

A prospective, randomized, controlled clinical study of a new subcutaneous, purified, urinary FSH preparation for controlled ovarian hyperstimulation in *in vitro* fertilization

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ABSTRACT

The aim of this study was to evaluate the clinical efficacy and safety of a new urinary follicle stimulating hormone (FSH) preparation (Fostimon®) in patients undergoing *in vitro* fertilization-embryo transfer or intracytoplasmic sperm injection. Metrodin HP® was adopted as a reference drug, as its purity and therapeutic efficacy are well known. Sixty normo-ovulatory patients aged 18-38 years with normal basal FSH and body mass index < 25 kg/m² were selected for the study. After gonadotropin releasing hormone analogue pituitary desensitization, patients were randomized to receive either Fostimon or Metrodin HP at the initial dosage of 225 IU for 6 days. Thereafter, the dosage was tailored according to the ovarian response. Both drugs were administered by the subcutaneous route. The primary end-points were: number of follicles larger than 15 mm, levels of 17β-estradiol on the day of human chorionic gonadotropin (hCG) injection and number of oocytes recovered. The secondary end-points were: number of FSH ampules

used, day of hCG injection and pregnancy rate. FSH kinetic curves were calculated during the treatment period with both products. Safety was evaluated by pre- and post-treatment blood biochemistry and hematology, and recording all side-effects. Local tolerance was investigated at each visit. None of the parameters evaluated showed a statistically significant difference between the two groups. Local tolerance was always recorded as good/excellent by both the patients and the physician. In conclusion, Fostimon proved to be an effective and safe drug for assisted reproductive cycles.

INTRODUCTION

The new generations of urinary gonadotropins, the highly purified human urinary follicle stimulating hormone (u-hFSH HP, Metrodin HP®; Serono Laboratories, Autbonne, Switzerland), which have been used with good results in the past decade,

have proved to be highly potent, safe and pure enough for subcutaneous administration¹. Recently, recombinant FSH (rFSH) has been proposed as being safer and clinically advantageous over u-hFSH HP^{2,3}. However, we should not assume that urinary gonadotropins will be entirely replaced by the much more expensive rFSH until clear-cut evidence of cost-effectiveness is available⁴⁻⁶.

While rumors exist concerning the possible shortcomings of the best-known urinary gonadotropin and its future, new preparations, not yet tested in clinical practice, continue to appear on the market.

Since 1997, a new urinary FSH (Fostimon[®], IBSA, Lugano, Switzerland) has been made available in some European countries, including Italy. This product is the result of a new purification ion-exchange chromatography column method. This preparation has a specific activity of > 6000 IU/mg protein and a purity grade of > 90%. These high levels of biological potency and safety allow Fostimon to be administered by both the intramuscular and subcutaneous routes.

In order to confirm the clinical efficacy and safety of Fostimon administered by the subcutaneous route, we carried out a randomized study in assisted reproductive cycles in parallel with a u-hFSH HP, still considered the 'gold standard' for controlled ovarian hyperstimulation (COH). Metrodin HP, which has been marketed in Italy since 1995, was selected as the reference drug.

MATERIALS AND METHODS

Patients

Between January 1998 and March 1999, 60 patients were recruited from couples scheduled for *in vitro* fertilization-embryo transfer (IVF-ET) or intracytoplasmic sperm injection (ICSI) in the Department of Obstetrics and Gynecology of the University of Genoa, Italy. The inclusion criteria were normo-ovulatory patients aged 18-38 years, body mass index lower than 25 kg/m², basal FSH lower than 12 mIU/ml and normoprolactinemia. Patients had to be free from ovulatory drugs for the 3 months preceding the study.

The exclusion criteria included impairment of liver and/or renal function, thyroid and/or adrenal

abnormalities, ovarian cyst and ascertained or presumed hypersensitivity to gonadotropins.

The study was approved by the Ethics Committee of the local hospital. All subjects gave their written informed consent.

Protocol and assessments

All patients received a gonadotropin releasing hormone (GnRH) analogue (buserelin nasal spray, 1 mg daily in five doses) from the luteal phase preceding the treatment cycle until the day of human chorionic gonadotropin (hCG) administration. After pituitary downregulation was reached (i.e. serum 17 β -estradiol < 30 pg/ml and no follicles or cystic structures larger than 6 mm in diameter), multiple follicular recruitment was induced with either Metrodin HP or Fostimon according to a computer-based randomization code. The starting dosage of FSH for both treatments was 225 IU for 6 days. From day 7, the dosage was individualized according to the ovarian response. Ovarian response was monitored every other day, starting from day 7, by means of 17 β -estradiol levels and vaginal ultrasound. Closer controls were established as needed. Human chorionic gonadotropin (Gonasi[®] HP, AMSA, Rome, Italy) was administered subcutaneously at a dosage of 10 000 IU in the presence of at least one follicle over 18 mm in diameter and two other follicles larger than 15 mm in diameter.

Serum FSH and luteinizing hormone (LH) levels were measured before starting gonadotropin administration, every other day from day 7 until hCG injection, and 22 days after the beginning of therapy. Routine blood biochemistry and hematology examinations were performed before starting the therapy and also during the week following the retrieval of oocytes. At each visit, any adverse events were recorded.

On the day of the first gonadotropin injection, patients were instructed by a nurse on subcutaneous administration and on the evaluation of local side-effects. Thereafter, therapy was self-administered by the patients and local tolerance was reported on each subsequent visit according to the following scale: excellent = no side-effects; good = only mild and transient (< 30 min) local discomfort (redness/itching or swelling); acceptable = mild discomfort (> 30 min); unacceptable = pain or any other symptoms not definable as

mild. The site of the most recent injection was checked at each visit by a physician. In order to obtain an objective evaluation, this physician was not aware of the preparation used.

End-points

The main efficacy criteria were: number of follicles larger than 15 mm in diameter, 17β -estradiol levels on the day of hCG injection and number of oocytes retrieved. The number of FSH vials used, the number of days needed for adequate follicular recruitment, pregnancy rate and number and causes of cancelled cycles were also taken into consideration, but only as secondary criteria.

Serum FSH measurements before, during and after the completion of treatment were used to calculate and compare areas under the curve (AUCs) for both preparations.

Safety was assessed in accordance with common laboratory parameters and incidence of side-effects. Local tolerance was evaluated by the scale previously described.

Assays

Blood biochemistry and hematology analyses were performed in the hospital according to the normal-range tests. All hormones were assayed using commercially available kits. FSH and LH were analyzed using an immunoenzymometric assay kit (Iema Well, Radim, Italy), the standards of which are calibrated against the IRP 78/549 2nd International Standard for FSH and against the IRP 68/40 1st International Standard for LH. Mean intra-assay coefficients of variation were 4.2% for FSH and 4.5% for LH. Mean inter-assay coefficients of variation were 7.2% for FSH and 7.74% for LH. For the determination of 17β -estradiol, a radioimmunoassay (RIA CT, Radim, Italy) was used with mean intra-assay and inter-assay coefficients of variation of 2.4% and 2.9%, respectively.

Sample size and statistical analysis

Sample size was calculated by considering the levels of 17β -estradiol recorded during the 7-day period preceding the end of treatment. The AUC was calculated using the trapezoidal rule, which was drawn by the curve of the hormone concentrations. According to the criteria of the

bioequivalence experimental design and choosing $\alpha = 0.05$ and $\beta = 0.20$, i.e. a potency equal to 80%, 30 patients per treatment group had to be enrolled so that the 90% confidence interval of the AUC for the drugs used in this study was between 80 and 120%.

Instat (Graphpad) software was used to perform statistical analysis on the different clinical parameters. For continuous variables, the inferential analysis was performed using a statistical model such as Student's *t* test or Bonferroni's analysis of variance with multiple comparisons. For discrete variables, non-parametric tests, such as Fisher's exact test, were used.

RESULTS

A total of 60 patients were recruited (30 patients per group); the demographic data and clinical characteristics are shown in Table 1. No statistical difference between groups was observed for any of the parameters considered.

One patient scheduled for Fostimon therapy abandoned the study after pituitary suppression because of personal reasons, while yet another patient in the same group dropped out after just 6 days of treatment due to a growing follicular cyst.

Among the remaining 58 patients, five cycles were cancelled before oocyte retrieval: three for inadequate ovarian response (two in the Fostimon group, one in the Metrodin HP group) and two because of a high risk of hyperstimulation (Metrodin HP group).

The clinical results obtained from the patients who received hCG (26 in the Fostimon group and 27 in the Metrodin HP group) are shown in Table 2. No statistically significant difference was found for any of the clinical parameters evaluated.

In two patients of the Metrodin HP group, no oocytes were recovered. ICSI was performed in 15 patients treated with Fostimon and in 16 patients treated with Metrodin HP, while IVF was performed in 11 and nine cases, respectively. In two patients in each group, no embryos for replacement were available because of failed fertilization. Five intrauterine pregnancies occurred in each group (the pregnancy rate per embryo transfer was 20.8% with Fostimon versus 21.7% with Metrodin HP).

In Table 3, the mean \pm SD values of FSH and LH levels observed before, during and after

Table 1 Demographic data and clinical characteristics of the patients

Parameter	Fostimon (n = 30)	Metrodin HP (n = 30)	p Value
Age (years) (mean ± SD)	35 ± 2	34 ± 2	NS
BMI (kg/m ²) (mean ± SD)	21 ± 2.7	21 ± 2.5	NS
No. of cases of primary infertility	28	27	NS
Years of infertility			
mean	5.5	5.4	NS
range	1–10	2–14	
Main causes of infertility (%)			
tubal–peritoneal factor	24.1	17.2	NS
unexplained	3.4	3.4	NS
male factor	41.3	51.7	NS
female + male factor	31	27.5	NS
No. of cases of ICSI indicated (%)	17 (56.6)	18 (60)	NS

BMI, body mass index; ICSI, intracytoplasmic sperm injection

Table 2 Clinical results obtained from the patients who received human chorionic gonadotropin (hCG)

Parameter	Fostimon (n = 26)	Metrodin HP (n = 27)	p Value
No. of FSH vials used (mean ± SD)	32 ± 8	31 ± 7	NS
Day of hCG injection (mean ± SD)	13 ± 1.3	12 ± 1.5	NS
17β-Estradiol (pg/ml) on the day of hCG injection (mean ± SD)	945 ± 502	1107 ± 470	NS
No. of follicles > 15 mm on the day of hCG injection (mean ± SD)	9 ± 2	11 ± 5	NS
No. of oocytes retrieved (mean ± SD)	7 ± 3	8 ± 6	NS

FSH, follicle stimulating hormone

Table 3 Serum levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) before (day 0), during (days 7, 9, 11, 13 and 14) and after (day 22) treatment. Values are given as mean ± SD

	Day 0	Day 7	Day 9	Day 11	Day 13	Day 14	Day 22
LH (mIU/ml)							
Fostimon	1.1 ± 1.0	0.8 ± 0.6	1.0 ± 0.8	1.0 ± 0.9	0.8 ± 0.7	0.5 ± 0.1	0.5 ± 0.1
Metrodin HP	1.4 ± 1.6	1.3 ± 1.1	1.4 ± 1.4	0.8 ± 0.6	0.7 ± 0.5	0.5 ± 0.1	0.6 ± 0.4
FSH (mIU/ml)							
Fostimon	4.0 ± 2.5	20 ± 7.8	19 ± 8.9	18 ± 9.8	15 ± 10.7	7.0 ± 7.1	1.0 ± 0.8
Metrodin HP	4.0 ± 3.5	19 ± 6.2	18 ± 6.5	17 ± 8.6	12 ± 6.2	10 ± 5.9	2.0 ± 3.2

treatment are reported. The time course of 17β-estradiol is graphically reported in Figure 1. No statistical differences were observed. The main FSH kinetic parameters are reported in Table 4. There was no statistical difference between the two different FSH preparations regarding the AUC, C_{max} (peak height) and T_{max} (time of peak concentration) observed after treatment. The increase in 17β-estradiol during FSH administration in the two groups was similar.

In both groups, a significant increase in the white blood cell count was recorded in the

post-treatment evaluation. All other blood biochemistry and hematology tests did not show any relevant clinical change from the baseline.

One patient in each group complained of mild and transient nausea and one in the Fostimon group had a headache for a few hours. We also recorded a case of cystitis in the Metrodin HP group. The local tolerance was always reported as good/excellent. The incidence of mild discomfort lasting for less than 30 min at the site of injection was comparable in both treatment groups.

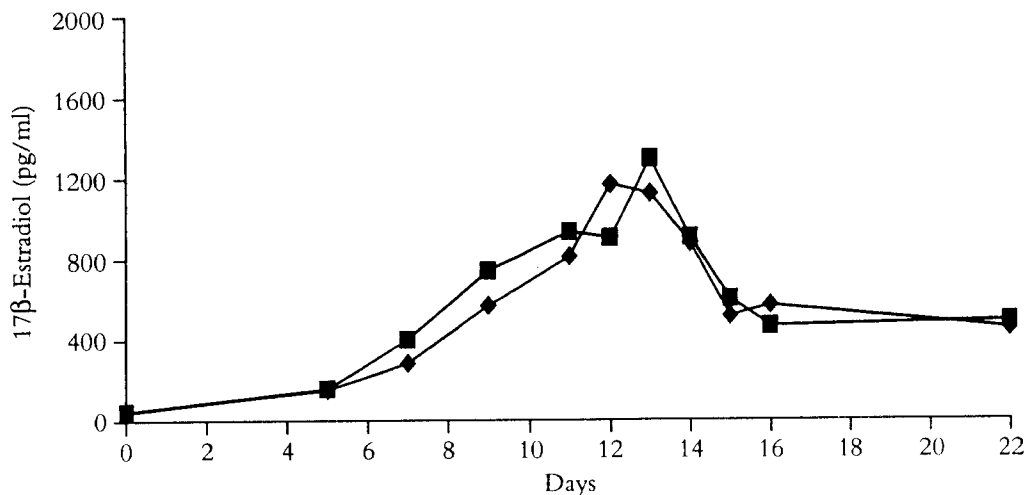


Figure 1 Mean serum levels of 17β-estradiol. —◆—, Fostimon; —■—, Metrodin HP

Table 4 Follicle stimulating hormone kinetic parameters of Fostimon and Metrodin HP

	Fostimon			Metrodin HP		
	Mean	Standard deviation	95% confidence interval	Mean	Standard deviation	95% confidence interval
AUC ₀₋₂₂ (mIU/ml)	215.2	88.5	176.9–253.5	219.1	74.9	183–255.2
C _{max} (mIU/ml)	23.4	8.8	19.5–27.2	21.7	6.6	18.5–24.9
T _{max} (days)	8.7	7*	7.8–9.6	9	9*	7.9–10.1

*Median; AUC₀₋₂₂, area under the curve between day 0 and day 22; C_{max}, peak height; T_{max}, time of peak concentration

DISCUSSION

COH protocols for IVF have been greatly modified in recent decades. The original urinary human menopausal gonadotropin was gradually replaced by preparations with higher FSH activity and a lower content of non-specific co-purified urinary proteins. The use of GnRH analogues in long and short COH protocols allowed the spontaneous LH peak to be avoided and oocyte retrieval to be programmed. Even if the introduction of each new drug yielded both clinical and practical advantages in infertility COH protocols, it was always linked to a cost increase. As there is nowadays an ever increasing and justifiable concern about the burden of infertility treatment cost for public health services and patients, an effort should also be made to evaluate cost-effectiveness, along with efficacy and tolerability, before replacing old drugs with new ones.

Thus, there seems to be a consensus that, for the time being, urinary gonadotropins should not be completely abandoned in favor of the much more

expensive recombinant preparations⁵⁻⁷. On the other hand, while the use of different new urinary gonadotropin preparations recently available on the market should be encouraged due to the lower cost, by no means should therapeutic strategies be driven only by cost without taking safety and efficacy into account.

Metrodin HP is considered to be the gold standard extractive product for COH because of its high level of purity (> 95%) and specific activity (> 9000 IU FSH/mg protein). This study aimed to assess the efficacy and safety of Fostimon, a new urinary FSH preparation, using as a reference drug a widely tested commercial preparation.

The efficacy results of this study show that Fostimon does not differ from the reference drug in terms of number of FSH vials used (Fostimon 32, Metrodin HP 31), day of hCG injection (Fostimon 13, Metrodin HP 12), number of follicles > 15 mm in diameter (Fostimon nine, Metrodin HP 11) and number of oocytes retrieved (Fostimon seven, Metrodin HP eight). Fostimon

guarantees kinetic profiles of FSH absorption and 17β -estradiol stimulation comparable to the test drug. All safety parameters, including the incidence of side-effects, showed that Fostimon is as safe as Metrodin HP.

The subcutaneous administration of daily drugs is, indeed, a clinical advantage that should not be underestimated in evaluating new preparations. It lessens stress and costs associated with IVF treatment because patients can be instructed to self-administer. Moreover, because none of the patients complained of discomfort at the injection site that was more than mild and transient, the good local tolerance of subcutaneous injections may be considered as evidence of low impurity content if we assume that the incidence of local side-effects is triggered by non-active proteins⁸.

Hence, although definitive conclusions cannot be drawn because of the relatively low number of patients enrolled, this pilot study demonstrated Fostimon to be as effective and safe as Metrodin HP in patients undergoing COH cycles for IVF and ICSI.

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