Effects of high doses of recombinant human follicle-stimulating hormone in the treatment of male factor infertility: results of a pilot study

We performed a randomized double-blind placebo-controlled study on seminal parameters and endocrine profile of 30 normogonadotropic patients with male factor infertility to assess the efficacy of treatment with recombinant human FSH (rhFSH) at high doses (300 IU on alternate days) for a period of ≥4 months. The treatment induced a marked increase in sperm count, a slight increase in sperm motility, no change in sperm morphology, and an evident increase only in FSH serum levels, showing that a prolonged treatment with rhFSH at high doses led to an evident improvement of sperm count in normogonadotropic infertile patients with idiopathic oligoasthenozoospermia. (Fertil Steril 2006;86:728–31. ©2006 by American Society for Reproductive Medicine.)

The physiologic regulation of spermatogenesis is still not completely understood (1). The role of FSH, in particular, in regulating testicular function and in the initiation, regulation, and maintenance of spermatogenesis continues to be debated, whereas its role in regulating ovarian function is well accepted (1, 2). Various attempts have been made to increase sperm production in patients with severe male factor infertility and normal LH and T production by administering various formulations of FSH, which appeared to be the most obvious remedy. Several studies have been performed, but most showed no results in sperm parameters after both urinary purified (u) FSH (3–6) and recombinant human (rh) FSH (7) administration. Thus, there is no scientific basis for recommending FSH treatment for idiopathic male factor infertility, because all studies do not support the effectiveness of FSH in improving sperm parameters or pregnancy rates (1, 8).

However, Acosta et al. (3) admitted that the dose of uFSH used could not be enough to modify the main endocrine or classic semen parameters. Furthermore, although FSH treatment of oligospermic men has not consistently produced robust increases in sperm count, certain patients have been reported to respond to FSH stimulation, and therefore the value of FSH treatment in subsets of subjects with idiopathic infertility should not be dismissed (9). In addition, in some of these studies with no results in sperm parameters after FSH treatment, an increase in both fertilization and pregnancy rates in couples with male factor infertility undergoing IVF-ICSI cycles was documented (4, 6, 10) suggesting that FSH may be an adjuvant and valuable therapy for the male partner in these couples (10). On the other hand, FSH therapy in infertile men improved significantly certain ultramorphologic parameters (6, 11). In vitro culture in rhFSH-supplemented medium increased motility of testicular spermatozoa (12). Finally, some more recent studies showed some direct effects on basic sperm parameters with both uFSH (13) and rhFSH (14, 15).

Considering these discordant results, in this randomized double-blind, placebo-controlled study we investigated the clinical effects of high doses (300 IU SC every other day for ≥4 months) of rhFSH on the endocrine profile and seminal parameters in patients with male factor infertility.

This study was approved by our institutional review board, and all the patients gave written informed consent. Thirty subjects entered the study and were divided into two age-, weight-, and height-matched groups, which were blinded for the examiners according to a computer-generated randomization list. Fifteen patients were treated with rhFSH and 15 with placebo. All the subjects had a history of unexplained male factor infertility of ≥2 years duration with no indication of hormonal (normal basal FSH and T values), infective (negative spermicoculture), or physical causes for their infertility and having a partner with no endocrine and/or obstructive disorders. All the subjects had poor semen quality (moderate to severe oligoasthenozoospermia, range 1 to 15 × 10⁶/mL sperm concentration). Exclusion criteria from the study were testicular tumor, hypergonadotropic hypogonadism, hypogonadotropic hypogonadism, isolated gonadotropin deficiency, hyperprolactinemia, severe scrotal varicocele, history of cryptorchidism, leucocytosperma, acute orchitis and other genital infections, positivity to seminal sperm antibodies, presence of Y chromosome microdeletions, obesity, and other systemic severe chronic illness.

The rhFSH (Gonal-F) and placebo were supplied by Serono (Rome, Italy) in identical vials which are also

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labeled identically. The rhFSH was supplied in vials containing 150 IU lyophilized FSH with 30 mg saccharose. Matching placebo was supplied in vials containing 30 mg saccharose only. Two vials of study drug (300 IU of rhFSH or placebo) were administered by SC injection every other day for ≥4 months.

The SC injections were delivered into the abdominal wall, alternating the injection site between the right and left sides. The SC mode of injection was deliberately chosen over IM because the possibility of self-administration and less painful injections make long-term treatment more acceptable for the patient, thus improving compliance (16). No difference in steroidogenic response and pharmacokinetic parameters after SC and IM administration in healthy volunteers of both genders have been shown (17). No other medications were administered in all the subjects during the study period.

Semen and blood samples were obtained simultaneously in all the subjects before treatment was started and in days immediately after treatment was discontinued. Semen samples were collected by masturbation and were treated as previously reported (18). An aliquot was used for routine sperm count (sperm concentration, motility, morphology, and viability, according to the World Health Organization guidelines [19]), spermioiculure, and the evaluation of antisperm antibodies (immunobead binding test). A leukocyte count at light microscopy was also performed counting all the round cells negative for specific sperm stains. The main sperm parameters detected at semen analysis for both groups are reported in Table 1.

Blood samples were collected by venipuncture. Serum was stored frozen at ~20°C until assayed. The radioimmunoassay techniques used for hormonal measurements were: FSH, LH, and PRL by rapid double antibody (kits from Biodata, Rome, Italy); T by thin-layer chromatography on silica gel 60 F254 as previously described (20); and free T by the Coat-A-count procedure (Diagnostic Product Corp., Los Angeles, CA).

Data analysis was performed by nonparametric Mann-Whitney U test and confirmed by paired t test. A value of $P<.05$ was accepted as statistically significant. Results are expressed as mean ± 1 SD.

Averages of the peripheral hormone serum levels before and after rhFSH and placebo treatments are reported in Table 1. In both instances, differences between the serum levels of LH, prolactin, free T, and total T were not statistically significant (NS). Only FSH serum levels increased significantly ($P<.02$) during the treatment with rhFSH and not with placebo. No side effects were seen during and
after both treatments, nor did local reactions at the injection site occur. Although pregnancies were considered as only secondary efficacy endpoints, four and no pregnancies in the months after rhFSH and placebo treatment, respectively, were observed. All of them went to term successfully. The low number of pregnancies did not allow for a meaningful statistical evaluation.

Averages of the main sperm parameters before and after rhFSH and placebo treatments, considered as primary efficacy endpoints, are reported in Table 1. Sperm concentration nearly doubled (P<0.01) after rhFSH but remained unchanged (P=NS) after placebo. Total sperm number was significantly higher (P<0.05) after rhFSH administration. No change was observed in placebo group. The percentage of forward progression up of sperm with rapid linear movement (type 1 motility) was not significantly different in both treatments, and that with slow linear movement (type 2 motility) increased significantly (P<0.02) after rhFSH only. No differences were observed in both treatments in the percentages of in situ motility. The rhFSH administration showed a slight decrease (P<0.05) in the percentage of immobile sperm, whereas placebo administration showed no effect. The percentages of normal sperm morphology as well as leukocyte counts remained unmodified (P=NS) after both treatments.

The exact role of FSH and LH in male reproduction remains an enigma of andrologic research (1). Considering the discordant results obtained in seminal parameters of infertile subjects by FSH therapy reported in literature, in this study we have deliberately chosen to modify some treatment parameters, in particular [1] the amount of the treatment, [2] the interval time between two injections, and [3] the duration of the treatment.

Based on findings that elimination half-lives of the existing formulations of FSH are similar and approximately 32–37 hours, as shown by us (21) and others (22), and that the half-life of bioactive FSH is considerably shorter (13.4 hours) (23), it was concluded that most of the dosing regimens currently used are inadequate (7, 23). Therefore, we administered 1,050 IU rhFSH per week, which is more than twice as high as the doses (450 IU FSH/wk) commonly used by most investigators with both uFSH (4, 5, 10, 11, 13, 24, 25) and rhFSH (14, 15, 26) but similar to those used by Ben Rafael et al. (6) with uFSH and by Kamischke et al. (7) with rhFSH.

The choice of administering rhFSH every other day was established on the basis of improving the patients’ compliance through a long period of treatment. No difference between 150 IU rhFSH daily and 300 IU rhFSH every other day was likely in immunoreactive serum FSH, because rhFSH treatment increases serum FSH in a dose-proportional fashion (27) and reaches the maximal pharmacologic effect within 3–4 days (21, 28), maintain-

ing thereafter new steady-state levels of serum FSH throughout the whole treatment.

Concerning the duration of treatment, considering that the cycle of spermatogenesis lasts approximately 72 days (25), we chose a 4-month therapy to affect all the stages of a new whole spermatogenic and spermigenic cycle and its normalization. This was more than the ≤3 months commonly used in most other studies (29). Shorter treatment periods may lead to unsatisfactory and unfounded results (7, 16).

No side effects and no manifestations of FSH overdose were exhibited in the study, and we may confirm that rhFSH is well tolerated locally and systematically at our higher doses, in contrast to the frequently observed female ovarian hyperstimulation (26).

From an endocrine point of view, treatment with high doses of rhFSH showed a significant increase of serum FSH concentration, even though a higher effect on serum FSH was expected. None of the other reproductive hormone concentrations (LH, PRL, total T, and free T) were affected. Endogenous LH remained unchanged, because rhFSH lacks intrinsic LH activity. The unmodified total and free T levels confirmed that at high doses rhFSH did not affect androgen biosynthesis either.

Concerning semen parameters, the study indicates that rhFSH improves sperm count and, to a much less extent, sperm motility, confirming a key role of FSH in stimulating human spermatogenesis. In particular, the treatment with high doses of rhFSH showed an increase in both sperm concentration and total sperm number, whereas poor results in motility and practically no results in morphology were observed, these two parameters remaining within pathologic limits. It seems as if new spermatogenesis is activated but with the persistence of the pathologic alterations concerning mobility and morphology typical of the germ cell of infertile subjects. The improvement appears more quantitative than qualitative, confirming that FSH is classically regarded as necessary for quantitative restoration of spermatogenesis (26).

These results are consistent in part with those of Foresta et al. (14) and Caroppo et al. (15) but not with those of Kamischke et al. (7). In a similar study, in fact, Kamischke et al. (7) failed to find a significant increase of the conventional semen parameters, because, as they themselves suggested, the treatment was probably not long enough (12 weeks). Patients who failed to produce sperm during short-term gonadotropin treatment should not be considered as nonresponders (16). We began to see significant increases in sperm count after ≥4 months.

In conclusion, the striking feature of this randomized double-blind placebo-controlled study is that rhFSH, at the high doses used and duration of treatment, may lead to
an improvement of sperm concentration, indicating that rhFSH is exerting an effect on spermatogenesis.

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REFERENCES