as 4. Embryos of better and worse intermediate quality were given embryo scores of 2 and 3, respectively.

In the case of a positive urine pregnancy test (biochemical pregnancy), an ultrasound scan was carried out 5–6 wk after oocyte retrieval to determine the viability of the pregnancy. A second ultrasound was performed at 12 wk gestation to confirm an ongoing pregnancy (positive heart beat).

Hormone assays

Blood samples were centrifuged within 2 h after withdrawal and stored at –20 C until assayed. Serum FSH, LH, and P levels were assessed by chemiluminescent immunoassay (Immulite, Diagnostic Products, Los Angeles, CA). Serum E2 levels were measured using RIA kits (Diagnostic Products), as described previously (21). Dimeric inhibin B levels were determined using an immunoenzymometric assay (Serotec, Oxford, UK), as described previously (22). Intra- and interassay coefficients of variation were less than 5% and 7% for FSH, less than 5% and 6% for LH, less than 10% and 10% for P, less than 5% and 7% for E2, and less than 8% and 14% for inhibin B, respectively.

Data analysis

The power calculation for this study was based on E2 levels on the day of hCG administration, because E2 provides a measure of ovarian response and correlates with the number of follicles and oocytes. Previous studies from our own group have indicated that late follicular phase mean E2 levels between 2800 and 4500 nmol/liter do not represent clinically important differences in ovarian response (18, 23), whereas concentrations below this range are associated with poor outcome. To detect whether a given stimulation protocol was associated with reduced ovarian response compared with the other stimulation protocols, represented by E2 levels below 2800 nmol/liter (with 90% power and $P < 0.05$), at least 120 patients (40/group) were needed. Eligibility for analysis after inclusion or protocol violations was decided by a third party, blinded for the randomization protocol.

Results are presented as the median and range unless otherwise indicated. Comparisons of outcome measures among the three randomized groups were performed using the Kruskal-Wallis H test for continuous data and the χ^2 test for binary variables. Two-group comparisons were made using the Mann-Whitney U test. Pearson's correlation coefficients were calculated. P values are two-sided, and $P < 0.05$ was considered the limit of statistical significance. Data were analyzed using the commercially available software package SPSS (SPSS, Inc., Chicago, IL).

Results

Subjects and baseline characteristics

Of the 169 patients randomized, 13 patients failed to start IVF treatment within the study period. Fourteen patients were excluded from analysis because of inclusion (n = 4) and protocol (n = 10) violations (4 of whom conceived). The remaining 142 patients (45, 48, and 49 patients for groups A, B, and C, respectively) were included in the data analysis.

With regard to the distribution of age, body mass index,

TABLE 1. Patient characteristics and clinical IVF outcome (median and range) of a randomized comparison between a GnRH agonist protocol and two GnRH antagonist protocols commencing rFSH on cycle d 2 or cycle d 5, respectively

	GnRH agonist long protocol (n = 45)	GnRH antagonist rFSH d 2 start (n = 48)	GnRH antagonist rFSH d 5 start (n = 49)	P^a
Included patients (n = 142)				
Age (yr)	33 (25–39)	33 (26–38)	33 (24–39)	0.36
Body mass index (kg/m^2)	23.0 (19.6–28.1)	24.2 (19.7–28.4)	22.9 (19.7–29.0)	0.08
$FSH_{day\ 2/3}$ (IU/liter)	5.5 (1.0–10.8)	6.3 (2.0–16.0)	6.3 (2.1–29.4)	0.38
Inhibin $B_{day\ 2/3}$ (ng/liter)	124 (21–292)	104 (11–260)	104 (18–727)	0.12
Patients undergoing oocyte retrieval (n = 104)				
n (% per started cycle)	38 (84%)	35 (73%)	31 (63%)	0.02
Cycle day start cetrorelix[b]		8 (5–12)	10 (8–14)	0.007
Day hCG				
FSH (IU/liter)	6.5 (2.8–11.3)	6.6 (4.6–9.3)	6.8 (3.5–12.7)	0.66
LH (IU/liter)	1.0 (0.1–2.6)	0.7 (0.1–5.3)	1.5 (0.1–5.8)	0.09
E2 (nmol/liter)	3,407 (850–10,347)	2,555 (653–8,045)	3,193 (901–14,992)	0.23
P (nmol/liter)	2.5 (0.6–6.1)	2.8 (0.8–6.1)	3.1 (0.7–7.3)	0.38
No. of follicles (≥10 mm)$_{day\ hCG}$	10 (4–21)	8 (3–27)	8 (3–22)	0.07
No. of follicles (≥15 mm)$_{day\ hCG}$	5 (3–17)	5 (3–11)	4 (3–10)	0.20
No. of oocytes retrieved	9 (1–25)	8 (2–31)	7 (1–27)	0.57
No. of embryos	4 (0–16)	4 (0–13)	3 (0–19)	0.99
Fertilization rate per subject (%)	50 (0–100)	54 (0–100)	68 (0–100)	0.12
No. of pregnancies (%)[c]	10 (22%)	10 (20%)	10 (20%)	0.96
No. of ongoing pregnancies (%)[d]	8 (18%)	8 (17%)	8 (16%)	0.98
No. of twin pregnancies (%)[e]	3 (38%)	2 (25%)	3 (38%)	0.83

[a] Kruskal-Wallis H test for continuous data; χ^2 test for binary variables.
[b] Largest follicle ≥14 mm.
[c] Defined as a positive urine hCG test (percentage per started cycle).
[d] Defined as positive heart beat on ultrasound at 12 wk gestation (percentage per started cycle).
[e] Percentage of ongoing pregnancies.

and baseline serum concentrations for FSH and inhibin B, no significant differences were found among the three groups (Table 1). There was no difference among the three groups in cycle length [median cycle lengths in groups A, B, and C, 28 d (range, 25–35), 28 d (range, 25–33), and 28 d (range, 25–33), respectively; $P = 0.69$] or the duration of infertility [median durations in groups A, B, and C, 3.5 yr (range, 0.6–12.0), 3.2 yr (range, 0.5–8.1), and 3.3 yr (range, 0.3–14.0), respectively; $P = 0.19$]. Moreover, no difference was observed in the percentage with primary infertility (for groups A, B, and C, 71%, 73%, and 65%, respectively; $P = 0.70$) or the percentage of patients undergoing IVF/ICSI (for groups A, B, and C, 22%, 15%, and 18%, respectively; $P = 0.64$). In the IVF (without ICSI) patients no difference was found in the sperm quality of the partner [median total motile sperm count per ejaculate for groups A, B, and C, 45×10^6 (range, 1.1–420.0), 40.0×10^6 (range, 0.3–220.0), and 38.8×10^6 (range, 2.0–180.0), respectively; $P = 0.94$], the incidence of males with a total motile sperm count below 5.0×10^6 (by χ^2 test, $P = 0.57$; data not shown), or the distribution of causes of infertility (by χ^2 test, $P = 0.55$; data not shown).

Outcome

Clinical outcome parameters comparing groups A, B, and C are shown in Table 1 and Fig. 2. A high cancellation rate because of failure to meet criteria for oocyte retrieval was observed in group C. Low response leading to cancellation of the cycle in this group correlated with an increased age and higher early follicular phase FSH concentrations [median age, 35 yr (range, 28–39) *vs.* 33 yr (range, 24–39); $P = 0.001$; median FSH, 8.0 IU/liter (range, 2.9–29.4) *vs.* 5.8 IU/liter (range, 2.1–21.0); $P = 0.02$; for low and normal response patients, respectively]. In patients with successful stimulation resulting in oocyte retrieval, the total amount of rFSH used and the duration of FSH stimulation decreased from group A to group C [Fig. 3; median total amount of rFSH used for groups A, B, and C, 1650 IU (range, 1050–2250), 1350 IU (900–2100), and 1200 IU (900–1950), respectively; $P < 0.001$; groups B and C, 18% and 27% decreases, respectively, *vs.* group A]. This difference in total amount of rFSH was more pronounced when the cancelled cycles were included according to an intention to treat analysis [median total amount of rFSH used for groups A, B, and C, 1650 IU (range, 1050–2250), 1425 IU (range, 900-2100), and 1050 IU (range, 450-1950), respectively; $P < 0.001$; groups B and C, 14% and 36% decreases, respectively, *vs.* group A]. Although none of the participants reaching criteria for oocyte retrieval demonstrated a premature LH surge (LH >10 IU/liter), three patients (two from group B and one from group C) ovulated after hCG administration before oocyte retrieval. In one patient (group C) ET was not carried out due to an imminent ovarian hyperstimulation syndrome.

The number of follicles on the day of hCG administration, the number of oocytes retrieved, and the number of embryos in patients in whom oocytes could be retrieved are given in Table 1. After oocyte retrieval, the best embryo was more

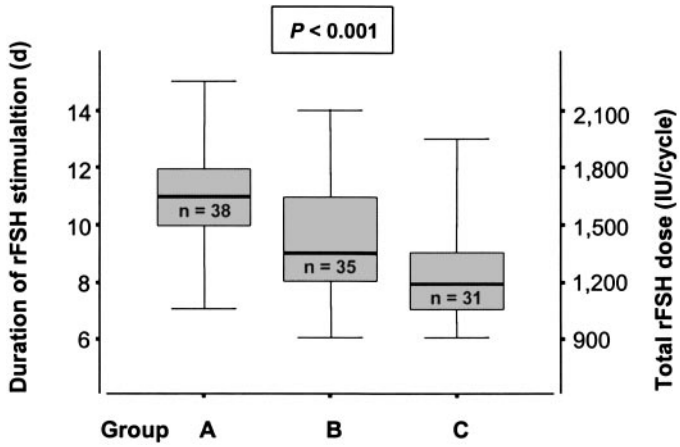

FIG. 3. Duration of rFSH stimulation (in days) and the total dose of rFSH used (in international units per cycle) in 104 patients undergoing oocyte retrieval, comparing three different ovarian stimulation protocols for IVF: a GnRH agonist long protocol (group A) *vs.* two GnRH antagonist protocols, starting rFSH on either cycle d 2 (group B) or cycle d 5 (group C). As a fixed dose of rFSH (150 IU/d) was used, the total amount of rFSH used was directly related to the length of stimulation. *Boxes* indicate 25th and 75th percentiles, with the *horizontal line* representing the median value. Whiskers span the range observed. Comparisons were performed using the Kruskal-Wallis H test.

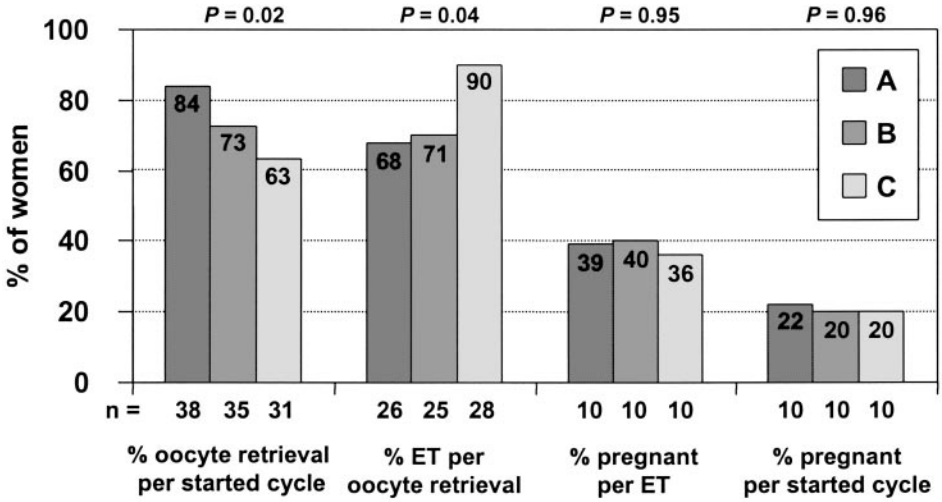

FIG. 2. Percentage of women undergoing oocyte retrieval per started IVF cycle, percentage of women undergoing ET per oocyte retrieval and per started cycle, pregnancies per ET, and pregnancies per started IVF cycle, comparing a GnRH agonist long protocol (group A) with two GnRH antagonist protocols with start of exogenous FSH on cycle d 2 (group B) or cycle d 5 (group C). Comparisons were performed using the χ^2 test.

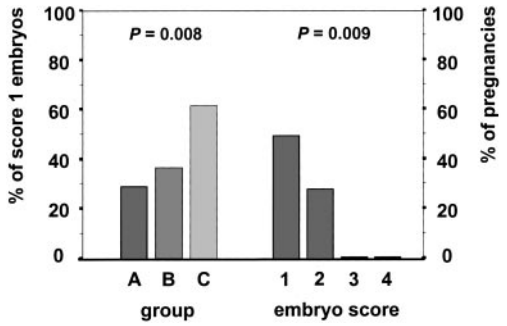

FIG. 4. Bar diagrams representing the percentage of embryos with a score of 1 (see *Subjects and Methods* for definitions) after oocyte retrieval in 104 patients, comparing a GnRH agonist long protocol (group A; n = 38) with two GnRH antagonist protocols commencing rFSH on cycle d 2 (group B; n = 35) or cycle d 5 (group C; n = 31; *left panel*) and the pregnancy rate per embryo score of the best embryo transferred in 79 patients undergoing ET (*right panel*). Comparisons were performed using the χ^2 test.

frequently scored as 1 in group C (Fig. 4; groups A, B, and C, 29%, 37%, and 61%, respectively; $P = 0.008$). For the overall group the quality of the best embryo available for transfer was directly related to the pregnancy rate (Fig. 4; $P = 0.009$), independent from the use of a GnRH agonist or antagonist (implantation rate when embryo score of best embryo is 1 or 2, 40% for GnRH agonist *vs.* 39% for GnRH antagonist; $P = 1.0$). Fewer cycles in group C were cancelled after oocyte retrieval because of total fertilization failure or abnormal embryo development (groups A, B, and C, 32%, 29%, and 7%, respectively; $P = 0.03$). For IVF without ICSI, the occurrence of total fertilization failure was related to sperm quality [median total motile sperm count, 47.5×10^6 (range, 2.0–420.0) *vs.* 17.0×10^6 (range, 0.3–75.0), respectively; $P = 0.008$]. Abnormal embryo development was not related to sperm quality (data not shown). The median day of ET was not different among the three groups ($P = 0.93$; data not shown). There was no statistically significant difference in the quality of the best embryo transferred among the three groups (percentage of embryo score 1 of best embryo for groups A, B, and C, 42%, 52%, and 64%, respectively; $P = 0.11$).

Figure 5 shows the number of pregnant or nonpregnant patients per number of oocytes retrieved, comparing groups A, B, and C. In 6 of 10 pregnant women in group C, 4 or fewer oocytes were retrieved, whereas this occurred in none of the pregnant women in group A and in just 1 patient in group B ($P < 0.01$). With regard to (ongoing) pregnancy rate per started cycle, no differences were found among the groups (Table 1 and Fig. 2). The overall pregnancy rates of the 142 study patients were 21%/started cycle, 29%/oocyte retrieval, and 38%/ET.

Endocrinology

Table 1 shows E2 levels on the day of hCG in patients reaching oocyte retrieval. Figure 6 shows the serum FSH concentrations during the follicular phase of the treatment cycle in all patients reaching criteria for oocyte retrieval, comparing groups A, B, and C. In groups A and B, FSH concentrations increased between cycle d 2 and 5 [median

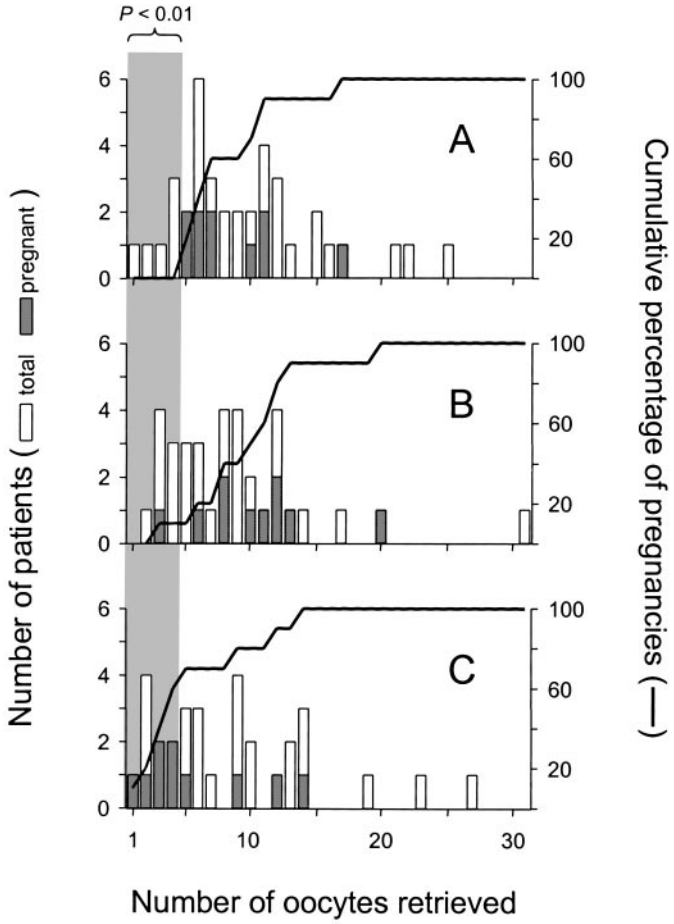

FIG. 5. Relation between the number of oocytes retrieved and pregnancy, comparing a GnRH agonist long protocol (group A; n = 38) with two GnRH antagonist protocols with start of rFSH on cycle d 2 (group B; n = 35) or cycle d 5 (group C; n = 31). *Bars* represent the total number of patients (□) and pregnant patients separately (■) per number of oocytes retrieved. *Lines* represent the percentage of pregnant women, cumulative over the number of oocytes obtained. The *shaded area* represents patients in whom four or fewer oocytes were obtained and for whom the chance of pregnancy is significantly different comparing groups A, B, and C (by χ^2 test, $P < 0.01$).

FSH d 2 *vs.* d 5 for group A, 5.1 IU/liter (range, 2.6–9.9) *vs.* 7.3 IU/liter (range, 3.5–13.0); $P < 0.001$); for group B, 6.2 IU/liter (range, 2.0–10.9) *vs.* 6.9 IU/liter (range, 3.8–14.4); $P = 0.02$] and decreased between cycle d 5 and day of hCG administration (median FSH d 5 *vs.* day of hCG, data presented above and in Table 1; $P = 0.04$ for group A; $P = 0.02$ for group B). However, in group C, FSH concentrations remained constant during the follicular phase [median FSH on d 2, 5.9 IU/liter (range, 2.1–21.0); on d 5, 5.7 IU/liter (range, 3.2–15.9); $P = 0.98$; day of hCG, 6.8 IU/liter (range, 3.5–12.7); $P = 0.08$].

Discussion

The concept of extending the FSH window to induce the development of multiple dominant follicles for IVF by administering exogenous FSH from the midfollicular phase onward combined with the use of GnRH antagonists constitutes a novel strategy for mild ovarian stimulation. As

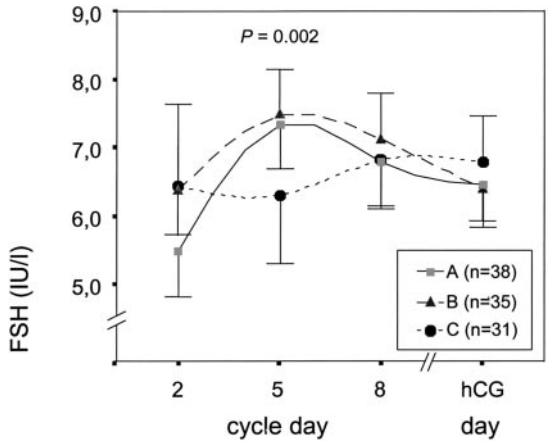

FIG. 6. FSH serum concentrations (mean ± SEM) in the follicular phase of an IVF stimulation cycle in 104 patients undergoing oocyte retrieval, comparing a GnRH agonist long protocol (group A) with two GnRH antagonist protocols, with start of rFSH on cycle d 2 (group B) or cycle d 5 (group C). The time scale on the x-axis is divided into cycle days (group A: day of start rFSH = cycle d 1; groups B and C: day of onset of menses = cycle d 1) and day of administration of hCG. FSH on cycle d 5 is statistically different comparing groups A, B, and C.

GnRH antagonist action is characterized by an immediate suppression of pituitary gonadotropin release (5, 24), these compounds offer the opportunity to commence the IVF treatment cycle in an undisturbed menstrual cycle. This study shows that the stimulating capacity of endogenous FSH can be exploited during the early follicular phase for the cyclic recruitment of a cohort of early antral follicles. Commencing exogenous FSH during the midfollicular phase results in multiple dominant follicle development despite a substantial reduction in the amount of exogenous FSH required.

In the GnRH antagonist d 5 group, significantly more cancellations due to low response were observed compared with the GnRH agonist and GnRH antagonist d 2 groups. We observed a significant difference in age and baseline FSH, between the cancelled patients and those who met criteria for oocyte retrieval in the GnRH antagonist d 5 group. This may indicate that a subgroup of women likely to have a low response to mild stimulation can be identified before the onset of the IVF. In contrast to previous reports, this difference in age and FSH between low and normal response patients was not found in the GnRH agonist or the GnRH antagonist d 2 groups. This is probably due to the relatively low number of patients involved. Compared with previous studies (17, 25, 26), we observed a relatively high cancellation rate (16% and 27%) before oocyte retrieval in both the GnRH agonist and the GnRH antagonist d 2 groups. This may be explained by differences in patient selection for IVF and differences in treatment protocols, as we used a fixed dose of exogenous FSH. In addition, cancellation criteria in minimal stimulation protocols may need to be revised due to the observed pregnancies in women with a low oocyte yield.

For those patients in the GnRH antagonist d 5 group who met criteria for oocyte retrieval, the duration of stimulation was shorter than in both other groups, resulting in a 27% reduction in the total amount of exogenous FSH required. The 14% reduction in the total amount of exogenous FSH used comparing the GnRH antagonist d 2 *vs.* the GnRH agonist group was comparable to differences observed in previous studies (13–17). A reduction in exogenous FSH may affect ovarian response, usually assessed by the number of follicles that have developed and the E2 levels during the late follicular phase. A reduced ovarian response will result in fewer oocytes after retrieval and probably a reduction in the number of resulting embryos. Given the close correlation between the number of embryos from which to select and the chance of pregnancy (1), pregnancy rates may suffer from less profound stimulation protocols. We did observe a tendency toward the development of fewer dominant follicles (≥10 mm) in the GnRH antagonist d 5 group. However, as no difference was found between the development of larger follicles (≥15 mm), mainly contributing to the production of E2 (27), no difference was observed in E2 levels on the day of hCG administration among the three studied stimulation protocols. Thus, in those patients achieving multifollicular growth after extending the FSH window, a normal ovarian response was seen. The concern that fewer oocytes will be obtained after milder stimulated cycles resulting in fewer pregnancies is therefore not supported by our findings. Despite a tendency toward the development of fewer dominant follicles, no difference was seen in the number of oocytes retrieved.

The median fertilization rate per subject did not differ among the three groups, and consequently, the median numbers of embryos obtained were comparable. However, as more patients in the GnRH agonist and GnRH antagonist d 2 groups were cancelled because of a total fertilization failure or abnormal embryo development, patients in the GnRH antagonist d 5 group presented with a better chance of undergoing ET, compensating for the observed increased cancellation rate. Compared with previous studies reporting transfer rates after oocyte retrieval between 80–95% (25, 26), 68–90% of the patients participating in the current study underwent ET. This difference may be explained by the relatively small proportion of patients undergoing ICSI (18%) and differences in patient inclusion, laboratory performance, and the small number of patients in the current study. When the criteria for oocyte retrieval were met in the current study, the chance of subsequently producing good quality embryos was significantly increased in the GnRH antagonist d 5 group. Milder stimulation may result in selection of good quality oocytes, which may result in better quality embryos. Although embryo quality is not the only factor determining implantation rate, embryo score is predictive of pregnancy (28). Our study provides further confirmation that high quality embryos are more likely to implant and result in pregnancy. Despite a better chance of retrieving high quality embryos, the patients in the GnRH antagonist d 5 group demonstrated a comparable pregnancy rate per ET. It has been suggested that the use of GnRH antagonists is associated with reduced implantation rates compared with conventional protocols (5). This could not be confirmed in the current study, which may be related to the flexible GnRH antagonist protocol applied.

Overall, the pregnancy rate per started IVF cycle was comparable in the three groups, with a pregnancy rate of 21%/started cycle for all patients together. Despite our relatively high cancellation rates during the stimulation phase or after

oocyte retrieval, these results are comparable to the percentages reported elsewhere. European studies report pregnancy rates per started cycle varying between 16–26%, with a mean of 21% (25). American studies tend to report higher percentages of pregnancies per started cycle (delivery rate per started cycle of 25%), but at the cost of extended, complex, and expensive stimulation protocols and increased chances for higher order multiple pregnancies due to the transfer of more than two embryos (26). Many studies from individual centers only mention pregnancy rates per oocyte retrieval or per ET, which are in the same range as the overall pregnancy rates observed in the current study (29%/oocyte retrieval and 38%/ET).

A low response during ovarian stimulation is currently believed to represent ovarian aging and poor oocyte quality (29–31). However, a low number of oocytes after mild stimulation may constitute a normal response, resulting in high quality oocytes and embryos. In our study the response of four or fewer oocytes after profound ovarian stimulation (group GnRH agonist or GnRH antagonist d 2) observed in 19% of the patients was indeed associated with impaired pregnancy outcome (only 7% of these patients conceived). However, after mild stimulation, the presence of four or fewer oocytes (observed in 29% of the patients) was associated with a good chance of pregnancy (67% of these patients conceived). This indicates that a low number of oocytes obtained after minimal stimulation may represent a selection of oocytes more likely to result in pregnancy. The low total dose of exogenous FSH may only stimulate the most mature follicles to ongoing growth, allowing a degree of selection of oocytes to occur.

The advantages of mild ovarian hyperstimulation for IVF are being increasingly recognized (2, 3, 32). The reduction in the duration of ovarian stimulation (fewer injections) combined with fewer side-effects (14) diminish patient discomfort and reduce costs. Milder stimulation may require less monitoring, because short-term complications and long-term risks are expected to be reduced, although this remains to be established in a larger series of patients. Despite these advantages, a lower ovarian response after milder stimulation resulting in an increased cancellation rate before oocyte retrieval has been put forward as an argument against the use of these protocols and restrained its introduction into clinical practice. However, despite the higher rate of cancellations in the GnRH antagonist d 5 group, the overall outcome did not differ among the three groups. This raises the question of whether this higher incidence of low ovarian response, and thus higher cancellation rate before oocyte retrieval, is, in fact, detrimental for overall IVF outcome. The cancelled patients in the GnRH antagonist d 5 group may represent those with a poor chance of a high quality ET, with impaired chances to conceive in IVF. As cancellation after mild stimulation can partly be predicted by age and early follicular FSH concentrations (two markers of ovarian reserve), low response after mild stimulation may allow patient selection before oocyte retrieval, avoiding subsequent procedures that are unlikely to lead to pregnancy. Moreover, cancellation of a cycle after a short stimulation should be viewed differently from cancellation after a prolonged stimulation period. This earlier selection of poor prognosis patients may improve overall health economics of IVF for the majority of patients.

The finding that a low number of oocytes obtained after minimal stimulation is associated with good pregnancy chances indicates that a large number of oocytes is not required for a successful IVF program. Although the criteria for oocyte retrieval did not differ among the protocols in this study, in retrospect these findings suggest the need for an adjustment of minimal criteria for oocyte retrieval after milder stimulation. A physiological reduction in the number of oocytes generated after mild ovarian stimulation distinctly differs from a pathological reduction associated with ovarian aging. The clinical introduction of GnRH antagonists allows a more physiological approach to ovarian stimulation. Moreover, the trend toward single ET, avoiding multiple pregnancies (33, 34), reduces further the need for high numbers of oocytes and embryos. The present study demonstrates the clinical applicability of the concept of extending the FSH window for ovarian stimulation in IVF. However, the full clinical potential of the described mild stimulation protocol requires confirmation in larger multicenter studies.

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