Comparison of Recombinant and Urinary Follicle-Stimulating Hormone Preparations in Short-Term Gonadotropin Releasing Hormone Agonist Protocol for In Vitro Fertilization-Embryo Transfer

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Submitted: August 10, 2000

Accepted: November 17, 2000

Purpose: To compare the efficiency and efficacy of two recombinant human FSH (r-FSH) and urinary (u-FSH) preparations in patients undergoing superovulation for IVF-ET using a short-term gonadotropin releasing hormone agonist (GnRH-a) (Triptorelin) protocol.

Methods: A total of 88 women undergoing IVF-ET were included in this prospective study. They were randomized to receive u-FSH (150 IU/d), follitropin- α (100 IU/d), or follitropin- β (100 IU/d) for 2 days, and dosages were subsequently adjusted according to the ovarian response.

Results: The FSH dose required for the overall stimulation was significantly lower in patients treated with r-FSH than in those treated with u-FSH while serum FSH values were higher in the latter group. There were no statistically significant differences in ovarian response and IVF outcome between r-FSH preparations.

Conclusions: Recombinant FSH preparations have a higher efficiency than urinary ones in patients undergoing IVF-ET using a short-term GnRH-a protocol. In this situation, the two recombinant follitropins have comparable effectiveness.

KEY WORDS: Follitropin- α ; follitropin- β ; in vitro fertilization; recombinant human follicle-stimulating hormone; urinary follicle-stimulating hormone.

INTRODUCTION

Since the first introduction of recombinant human follicle-stimulating hormone (r-FSH) produced from CHO cells in 1996 (1), many studies have been performed in Europe to compare the clinical effectiveness of these new compounds to that of FSH preparations derived from the urine of postmenopausal women (u-FSH). In patients involved in IVF-ET procedures with a long-term GnRH analog protocol (GnRH-a), r-FSH preparations have been shown to yield a higher number of oocytes and embryos with a lower total dose of FSH (2,3). Furthermore, the pregnancy rate per started cycle or per fresh embryo transfer was significantly improved (4). Similarly, in patients treated for chronic anovulation (group 2 of the World Health Organization classification), recombinant FSH preparations are able to induce similar follicular recruitment with a lower amount of FSH dose and in a shorter period of time (5,6). More recently, r-FSH has also been shown to be more effective than u-FSH in inducing multifollicular development and achieving pregnancy in young low responders (7). Collectively, these data show that r-FSH preparations are more potent than urinary ones. This difference has been related to number of factors: higher purity and bioavailability, increased specific activity, more consistent composition with a basic isoelectric profile of molecules derived from recombinant DNA technologies (8).

Consequently, it was suggested that the starting dose of r-FSH could be reduced. In fact, it has been shown in patients enrolled in a long-term GnRH analog protocol that treatment outcome of a fixed

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daily dose of 150 IU r-FSH is comparable to a fixed daily dose of 225 IU u-FSH-HP (9). Finally, very few studies have been performed so far to evaluate the respective efficacy of the two r-FSH preparations now available for clinical use: follitropin- α (Gonal F; Ares Serono, Geneva, Switzerland) and follitropin- β (Puregon; Organon, Oss, the Netherlands). In recent clinical studies, Gonal F and Puregon seemed to be equally suitable for use in patients whose hypophyseal LH secretion has been previously down-regulated by a long-term administration of GnRH analogs (10–12). However, endogenous LH secretion may also affect the ovarian response to FSH (13). Thus, assessing the efficacy of r-FSH preparations in situations where LH hypophyseal secretion is not as such suppressed as following a long-term GnRHa administration could be relevant. To our knowledge, no trials have been done to compare the respective efficacy of these r-FSH preparations in patients treated with a follicular phase GnRHa *flare* regimen. Therefore, this study presents the first direct comparison of the efficacy of both r-FSH and u-FSH in women undergoing ovarian stimulation, using a short-term GnRHa protocol for IVF or ICSI.

MATERIALS AND METHODS

Patients

A total of 88 infertile women aged 18–38 years was enrolled in this prospective, randomized, single-center study. In Vitro Fertilization with or without microinjection was indicated for tubal, male or unexplained infertility. The study was performed according to the declaration of Helsinki and the European Community note in the European Community on Good Clinical Practice for trials on medical products (14). All women had baseline FSH concentrations within the normal range (5–12 IU/l) and those with only one ovary or ovarian endometriosis were excluded from this study.

Treatment Protocol

A short-term GnRH agonist protocol was conducted as previously described (14): A progestogen pretreatment (Norethisterone 10 mg/day) was given for 10–20 days during the luteal phase of the cycle preceeding IVF in order to program the oocyte retrieval. A daily s.c. injection of 25 μ g Dtrp6 GnRH (decapeptyl; Ipsen Biotech. Paris, France) was started

on day 1 of the next cycle (the IVF cycle) and continued until hCG administration. Patients were then allocated according to a list of random numbers. Allocation was performed locally using concealed method (envelope) and was not blinded. Patients received either 150 IU u-FSH (Metrodin HP; Serono, Boulogne, France) or 100 IU r-FSH [Follitropin- α (Gonal F; Serono, Boulogne, France) or follitropin- β (Puregon; Organon, Neuilly, France)]. The design of the study was decided on the basis of previous reports stating an estimated 30% higher efficiency of r-FSH in comparison with u-FSH (6). These fixed FSH doses were given at days 4 and 5 of the IVF cycle and subsequent FSH doses were adjusted from day 6 to the time of hCG administration according to the hormonal plasma levels and ultrasound data. In order to avoid any bias in relation to the knowledge of patients' allocation, a rigid protocol for FSH adjustment was applied in all patients. 10,000 IU of hCG (Gonadotrophines Chorioniques Endo; Organon, Neuilly, France) were injected 36 h prior to oocyte retrieval once at least three follicles reached a diameter of 17 mm. Luteal support was provided by giving micronized progesterone (400 mg) daily from the time of oocyte retrieval and by administering hCG (2500 UI twice at 3-day interval) from the day following embryo transfer in absence of any risk of hyperstimulation (serum oestradiol values lower than 2500 pg/ml on the day of hCG administration).

Ovarian response to stimulation was monitored from day 6 of the IVF cycle by ultrasound scan and serum oestradiol (E2), LH and progesterone (P) determinations. After oocyte retrieval, both number and maturity of the oocytes were evaluated. The fertilization rate, the number, and the quality of the embryos were also recorded on the following days. Diagnosis of clinical pregnancy was established by measurements of serial serum β hCG concentrations and visualization of fetal sac on subsequent ultrasound scan.

Hormonal Assays

Radioimmunoassays were used to measure serum E2 and P (CoatRIA, Biomerieux, Lyon, France). The lower limit of sensitivity was 7 pg/ml for the E2 assay and 0.05 ng/ml for the P assay. The intraassay coefficients of variation ($CV_{\max}$) were 12 and 8.5%, respectively. Interassays variabilities were 16 and 11%, respectively. Serum FSH and LH concentrations were determined by a specific (125 I) radioimmunoassay without extraction (Bio-Merieux, Marcy l'Étoile, France). Intraassay and interassay variabilities for P, LH, and FSH were within 10%.

Table I. Demographic Characteristics in the Three Groups of Patients Receiving u-FSH or r-FSH

	u-FSH	Follitropin- α	Follitropin- β
No. of patients	30	22	26
Patient age (years)	34 $\pm$ 3	32 $\pm$ 4	31 $\pm$ 4
Infertility (%)			
Primary	73	63	92
Secondary	27	37	8
Causes of infertility (%)			
Tubal	11/30 (37%)	7/22 (32%)	6/26 (23%)
Male	9/30 (30%)	12/22 (54.5%)	13/26 (50%)
Unexplained	4/30 (13%)	2/22 (9%)	1/26 (4%)
Mixtes	6/30 (20%)	1/22 (4.5%)	6/26 (23%)

Statistical Analysis

Statistical comparisons were performed by using analysis of variance and student-*t* test. Results are expressed as mean $\pm$ SD. A *p*-value <0.05 was considered significant.

RESULTS

A total of 88 women were recruited to this study, being allocated to either u-FSH ($n = 32$), follitropin- α ($n = 28$) or follitropin- β ($n = 28$) treatment group. For personal reasons, four women in the follitropin- α group did not start the ovarian stimulation. Of the 84 treatment cycles, six (two in each group) were cancelled because of a poor ovarian response. Thus, ovarian stimulation ended up and oocyte retrieval was performed in 78 women. The three study groups were comparable with respect to the age of the female partner, duration and cause of infertility (Table I). Basal

serum FSH concentrations on days 3–5 of a natural menstrual cycle were within the normal range in all women. ICSI was performed in 26.7%, 36%, and 38.5% of treatment cycles with u-FSH, follitropin- α , and follitropin- β , respectively.

According to the design of the study, patients treated with r-FSH received a lower dose (100 IU less) during the first 2 days. But, if we consider the amount of FSH required during the whole stimulation, a significant higher need was observed in patients treated with u-FSH than in those treated with r-FSH ($p < 0.003$). Furthermore, no significant difference emerged between the three groups as regards the mean duration of ovarian stimulation. Consequently, the mean daily FSH dose was significantly lower in patients, using recombinant preparations. The cumulative dose of r-FSH was lower in patients treated with follitropin- α , but the difference did not achieve statistical significance (Table II).

As far as serum hormonal concentrations are concerned (Fig. 1), no significant difference in estradiol serum values could be observed at day 6 of the cycle while patients had received a fixed dose of 300 IU u-FSH or 200 IU r-FSH (126 $\pm$ 119, 125 $\pm$ 104, and 101 $\pm$ 63 pg/ml for u-FSH, follitropin- α , and follitropin- β , respectively). At the time of hCG administration, serum estradiol values were similar in the three groups (1607 $\pm$ 1069, 1614 $\pm$ 894, and 1419 $\pm$ 689 pg/ml for u-FSH, follitropin- α , and follitropin- β , respectively). However, serum FSH values were significantly higher in the u-FSH-treated group as compared with the two other groups ($p < 0.0004$) (Table II).

Table II. Stimulation Characteristics of Patients in the Three Groups of Patients and Main Results

	u-FSH	Follitropin- α	Follitropin- β	<i>p</i> -value
Duration of FSH treatment (d)	12.6	12.9	12.9	0.78
Cumulative FSH dose (IU)	1981 $\pm$ 972 ^a	1222 $\pm$ 569	1465 $\pm$ 760	0.003
Plasma FSH levels at day 1 (IU/l)	9.4 $\pm$ 3.8	8.5 $\pm$ 4.3	9.6 $\pm$ 3.8	0.62
Plasma FSH levels at day of hCG (IU/l)	21 $\pm$ 9.9 ^a	10.8 $\pm$ 3.4	13.7 $\pm$ 5.7	0.0004
No. of follicles >12 mm	10.1 $\pm$ 5.2	10 $\pm$ 3.2	8.3 $\pm$ 3.1	0.20
No. of retrieved oocytes	8.8 $\pm$ 5.7	8.6 $\pm$ 4.3	7.1 $\pm$ 5.3	0.44
No. of metaphase II oocytes	6.6 $\pm$ 4.7	6.3 $\pm$ 3.2	4.7 $\pm$ 2.9	0.15
Fertilization rate (%)	48	52	46	
No. of total embryos	3.4 $\pm$ 2.9	3.7 $\pm$ 2.9	3.1 $\pm$ 3	0.83
Cycle without transfer (%)	6/30 (20%)	3/22 (13.5%)	8/26 (30%)	NS
No. of replaced embryos	2.1	1.9	1.7	NS
Pregnancy rate/initiated cycle (%)	7/30 (23%)	5/22 (23%)	7/26 (27%)	NS
Pregnancy rate/transfer (%)	7/24 (29%)	5/19 (27%)	7/18 (38%)	NS
Ongoing pregnancy/transfer (%)	6/24 (25%)	4/19 (21%)	3/18 (17%)	NS

Note. Results are expressed as mean $\pm$ SD. Significance if $p < 0.05$.

^a Comparisons between patients treated with u-FSH and those treated with r-FSH.

Fig. 1. Comparisons of plasma oestradiol values before (day 1) and during (day 6; day hCG) FSH stimulation in the three groups of patients.

Considering the ovarian parameters, no significant differences could be observed in the mean number of large follicles (higher than 12 mm in diameter), nor in the number of total and mature collected oocytes. As shown in Table II, the overall fertilization rate was not significantly different between groups and was significantly higher in patients undergoing ICSI than in those having IVF without microinjection ($60 \pm 32\%$ vs. $42 \pm 35\%$; $p = 0.03$). Most cycles without transfer were observed in patients undergoing IVF, and the percentage of those cycles was slightly higher in patients treated with follitropin- β than in patients treated with follitropin- α (Table II). However, difference did not achieve significance ($p = 0.43$). In each group, reasons for poor or absence of fertilization were mainly related to male parameters (poor motility) or to the lack of spermatozoa binding on zona pellucida. The mean number of replaced embryo was similar in the three groups and the overall pregnancy rate per initiated cycle or per transfer was not significantly different. Finally, the rate of ongoing pregnancy per transfer was equivalent in the three groups. No ad-

verse events (ectopic pregnancy or hyperstimulation syndrome) were observed in any groups.

DISCUSSION

The present study was designed to compare the efficiency and efficacy of different FSH preparations in patients included in a short-term GnRH analog IVF protocol. This regimen, which includes an initial gonadotroph flare-up after GnRH analog administration in the early follicular phase, creates a state of endogenous LH secretion, which markedly differs from the long-term protocol characterized by an hypophyseal desensitization prior to ovarian stimulation. Our results clearly show that, in this situation, recombinant FSH preparations are more efficient than urinary ones as attested by the lower amount of FSH doses needed to achieve similar multifollicular development. Moreover, as serum oestradiol values were similar at day 6 of the IVF cycle following injection of fixed doses of FSH preparations (200 IU r-FSH and

300 IU u-FSH), the clinical efficacy of r-FSH proved to be greater than that of urinary preparations. These data are in line with other study performed in anovulatory patients (6) and in those treated with a long-term GnRH analog protocol for IVF (7,9).

Reasons for the higher efficiency of r-FSH preparations are not totally understood. It may be related to some properties of recombinant preparations. Indeed, it is presumed that subtle differences at the level of oligosaccharide moieties and isoelectric profiles may account for an increased biological activity and for a shorter half-life of r-FSH (17). The marked reduction in serum FSH levels observed in patients treated with r-FSH as compared with u-FSH may be related to the shorter half-life of recombinant preparations or to the lower amount of administered FSH. Finally, the relationship between half-life and biological activity is still unclear and deserves further investigation.

In addition, our study suggests that the superiority of r-FSH over u-FSH is unrelated to the LH environment. Although LH acts synergistically with FSH to stimulate folliculogenesis in patients completely deprived of hypophyseal secretion (13), there is no such evidence in patients treated with GnRH analogs. Indeed, the relative efficacy of r-FSH preparations seems to be similar in long-term and in short-term GnRH analog protocols attesting that, above a certain threshold, the level of endogenous LH does not play a crucial role for the effectiveness of r-FSH.

Finally, efficacy of the two r-FSH preparations seems to be very similar. Indeed, while ovarian parameters as well as the amount of required ampoules seem to indicate a higher efficacy of follitropin- α over follitropin- β , the difference did not achieve significance. The fertilization rate was comparable and the higher rate of cycles without transfer observed in patients treated with follitropin- β seems to be related to a poor semen quality rather than to the oocyte maturity itself. Also no difference in the IVF outcome could be observed. Although conclusions drawn from this study are limited because of the relative small number of patients, our observations are in good agreement with other recently published studies (10–12), attesting that subtle changes in carbohydrate structure are not reflected in clinical efficacy.

In conclusion, the present results demonstrate that, in patients involved in a short-term GnRH analog protocol, recombinant FSH preparations are more efficient than urinary ones and that both follitropins are equally effective in producing multiple follicular development. They also suggest that effectiveness of recombinant FSH preparations is not depend on

the degree of hypophyseal LH secretion induced by GnRH analogs.

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