Superovulation with a High Gonadotropin Dose for In Vitro Fertilization: Is It Effective?

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Purpose: *Our purpose was to investigate the effect on the ovarian response of increasing the gonadotropin dose.*
Methods: *We analyzed retrospectively the in vitro fertilization data for patients who had two cycles of treatment, with a higher dose in cycle 2. The patients were stratified according to age, ovarian response, and gonadotropin dose in the first cycle. The main outcome measure was the number of follicles, eggs, and embryos and the peak estradiol (E_2) level.*
Results: *The study included 244 patients. Patients in both age groups (n = 118, ≤33 years; n = 126, >33 years), low (n = 66) and intermediate (n = 145) responders, and patients who received <225 IU follicle-stimulating hormone (n = 175) in cycle 1 had a better response in cycle 2. However, the high responders (n = 33) and those who received 225 or 300 IU follicle-stimulating hormone (n = 69) in cycle 1 showed a similar response in both cycles, except for a significantly higher E_2 level in cycle 2.*
Conclusions: *Our results indicate that exceeding a daily dose of 300 IU is unrewarding.*

KEY WORDS: gonadotropin dose; in vitro fertilization; ovarian response.

INTRODUCTION

Controlled ovarian hyperstimulation is used in in vitro fertilization (IVF) to induce the development of multiple preovulatory follicles, allowing the subsequent collection of a number of mature oocytes. The starting dose of follicle-stimulating hormone (FSH) is generally determined on an individual patient basis, but the ability of known determinants of outcome, such as patient age and basal serum FSH concentration, to predict the ovarian response to a given dose of FSH is poor. The initial stimulatory dose of FSH is often increased in an attempt to improve the oocyte yield in subsequent cycles. In an early study, Lauffer *et al.* (1983) used a relatively high dose of human menopausal gonadotropin in their stimulation regimen in an effort to enhance pregnancy rates by increasing the number of oocytes obtained and thus embryos transferred per cycle (1). Although at least two cleaved embryos were obtained in more than 80% of the cycles, their pregnancy rate was comparable to that described previously with a more conservative approach (2). The relationship between the stimulating agent dose used and the number of eggs retrieved is nonlinear and remains one of the most difficult aspects of IVF cycle programming to predict accurately. Furthermore, in agreement with other reports of the value of increasing the dose of stimulating agent on the number of eggs and the IVF-embryo transfer (ET) outcome in poor responders (3), our own experience, and that described anecdotally from other large IVF units, has been that increasing the dose of FSH above a threshold dose has been largely unrewarding in this group of patients.

In this study we analyzed our IVF data from 1987 to 1996 to assess the effect of increasing the dose of stimulating agent on the ovarian response and the duration of stimulation in the patients who had two cycles of treatment at the Oxford Fertility Unit. Throughout the long period during which these data were collected, we maintained a uniform protocol of "long suppression" with intranasal gonadotropin-releasing hormone agonist (GnRHa) and a preferred low-dose (150 IU) start for IVF superovulation.

MATERIALS AND METHODS

We reviewed the database of the Oxford IVF Unit, from April 1987 to July 1996. Patients who had cycles

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canceled or who had failed fertilization and those whose second cycle of treatment was longer than 2 years from the start of the first cycle were excluded from the study, as were patients who required micromanipulation for oocyte fertilization. The patients who had step-up or step-down stimulation were not included in the study. All diagnostic categories were included in the study.

The IVF-ET protocol has been described elsewhere (4). We used intranasal GnRHa's given daily from day 21 of the cycle (or 21 days after the start of a gestagen induced bleed if the patient was severely oligo- or amenorrheic). Confirmation of pituitary down-regulation after the analogue induced withdrawal bleed required a serum estradiol (E_2) level of <20 pg/ml (72 pM). A variety of commercially available gonadotropin preparations derived from human menopausal urine was used during the study period, with an increase in the use of highly purified preparations (mainly Metrodin HP; Serono UK Ltd.) from 1993 onward. Recombinant FSH preparations were not used during the study period.

For this analysis, patients were stratified according to their age in the first IVF cycle, the number of follicles produced in the first cycle, and the daily dose of gonadotropin given in the first cycle.

Age in the First Cycle

Two groups were identified, according to the female age in the first cycle: group 1, ≤ 33 years; and group 2, >33 years. The age cutoff was chosen to give two groups that were under or over 35 years of age by the completion of cycle 2.

Number of Follicles Produced in the First Cycle

The three groups were as follows: group A, 1–5 follicles (low responders); group B, 6–12 follicles (average responders); and group C, >12 follicles (high responders).

Starting Dose of FSH in the First Cycle

The two groups were as follows: group I, those who received less than 225 IU FSH per day; and group II, those who received 225 or 300 IU FSH per day.

Outcome Measures

For each of the stratifications defined above, the first and second cycles were compared with regard to daily dose of gonadotropin, duration of stimulation (days), total dose of gonadotropin, number of follicles aspirated, number of eggs, number of embryos, and serum E_2 level on the day of human chorionic gonadotropin (hCG) administration. Furthermore, we compared the above-mentioned measures among the first cycles in the age groups specified above. The daily dose in cycle 1 was compared among the three groups stratified according to response.

We also compared the clinical pregnancy rate per cycle (CPR/C), live birth rate (LBR), and implantation rate, in cycle 2 in the groups of each stratification. The clinical pregnancy rate is defined as the presence of a fetal heartbeat on ultrasound scan. The live birth rate is defined as the number of women giving birth to a live baby (babies) at ≥28 weeks of gestation. The implantation rate is defined as the number of fetal heartbeats detected on ultrasound scan divided by the number of embryos transferred.

Statistical Analysis

The statistical analysis was carried out using the Minitab for Windows statistical package (Minitab Incorporation, PA). The normality of distribution of the data was tested and demonstrated before analysis. The paired *t* test was used, with analysis of variance (ANOVA) being used when more than two groups were compared. The results are expressed as the mean ± SE (95% confidence interval). The chi-square test was used to compare proportions. A *P* value of less than 0.05 was considered significant.

RESULTS

Two hundred forty-four patients were eligible for the study. Sixty percent of the patients received the same gonadotropin preparation in both cycles.

The overall live birth rate per started cycle during the study period was 22.8%.

Analysis of the Patients Stratified According to Age

One hundred eighteen patients were 33 years of age or younger and 126 were older than 33 years of age when they underwent their first cycle. The mean age ± SE (95% CI) of the patients was 31.1 ± 0.2 (30.8:31.4) and 37.4 ± 0.2 (37.1:37.7) in groups 1 and 2, respectively. In cycle 1 the patients in group 2 (older patients) received significantly higher daily and total doses ($P < 0.001$) than those in group 1, while the duration of stimulation was not significantly different.

Despite receiving lower daily and total doses in cycle 1, the patients in group 1 had a significantly higher number of follicles ($P = 0.02$) and embryos ($P = 0.03$) and higher E_2 levels ($P = 0.005$), while the number of eggs was not significantly different between the two groups ($P = 0.09$).

Both the younger and the older groups showed a similar response to an increased dose of gonadotropin in cycle 2 (Table I). The number of follicles, eggs, and embryos and the E_2 levels were all significantly higher in the second cycle. The duration of stimulation was not different between the two cycles of treatment for either age group. The clinical pregnancy rate per cycle (CPR/C), live birth rate (LBR), and implantation rate (IR) in cycle 2 were significantly higher in group 1 than group 2 ($P = 0.03$, 0.006, and 0.01, respectively (Table IV).

Analysis of Patients Stratified According to the Response in the First Cycle

The daily and total doses of gonadotropin were significantly higher in the second cycle in all three groups (Table II). However, the duration of treatment was shorter in cycle 2 in group B, while no significant difference was found in groups A and C with regard to the duration of stimulation in cycle 2. The number of follicles produced was significantly higher in the second cycle for all three groups, while the number of eggs and embryos and the E_2 levels were significantly higher in the second cycle in groups A and B but not in group C.

The CPR/C and LBR in cycle 2 for groups A, B, and C were not significantly different (Table IV). However, the implantation rate in group A was significantly higher than in groups B and C ($P = 0.02$), while groups B and C had similar IR in cycle 2. The three groups were significantly different with regard to age: A > B > C ($P < 0.001$). The mean age ± SE (95% CI) of the patients in the three groups was as follows: group A, 35.3 ± 0.4 (34.6:36); group B, 34.3 ± 0.2 (33.9:34.8); and group C, 32.7 ± 0.4 (31.8:33.5).

Analysis of Patients Stratified According to the Daily Dose in the First Cycle

Table III shows significant increases for all of the compared parameters in cycle 2 for the patients in group I (who were started on ≤150 IU/day in cycle 1). The starting daily doses of gonadotropin used in cycle 2 for group I patients were 75–187.5 IU/day (17 patients), 225 IU/day (96 patients), 300 IU/day (52 patients), and 375–450 IU/day (10 patients). Thus only 5.7% of group I patients received more than 300 IU/day in cycle 2.

In group II, 48 patients received 225 IU/day FSH and 21 patients received 300 IU/day in their first treatment cycles. The daily and total doses and E_2 levels were significantly higher in the second cycle but the other compared parameters, which reflect the number of oocytes and embryos and the duration of stimulation, showed no significant difference despite the higher dose of gonadotropin used in the second cycle. The CPR/C, LBR, and IR in cycle 2 were higher in group

Table I. Stratification by Age[a]

	Group 1 (<33 years) ($n = 118$)			Group 2 (>33 years) ($n = 126$)		
	Cycle 1	Cycle 2	P (1 vs 2)	Cycle 1	Cycle 2	P (1 vs 2)
Daily dose (IU of FSH)	158.3 ± 3.8 (151.6:165)	267.3 ± 8.2 (251.1:283.4)	<0.001	189.9 ± 5.2 (179.5:200.3)	305.9 ± 8.6 (289:322.9)	<0.001
Duration	9.9 ± 0.2 (9.6:10.2)	9.7 ± 0.2 (9.4:10.1)	ns[b]	10 ± 0.2 (9.7:10.3)	9.6 ± 0.1 (9.4:9.9)	0.07
Total dose (IU of FSH)	1566.7 ± 40.8 (1485.9:1647.4)	2595.2 ± 94.3 (2408.4:2781.9)	<0.001	1893.2 ± 60.3 (1773.9:2012.4)	2940.5 ± 92.4 (2757.6:3123.3)	<0.001
Follicles	8.7 ± 0.4 (7.9:9.5)	10.6 ± 0.5 (9.7:11.5)	0.001	7.5 ± 0.3 (6.9:8)	8.9 ± 0.4 (8:9.7)	<0.001
Eggs	5.9 ± 0.3 (5.3:6.6)	7.8 ± 0.3 (7:8.5)	<0.001	5.3 ± 0.2 (4.8:5.7)	6.4 ± 0.3 (5.7:7)	<0.001
Embryos	3.6 ± 0.2 (2.4:2.6)	4.3 ± 0.2 (2.6:2.8)	<0.001	3.1 ± 0.1 (2.3:2.5)	3.5 ± 0.1 (2.4:2.7)	0.003
Estradiol (pM)	3387 ± 173 (3045:3729)	4009 ± 199 (3615:4403)	0.02	2776 ± 130 (2519:3034)	3607 ± 184 (3243:3971)	<0.001

[a] Results expressed as mean ± SE (95% CI).
[b] Not significant.

Table II. Stratification by Response[a]

	Group A (1–5 follicles) (n = 66)			Group B (6–12 follicles) (n = 145)			Group C (>12 follicles) (n = 33)		
	Cycle 1	Cycle 2	P	Cycle 1	Cycle 2	P	Cycle 1	Cycle 2	P
Daily dose (IU of FSH)	176.7 ± 6.6 (163.5:189.9)	321 ± 13.9 (293.3:348.8)	<0.001	178.7 ± 4.4 (170:187.4)	284.2 ± 7.2 (270:298.4)	<0.001	152.3 ± 6.5 (139:165.5)	232.9 ± 9.5 (213.6:252.3)	<0.00
Duration	10 ± 0.2 (9.5:10.4)	9.9 ± 0.3 (9.3:10.4)	ns[b]	10 ± 0.1 (9.8:10.3)	9.6 ± 0.1 (9.4:9.8)	0.004	9.6 ± 0.3 (9:10.2)	9.8 ± 0.4 (9:10.5)	ns
Total dose (IU.of FSH)	1749.4 ± 74.3 (1601:1897.9)	3187 ± 170 (2848:3527)	<0.001	1787.8 ± 50.5 (1688:1887.6)	2696.1 ± 69.4 (2559:2833.2)	<0.001	1493 ± 84.3 (1311.3:1654.6)	2289 ± 136 (2011:2566)	<0.00
Follicles	4 ± 0.1 (3.7:4.2)	4.3 ± 0.2 (6:8.1)	<0.001	8.2 ± 0.1 (8:8.5)	10 ± 0.4 (9.4:10.8)	<0.001	15.7 ± 0.5 (14.7:16.6)	13.7 ± 0.9 (11.9:15.4)	0.03
Eggs	3 ± 0.2 (2.7:3.4)	5 ± 0.4 4.3:5.8	<0.001	5.5 ± 0.2 (5.2:5.8)	7.3 ± 0.3 (6.7:7.9)	<0.001	10.9 ± 0.5 (9.9:12)	10 ± 0.6 (8.8:11.3)	ns
Embryos	2.5 ± 0.2 (2.2:2.8)	3.7 ± 0.3 (3:4.3)	<0.001	4 ± 0.2 (3.7:4.4)	5.3 ± 0.2 (4.8:5.8)	<0.001	8.3 ± 0.7 (6.8:9.7)	7 ± 0.5 (6:8.1)	ns
Estradiol (pM)	2011 ± 127 (1757:2266)	3276 ± 256 (2764:3789)	<0.001	3113 ± 129 (2858:3368)	3922 ± 169 (3587:4257)	<0.001	5025 ± 279 (4457:5593)	4337 ± 410 (3501:5174)	ns

[a] Results expressed as mean ± SE (95% CI).
[b] Not significant.

I compared to group II (P = 0.007, P = 0.02, and $P < 0.001$, respectively) (Table IV). The mean age of the patients in the two groups was 33.5 ± 0.2 (33.2:33.9) and 36.3 ± 0.3 (35.6:37) for group I and II, respectively; patients in group II were significantly older than those in group I ($P < 0.001$).

Table III. Stratification by Gonadotropin Dose[a]

	Group I (<225 IU/day) (n = 175)			Group II (225 or 300 IU/day) (n = 69)[b]		
	Cycle 1	Cycle 2	P (1 vs 2)	Cycle 1	Cycle 2	P (1 vs 2)
Daily dose (IU of FSH)	145.7 ± 1.3 (143.1:148.3)	252.2 ± 5.6 (241:263.3)	<0.001	247.8 ± 4.2 (239.5:256.2)	276 ± 9.7 (356.7:395.5)	<0.001
Duration	10 ± 0.1 (9.7:10.2)	9.6 ± 0.1 (9.3:9.8)	0.01	9.9 ± 0.2 (9.5:10.2)	10 ± 0.22 (9.5:10.4)	ns[c]
Total dose (IU of FSH)	1454.4 ± 23.6 (1407.8:1500.9)	2387.7 ± 54.9 (2279.3:2496.2)	<0.001	2460.7 ± 65.7 (2329.5:2591.8)	3749 ± 131 (3488:4009)	<0.001
Follicles	8.5 ± 0.3 (7.8:9)	10.5 ± 0.4 (9.8:11.2)	<0.001	7.2 ± 0.4 (6.4:8)	7.8 ± 0.5 (6.7:9)	ns
Eggs	5.8 ± 0.3 (5.3:6.3)	7.8 ± 0.3 (7.2:8.3)	<0.001	5 ± 0.3 (4.4:5.4)	5.3 ± 0.4 (4.6:6.1)	ns
Embryos	4.5 ± 0.2 (4:5)	5.7 ± 0.2 (5.3:6.2)	<0.001	3.6 ± 0.2 (3.2:4)	3.6 ± 0.3 (3:4)	ns
Estradiol (pM)	3144 ± 135 (2877:3411)	3921 ± 167 (3591:4252)	<0.001	2899 ± 176 (2548:3250)	3501 ± 222 (3059:3943)	0.001

[a] Results expressed as mean ± SE (95% CI).
[b] Twenty-one patients received 300 IU and 48 patients received 225 IU.
[c] Not significant.

Table IV. IVF Outcome in Cycle 2[a]

	Group A	Group B	Group C	Group 1	Group 2	Group I	Group II
CPR/C	20/66 (30%)	34/145 (23%)	7/33 (21%)	37/118 (31%)	24/126 (19%)	53/179 (30%)	9/70 (13%)
LBR	12/66 (18%)	28/145 (19%)	4/33 (12%)	31/118 (26%)	16/128 (13%)	41/179 (23%)	7/70 (10%)
IR	30/150 (20%)	47/389 (12%)	8/91 (9%)	54/318 (17%)	31/312 (10%)	78/471 (17%)	9/164 (5%)

[a] CPR/C, clinical pregnancy rate per cycle; LBR, live birth rate; IR, implantation rate.

DISCUSSION

The introduction of gonadotropin-induced superovulation in IVF treatment dramatically improved success rates above those obtained in the early days of oocyte collection in natural cycles. Increasing the number of oocytes generally allows for better selection of embryos for transfer and for possible cryopreservation.

At any time, the majority of follicles in the ovary are in the primordial resting state, with only a small number entering the pool of growing follicles. The initial growth phase, from the primordial stage until the antral stage (2–4 mm in diameter) is FSH independent (5). As the antral follicles grow, they develop the FSH receptors on their granulosa cells, entering the FSH-dependent phase (6). The aim of superovulation for IVF is to surpass the FSH threshold level of all follicles within the cohort, producing a group of mature, synchronous follicles after 10–15 days of treatment. The number of large follicles obtained will then depend on the number available in the growing pool at the start of the cycle (7). In an attempt to improve the outcome in poor responders (those who produced fewer than five dominant follicles measuring ≥16 mm in diameter), Land *et al.* (1996) doubled the dose in the second cycle. They reported a significant increase in the number of eggs but not in the number of embryos (3). However, Pantos *et al.* (1990) reported that increasing the human menopausal gonadotropin dose above 150 IU/day does not increase the number of eggs retrieved in poor responders (those who produced four or fewer eggs in their first cycles) (8).

We found that both the low responders (<6 follicles in their first cycles) and the average responders (6–12 follicles in their first cycles) had significantly higher numbers of eggs and embryos and higher E_2 levels in their second cycles, in which the dose of gonadotropin was increased. The high responders (>12 follicles in the first cycle) did not demonstrate any increase, in oocyte or embryo number or E_2 level after the dose increase presumably because the FSH threshold in the entire cohort of follicles was already exceeded in the first cycle. Nevertheless, it was interesting to find out that the clinical pregnancy rate per cycle and the live birth rate were similar in the three groups, despite the fact that they remained distinctively different in their response in the second cycle. Nonetheless, the implantation rate was significantly higher in the low responders compared to the moderate and high responders, which may reflect the better quality of the embryos in this group. This can be interpreted as that a higher number of eggs may be associated with a lower quality.

Both the younger and the older age groups responded in a similar manner when the dose of gonadotropin was increased in the second cycle, suggesting that the FSH threshold itself does not alter with increasing age. Rather, it seems that the number of recruitable preantral follicles present in the FSH-recruitable cohort at the start of the cycle declines with age, resulting in a smaller cohort size. Considering that the study is not randomized and the patients who achieved a live birth in their first cycle are not included, we are unable to comment on the effect of the dose increase on the clinical pregnancy and live birth rates. Nevertheless, it seems from the clinical pregnancy and live birth rates in cycle 2 that age has more impact than ovarian response (number of eggs) on the pregnancy outcome in IVF.

It is also notable that an improved response in cycle 2 was seen only if the starting dose of gonadotropin in cycle 1 was ≤150 IU. Those who were given 225 or 300 IU in cycle 1 did not perform any better in the subsequent cycle despite a dose increase, and the low implantation rate in these patients indicates that the embryo quality did not improve by the dose increase. This suggests that for most patients, 225–300 IU/day of FSH is the maximum effective dose for controlled ovarian hyperstimulation and that increasing the FSH dose above 300 IU/day after a poor response in the first cycle is probably pointless. This observation is of particular relevance at a time when recombinant FSH is being introduced, with concomitant large increases in the cost of gonadotropin treatment to the patient or the health-care system. We suggest that a national or international analysis of data from many centers using recombinant gonadotropin in IVF might establish a maximum effective dose for this preparation (probably 300 IU/day), which could then be used to rationalize funding for IVF treatment. Furthermore, because we have maintained high pregnancy rates with a few cases of moderate or severe ovarian hyperstimulation syndrome (one or two hospitalized cases per year throughout the study period), we argue that a starting dose of 150 IU per day in the first IVF cycle represents the best compromise between safety and efficacy for the younger IVF patient with a good prognosis for conception within one to three cycles of IVF treatment.

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