Endogenous LH Surge Versus hCG as Ovulation Trigger After Low-Dose Highly Purified FSH in IUI: A Comparison of 761 Cycles

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Purpose: *The results obtained with a protocol consisting of ovarian stimulation with low doses of highly purified FSH (FSH HP), administration of a GnRH analogue to induce an endogenous surge of gonadotropins, and IUI were evaluated. These results were compared with those seen with similar FSH stimulation and hCG administration followed by IUI.*
Methods: *Three hundred sixty-four patients scheduled for IUI, after inclusion in a total of 345 FSH HP/GnRH-stimulated cycles and 416 FSH HP/HCG-stimulated cycles, were studied. The stimulation protocol consisted of daily subcutaneous injection of 75 IU of FSH HP from day 3 or 5 of the cycle, depending on the duration of the spontaneous cycle. hCG was administered on days 0, +2, and +5 to support the luteal phase. Monitoring was conducted using circulating estradiol levels and vaginal ultrasonography. Administration of two s.c. doses of leuprolide acetate (LA) or 7500 IU of i.m. hCG when at least one 18-mm-diameter follicle was seen and estradiol levels reached 120 pg/ml per follicle with a diameter ≥16 mm. Intrauterine insemination was with semen capacitated by swim-up, thawed at room temperature if previously frozen.*
Results: *The ovulation rate was 99.28 after hCG and 99.23 with LA. No significant differences were seen between the estradiol and progesterone levels of both groups or in the estradiol/progesterone ratio. The duration of the luteal phase was similar in both groups. Pregnancy rates per cycle were 17.31% (hCG) and 27.25% (LA), respectively (P = 0.0007), and abortion rates 22.22% (hCG) and 24.47% (LA), respectively. No cases of ovarian hyperstimulation were seen.*
Conclusions: *After FSH HP administration according to a low-dose protocol, the use of LA to trigger a gonadotropin surge as a means of inducing ovulation in FSH-stimulated women could be a good alternative to improve the results and prevent ovarian hyperstimulation in IUI cycles.*

KEY WORDS: ovulation; highly purified follicle stimulating hormone; human chorionic gonadotropin; leuprolide acetate; intrauterine insemination.

INTRODUCTION

Triggering ovulation is a crucial aspect of ovarian stimulation when the latter precedes intrauterine insemination. The medication administered must be effective for inducing both the culmination of meiosis and the disintegration of the cumulus and rupture of the follicular wall, thus enhancing the release of fertilizable oocytes, allowing them to reach the fallopian tubes and be within reach of the sperm.

On the other hand, it is essential to avoid ovarian hyperstimulation—always a difficult complication in drug-induced ovulation. Ovarian hyperstimulation syndrome is an abnormal condition basically due to the development of a high number of follicles but usually present after hCG administration (1,2), particularly in cases in which an established pregnancy maintains this situation. Nonetheless, it can be seen after endogenous surges of LH.

Nevertheless, the above statement is not strictly accurate because, although it is true that hyperstimulation is practically dependent on the use of hCG, it is also true that it does not usually occur if prior ovarian stimulation with clomiphene, hMG, or FSH has not resulted in the development of multiple preovulatory follicles. The use of low-dose FSH has proved to be effective (3–5) to this end.

Finally, both FSH-induced follicular development and maturation and pharmacologic ovulation induction must allow the formation of a corpus luteum able

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to produce suitable progesterone levels. Nevertheless, pharmacologic support of the luteal phase could be useful in stimulated cycles to prevent an inadequate luteal phase.

Physiologically, pituitarian LH is the hormone responsible for the events associated with ovulation, but its pharmacologic use is not possible, because there is no suitable preparation available. hCG, a hormone that causes very similar events in the ovaries, is used as a drug substitute. Nevertheless, owing to certain structural differences between the two hormones (6), hCG action is more intense and prolonged than that of LH (7). Previous studies by our group (8–10) have shown, by comparing the effects of hCG and recombinant LH in rabbits, that the use of LH improved the quality of oocytes and embryos as well as the periovulatory endocrine environment. Some publications support the idea that triggering ovulation in women previously stimulated with gonadotropins by inducing an endogenous surge of LH, after the administration of GnRH or one of its agonists, can be a good alternative in assisted reproduction (11–13). All these were reasons to undertake this study, the objectives of which were (a) to evaluate the results of triggering ovulation by leuprolide acetate (LA) administration in FSH-stimulated cycles for intrauterine insemination (IUI); (b) to determine the circulating levels of FSH and LH after inducing the gonadotropin surge with LA in stimulated cycles, and (c) to compare them with those seen after hCG administration with the same purpose, in terms of estradiol and progesterone levels during the luteal phase as well as in terms of pregnancy and abortion rates.

MATERIALS AND METHODS

Patients

The minimum sample size required was calculated considering the null hypothesis that there were no differences in pregnancy rates between the treatment groups. Assuming a 15% pregnancy rate and 10% improvement in any of the groups after treatment, the null hypothesis would be rejected after the inclusion in each group of 270 evaluable treatment cycles (α = 0.05; β = 0.02; two-sided test and continuity correction). During the period September 1994–May 1996, a total of 364 consecutive patients entered this prospective nonrandomized study, which included 836 consecutive attempts (initiated cycles) and 761 stimulated cycles in all, defined as those in which ovulation-triggering therapy was administered. Seventy-five cycles were canceled (8.97%) and were not included in the study. The reasons for cancellation were nonresponse (23 cycles), spontaneous ovulation during stimulation (21 cases), personal decision (7 cycles), concomitant illness (6 cycles), growing ovarian cysts (5 cycles), an excessive number of follicles (4 cycles), patient mistake in following the treatment (3 cycles), and a developing follicle in the contralateral nonpatent tube (3 cycles). Three additional cases in which it was not possible to obtain a semen sample were also considered as cancellations and were not included in the study. The only criterion for inclusion was to have been scheduled for IUI at the infertility clinic. All the patients gave written informed consent. In 345 cycles, a GnRH agonist, leuprolide acetate (GnRHa), was administered; in the remaining 416, hCG was administered. Even though the protocol used was not randomized, no clinical criteria were considered to assign hCG or GnRHa administration.

Neither patients nor cycles were excluded from the study unless the cycle was canceled before triggering ovulation. Particularly, endometrial characteristics at ultrasound scan (thickness or type) were not a reason for cancellation.

The diagnosis of male factor was established according to the WHO criteria after the following tests: semen bacteriology, computerized semen analysis (Cellsoft CASA) and capacitation test (eventually hamster test and hemizona assay), morphology test according to Kruger's criteria, and MAR test (eventually serum antisperm antibodies). A computerized semen analyzer is included in a quality control program monthly and shows a good correlation with manual analysis. The diagnosis of female factor was established after the following tests: hematology and blood biochemistry, sexually transmitted disease serology, hormonal assessment of the cycle (FSH, LH, PRL, and SHBG in the early follicular phase and estradiol and progesterone in the midluteal phase), endometrial biopsy, antisperm antibody determination, histerosalpingography (in all patients), and laparoscopy (295 patients; 81.21%). The diagnosis of policystic ovary syndrome (PCOS) was established when patients showed chronic anovulation, abnormally high LH/FSH ratios on day 3 of the cycle, and/or abnormally high LH levels after the administration of 10 μg of i.v. GnRH (>34 μg/mL, as assessed by a control group), and ultrasound criteria (vaginal ultrasonography). Besides these criteria, others considered were normal androgenic adrenal function, assessed by SDHEA and T levels after ACTH stimulation. The clinical charac-

Table I. Clinical Characteristics of the Patients in Both Groups: Percentage or as Mean ± SE

	FSH HP/hCG	FSH HP/AL
No. of cycles	416	345
Age (X ± SE)	33.0 ± 3.6	32.2 ± 3.5
Years of sterility (X ± SE)	6.1 ± 3.1	6.2 ± 3.2
Primary sterility (%)	83.17	87.25
Secondary sterility (%)	16.83	12.75
Cycle ≤35 days (%)	81.01	80.29
Cycle >35 days (%)	18.99	20.71
BMI	22.9 ± 3.6	22.9 ± 3.3
Diagnosis		
Anovulation (%)	19.8	21.1
Cervical f. (%)	1.44	2.61
Idiopathic (%)	3.36	2.61
Male f. (%)	77.9	80.1

teristics of the patients are given in Table I, showing that there was no statistically significant difference in the parameters between the two groups (FSH HP/hCG and FSH HP/GnRHa). Minimal endometriosis was present in 41 cycles (5.39%) but it was never considered the main diagnosis.

Protocol for Stimulation and Ovulation Induction (Table II)

None of the patients had any treatment during the menstrual cycle previous to the stimulated cycle. After an ultrasound scan and estradiol determination, ovarian stimulation was routinely begun on the third day of the cycle in those patients whose menstrual cycles usually lasted less than 35 days and on the fifth day of the cycle in those with a cycle usually lasting longer than 35 days. Stimulation consisted of a daily subcutaneous injection of 75 IU of highly purified FSH (Neofertinorm, Laboratorios Serono, S.A. Madrid, Spain). In some cases of PCO patients having shown a hyperstimulation risk in a previously stimulated cycle, half an ampoule of FSH was administered daily at the beginning of the treatment. This dose could subsequently be increased or decreased by half an ampoule, depending on the ovarian response.

Table II. Number of Ampoules and Days Taken to Achieve Adequate Ovarian Stimulation: Parameters of Follicular Development (X ± SE)

	FSH HP/hCG	FSH HP/AL
No. of ampoules	9.56 ± 0.23	9.35 ± 0.22
Days' stimulation	8.70 ± 0.17	8.60 ± 0.15
No. of follicles ≥10 mm	3.38 ± 0.09	3.77 ± 0.11
No. of follicles ≥16 mm	1.81 ± 0.05	1.87 ± 0.05
Follicle diam. (mm)	19.43 ± 0.07	19.51 ± 0.07

Ovarian response to stimulation was controlled by measuring estradiol serum levels by RIA. The size of the ovaries, the number of follicles developed in each ovary, and the greatest diameter were all assessed by vaginal ultrasound. When considered necessary, the mean of two diameters of each follicle was evaluated. The amount, appearance, threadiness, and crystallization of the cervical mucus; the opening of the external cervical orifice; and the caryopicnotic index were also assessed. Endometrial development was studied as well. An ultrasound endometrial thickness greater than 8 mm was considered adequate.

Ovulation was triggered by administering two subcutaneous doses of 1.5 mg of leuprolide acetate (Procrín, Abbott, Madrid, Spain) 12 hr apart in 345 cycles (FSH/GnRHa) or by the intramuscular administration of 7500 to 10,000 IU of hCG (Profasi HP, Laboratorios Serono, S.A. Madrid, Spain) in 416 cycles. LA and hCG were administered when at least one follicle with a diameter ≥18 mm was seen, circulating estradiol levels reached the value of 120 pg/ml per follicle ≥16 mm in diameter and there was an adequate estrogenic effect, as judged by the cervicovaginal parameters and endometrial development. During the luteal phase, 1000 or 2500 IU of hCG were administered on days 0, +2, and +5, depending on the estradiol level on day −2, in the FSH/GnRHa cycles. The same procedure was followed on days +2 and +5 in the FSH/hCG cycles. Only 1000 IU of hCG was administered when estradiol levels on day −2 were higher than 1000 pg/ml.

Semen Preparation and Insemination

For insemination either fresh semen, obtained from the partner, or frozen semen, from a semen bank, was used. The cryopreserved samples were thawed at room temperature. All the samples were capacitated by swim-up. The percentage of fresh and cryopreserved samples and seminal parameters before and after capacitation are shown in Table III. Intrauterine insemination was systematically practiced 36 hr after the hCG injection or after the first LA injection. Therefore the day of ovulation induction was day −2, considering day 0 the day of ovulation.

Cycle, Ovulation, Pregnancy, and Abortion

Because FSH stimulation was common to both study groups, the main objective of the study was to assess

Table III. Seminal Parameters ($X \pm$ SE) and Percentage of Inseminations with Homologous (Partner) and Cryopreserved (Donor) Semen

	FSH HP/hCG	FSH HP/AL
Fresh semen		
Concentration (mol/ml)	47.50 ± 1.43	45.62 ± 1.61
% progressive motile sperm	31.77 ± 0.82	30.79 ± 0.95
Capacitated semen		
Concentration (mol/ml)	28.84 ± 1.22	26.27 ± 1.23
% progressive motile sperm	71.06 ± 0.79	67.31 ± 0.95
Cycles with homologous semen (%)	294 (70.7%)	229 (66.4%)
Cycles with cryopreserved semen (%)	122 (29.3%)	116 (33.6%)
REM (ins)	10.74 ± 0.52	9.44 ± 0.61

the two treatments used to trigger ovulation (hCG and GnRH) and the main parameters to be assessed were ovulation, pregnancy, and abortion rates. A cycle was defined as that in which hCG or GnRH were injected to trigger ovulation. Therefore treatments canceled before this occurred were not considered for the study; additionally three cycles in which the ovulation was triggered but insemination was not practiced were also excluded. No cycles were canceled, nor were any patients dropped from the study if the endometrial thickness was less than 8 mm.

Ovulation was considered to have occurred whenever there was pregnancy or when circulating progesterone levels exceeded 6 ng/ml during the luteal phase. The existence of pregnancy was established whenever at least one embryonary sac was seen in the ultrasound scan obtained 14 days after a β-hCG plasma level of more than 20 mU/ml. Biochemical pregnancies were thus excluded from the pregnancy and abortion rates.

Statistical Analysis

The different parameters obtained were reduced to their simple statistics: mean, standard deviation, and sample size for the quantitative variables and percentage and sample size for the qualitative variables. The values shown in the tables are given as means and standard errors of the mean, unless otherwise indicated.

The statistical analysis was performed on a Macintosh computer, using the SPSS 6.0 program. Statistically significant differences were established when there was a P value of less than 0.05. Student's t test was used for paired and nonpaired data, and Kolmogorov–Smirnoff's and Wilcoxon's tests and contingency tables were also used depending on the variables and their distribution. The chi-square test was used for independent groups and qualitative data.

Table IV. Circulating Levels of Estradiol (pg/ml; $X \pm$ SE)

Day of the cycle	FSH HP/hCG	FSH HP/AL
−10	49.35 ± 2.09	51.36 ± 1.60
−5	119.11 ± 3.25	130.76 ± 7.20
−2	297.93 ± 6.40	313.24 ± 9.98
−1	373.87 ± 9.14	442.12 ± 15.08
0	200.62 ± 6.27	219.57 ± 8.71
+2	130.31 ± 5.32	143.24 ± 7.52
+5	231.73 ± 9.68	252.71 ± 12.10
+8	237.78 ± 1.26	268.65 ± 48.56

RESULTS

Clinical Aspects

Ovarian stimulation was considered adequate in the FSH/GnRHa group after 8.60 ± 0.15 days of therapy and the injection of 9.35 ± 0.22 ampoules of Neofertinorm, after which, on the day LA was administered (day −2), 3.77 ± 0.11 ovarian follicles with a diameter of 10 mm or greater and 1.87 ± 0.05 follicles with a diameter of 16 mm or greater were seen; on this day, the diameter of the largest follicle was 19.51 ± 0.07 mm. In the FSH/hCG group, stimulation lasted 8.70 ± 0.17 days, using 9.56 ± 0.23 ampoules of Neofertinorm. At the end of stimulation, 3.38 ± 0.09 follicles of 10 mm or greater and 1.81 ± 0.05 follicles of 16 mm or greater were seen; the diameter of the largest follicle on day −2 was 19.43 ± 0.07 mm. No statistically significant differences were seen between the groups on comparing the various parameters.

Hormone Levels

The results of stimulation in terms of circulating estradiol and progesterone levels (Tables IV and V)

Table V. Circulating Levels of Progesterone (ng/ml; $X \pm$ SE)

Day of the cycle	FSH HP/hCG	FSH HP/AL
−1	0.77 ± 0.05	0.87 ± 0.05
0	1.52 ± 0.09	1.20 ± 0.07
+2	10.17 ± 0.36	9.97 ± 1.26
+5	24.07 ± 0.71	23.63 ± 0.85
+8	25.79 ± 1.26	25.48 ± 3.87

Table VI. Estradiol/Progesterone Ratio (X) Seen in the Two Groups Studied

Day of the cycle	FSH HP/hCG	FSH HP/AL
−1	849.37 ± 45.96	753.61 ± 39.27
0	215.00 ± 10.09	282.31 ± 15.72
±2	21.90 ± 2.70	25.55 ± 2.69
±5	11.60 ± 0.72	13.35 ± 1.10
±8	10.67 ± 0.74	78.10 ± 68.37

were almost superimposable. The difference seen in the estradiol/progesterone ratio (Table VI) was not statistically significant. The circulating levels of FSH and LH seen in the FSH/hCG group, both at baseline and at the end of stimulation, are shown in Table VII. A significant decrease ($P < 0.05$) in FSH levels was seen on days −1 and 0, whereas the LH values showed a small, but significant, increase on the same days. The behavior of the FSH and LH in the FSH/GnRHa group was substantially different. The circulating levels of these hormones and the LH/FSH ratio are also shown in Tables VII, VIII and IX. There was a sharp increase, which was significant, in both FSH and LH and an increase in the LH/FSH ratio. This increase corresponded to the pituitary surge of both hormones, where the LH surge was prevalent.

Table VII. Circulating Periovulatory Levels of LH (mUI/ml; $X \pm SE$; $n = 170$)

Day of the cycle	FSH HP/hCG ($n = 14$)	FSH HP/AL ($n = 170$)	P
−2	4.73 ± 3.27	5 ± 3.74	NS[a]
−1	10.42 ± 9.43	93.83 ± 40.93	<0.001
0	15.61 ± 7.68	17.67 ± 9.04	=0.054

[a] NS: not significant

Table VIII. Circulating Periovulatory Levels of FSH (mUI/ml; $X \pm SE$)

Day of the cycle	FSH HP/hCG ($n = 14$)	FSH HP/AL ($n = 170$)	P
−2	6.76 ± 2.11	6.68 ± 2.34	NS
−1	5.84 ± 2.56	22.55 ± 9.44	<0.01
0	5.80 ± 2.78	7.99 ± 3.40	=0.011

Table IX. Periovulatory Ratio of LH/FSH ($X \pm SE$)

Day of the cycle	FSH HP/hCG ($n = 14$)	FSH HP/AL ($n = 170$)	P
−2	0.72 ± 0.54	0.82 ± 0.77	NS
−1	1.99 ± 1.18	4.58 ± 2.37	<0.001
0	2.94 ± 1.83	2.36 ± 1.16	NS

Table X. Ovulation, Pregnancy, and Abortion Rates [n (%)] Seen in the Two Groups Studied

	FSH HP/hCG	FSH HP/AL
Attempts	457	379
Cancellations	41 (8.97%)	34 (8.97%)
Cycles	416	345
Ovulation rate	99.23	99.28
Pregnancy rate/cycle	72/426 (17.31%)*	94/345 (27.24%)*
1 gestational sac	66 (91.66%)	81 (86.17%)
2 gestational sacs	6 (8.33%)	13 (13.82%)
Abortion rate	16/72 (22.22%)	23/94 (24.46%)

* $P = 0.0011$.

Ovulation, Pregnancy, and Abortion Rates

Ovulation, pregnancy, and abortion rates are shown in Table X. The pregnancy rate per cycle was significantly higher in the FSH HP/GnRHa group (27.24%) than in the FSH HP/hCG group (17.31%) ($P < 0.01$). Regarding the remaining parameters, the differences seen between the groups were not statistically significant.

REMARKS, DISCUSSION, AND CONCLUSIONS

Various publications reporting triggering an endogenous surge of gonadotropins with the administration of a GnRH agonist have established the efficacy of this surge both for completing oocyte maturation (14–16) and for breaking the follicle wall (12,13). Experimental work by our own group (8–10) has also shown that both the quality of the oocytes and embryos and the endocrine environment can improve when pure urinary LH or recombinant human LH is used to trigger ovulation, compared to hCG administration. There is no need to discuss these aspects any further, as they have been more than sufficiently confirmed and would be explained by the qualitative differences between the biological actions of hCG and LH. These differences may, in turn, be due to the structural differences between both hormones.

This is, however, the first paper reporting on a large series in which this protocol was used and, in the light of the results obtained, some aspects are worth discussing. Like Tulchinsky *et al.* (13), we have used

two consecutive doses of the agonist, considering that with this condition, the gonadotropin surge would be more similar to the physiological surge halfway through the cycle. We have no objective support for this behavior, but it should be stressed that pregnancy rates are higher in Tulchinsky's series of 13 patients (31%) and in our series than in those of other authors who used a single dose (11).

The behavior of the circulating estradiol and progesterone levels seen in this study is similar to that seen in prior experimental work (10), although, as in the latter, the differences seen, which could be considered advantageous, fail to achieve statistical significance. In the FSH/hCG group, decreased FSH levels were seen on days −1 and 0. This decrease is most likely due to the discontinuation of therapy with this hormone. Nevertheless, in the FSH/GnRHa group, an increase was seen on these same days of the cycle. It has been suggested that the physiologic increase in FSH in the preovulation phase of the spontaneous cycle could play a beneficial role in the expansion of the cumulus, thus promoting the passage of the spermatozoa (17, 18).

In both groups, an increase in circulating LH levels was seen on days −1 and 0, after hCG or GnRHa administration. In the GnRHa group, this marked increase is obviously due to the pituitary surge of the hormone. Very plausibly, the discreet increase seen in the hCG group is also the result of an increase in the extent of the pituitary LH pulses, related to the high circulating estradiol levels seen at this point of the cycle. Increasing levels of LH are also seen in the late follicular phase of the natural cycle (19), and GnRH induces higher levels of LH during the late follicular phase (20, 21).

Obviously, ovulation may not occur if the GnRHa administered fails to induce the pituitary surge of gonadotropins. This occurred in three of the cycles included in our study (0.87%). We have no data available that would make it possible to evaluate these patients' pituitary susceptibility to GnRH, therefore we cannot elucidate whether the agonist was administered too early in these cases or whether there was really some disorder in gonadotropin release. Finally, ovulation rates were similar in both groups (>99%).

Pregnancy rates were significantly higher in the FSH HP/GnRHa group ($P < 0.001$) than in the FSH HP/hCG group. Because the clinical characteristics of the patients included in this large study were similar in both treatment groups and the follicular stimulation used was identical, showing no differences in terms of the total dose of FSH administered, length of the stimulation period, follicular dynamics, and steroid profiles during the follicular phase, it is possible to speculate that the higher implantation rate that led to the higher pregnancy rate observed in the FSH HP/GnRHa group was due to better oocyte/embryo quality in this group, or/and to better endometrial receptivity, as a consequence of a more physiologic endogenous surge of gonadotropins and steroid balance.

A high number of cycles has been seen in which the luteal phase was inadequate, after GnRHa administration to trigger ovulation (22–24). In our series this situation was not seen since in all the cycles, the luteal phase was supported by hCG, which we have always considered necessary.

Different publications have stated that the use of GnRHa to trigger ovulation in women previously stimulated with gonadotropins could help to prevent ovarian hyperstimulation. We have defended this approach (12), but we cannot support this hypothesis with any objective data. Probably, the fact that in our series there were no cases of hyperstimulation may be due to extremely careful management of subcutaneous FSH HP administration.

This large series of patients treated with FSH HP administered subcutaneously according to a slow-dose protocol prior of IUI confirms the low rate of complications (HOOS) to be expected. Furthermore, it appears that the use of GnRHa as described here can be considered a good alternative for drug induction of ovulation. Moreover, these findings should help to improve our knowledge of the physiology of ovulation.

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