Recombinant human follicle stimulating hormone for treatment of male idiopathic infertility: a randomized, double-blind, placebo-controlled, clinical trial

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To examine the role of recombinant human follicle stimulating hormone (rhFSH) in male idiopathic infertility a randomized, double-blind, placebo-controlled study was performed. Of 211 patients screened, 67 were finally included. After two pre-examinations, patients were randomized and treated for 12 weeks, either with 150 IU rhFSH or with placebo. Examinations (physical examination, scrotal ultrasonography, semen analysis, hormone measurements, and in 31 patients electron microscopy (EM) of spermatozoa were performed 6 and 12 weeks after treatment initiation and 6 and 12 weeks after completion of treatment. Pregnancies were recorded for a further 3 months after the last examination. Of the 67 patients included in the study, 34 treated and 31 placebo patients could be analysed. In the treated group, FSH was elevated compared to baseline values ($P < 0.001$). At the end of treatment testicular volume in the treated group was increased compared to placebo ($P < 0.05$) and baseline ($P < 0.001$). Apart from an increase in sperm motility ($P < 0.05$) in the placebo group and in sperm DNA condensation ($P < 0.001$) in the treated group no significant changes were observed in semen parameters. Two spontaneous pregnancies in partners of men in the treated group and none in the placebo group occurred. However, two pregnancies occurred in partners of men in the placebo group induced by intrauterine insemination or intracytoplasmic sperm injection. In conclusion, at the chosen dose and duration, rhFSH did not lead to an improvement of conventional or EM sperm parameters nor to an increase in pregnancy rates. However, the increased testicular volume and sperm DNA condensation give reason for further investigations.
Key words: electron microscopy/male idiopathic infertility/ randomized controlled clinical trial/recombinant human FSH/ semen parameters

Introduction

In about 30% of cases of male infertility, representing the largest group of those consulting for infertility, no obvious cause for subnormal semen parameters and possibly for patho-logical hormone concentrations can be found. This condition is classified as 'idiopathic infertility' and to date rational approaches to treatment are lacking (Nieschlag and Leifke, 1997). For the treatment of male idiopathic infertility, testoster-one (Vandekerckhove *et al.*, 1996), anti-oestrogens [World Health Organization (WHO), 1992b; Rolf *et al.*, 1996] and various other non-hormonal substances like kallikrein (Keck *et al.*, 1994; Vandekerckhove *et al.*, 1995) have been evaluated and all have proved to be ineffective in controlled clinical trials (Leifke and Nieschlag, 1996).

Since gonadotrophins and gonadotrophin-releasing hormone (GnRH) are required for normal testicular function (Weinbauer *et al.*, 1997) and are effective in the treatment of hypogonadism (Kliesch *et al.*, 1994, 1995), they were applied in idiopathic male infertility on the hypothesis that elevation of GnRH or gonadotrophin concentrations may lead to stimulation of spermatogenesis. But neither GnRH (Bals-Pratsch *et al.*, 1989) nor human chorionic gonadotrophin (HCG)/human menopausal gonadotrophin (HMG) treatment (Knuth *et al.*, 1987) had any beneficial effect on sperm parameters or pregnancy rates in controlled clinical trials.

In monkeys, however, it has been shown that administering follicle stimulating hormone (FSH) alone stimulated spermato-genesis (Van Alphen *et al.*, 1988; Weinbauer *et al.*, 1992). In men with pituitary adenomas secreting FSH, testicular enlargement has been observed (Heseltine *et al.*, 1989) and an activating mutation of the FSH receptor in a hypophysectom-ized man was able to sustain spermatogenesis autonomously and to maintain testicular volume in the upper normal range (Gromoll *et al.*, 1996).

As the failure of previous HMG treatments was attributed to a possible loss of FSH biopotency and residual HCG activity, the availability of recombinant FSH (rhFSH), together with an increased pregnancy rate in the partners of infertile men, reported in an uncontrolled study with pure FSH, has led to a reconsideration of FSH treatment in male infertility (Acosta *et al.*, 1992). The efficacy of rhFSH was said to be due to the better electron microscopical appearance of the sperm head subcellular organelles (Bartoov *et al.*, 1994; Ben-Rafael *et al.*, 1995).

The initial findings of an increased partner pregnancy rate with no increase in conventional semen parameters were further confirmed by various other groups in uncontrolled studies (Ranieri *et al.*, 1994; Ballesca *et al.*, 1995; Montag *et al.*, 1995). Other uncontrolled studies even showed an increased pregnancy rate and improved classical semen para-meters (Ben-Rafael *et al.*, 1995) or only improved semen parameters (Sigg and Baciu, 1994; Merino *et al.*, 1996; Glander and Kratzsch, 1997). In randomized controlled studies, in

contrast, no difference in pregnancy rates or classical semen parameters compared to no treatment (Matorras *et al.*, 1997) or placebo (Comodo *et al.*, 1996) was seen when patients were given 150 IU purified urinary FSH three times a week or 300 IU urinary FSH every second day respectively.

As most studies published so far were not properly controlled, the need for double-blind, placebo-controlled studies has been stressed (Baker *et al.*, 1992; Simoni and Nieschlag, 1995). In order to clarify the question whether rhFSH has an effect on seminal parameters of patients with idiopathic male infertility or on pregnancies of their partners, a placebo-controlled, randomized, double-blind clinical trial was performed.

Materials and methods

Study design

The study protocol was approved by the Ethics Committee of the University and the State Medical Board, Münster. Written informed consent was obtained from each patient before initiation of treatment. The study was performed between March 1994 and November 1996, following the guidelines of good laboratory [Organisation for Economic Co-operation and Development (OECD), 1992] and clinical practice [WHO, 1993b; International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 1996].

A total of 211 potential participants was examined twice before recruitment for on the study. At the first screening examination, a complete physical, hormonal and semen examination was performed. If the results of the first screening examination were in accordance with the inclusion criteria, a second pre-examination was performed including detailed medical histories of the patient and female partner, physical examination, clinical chemistry, red blood cell count, clotting factors, hormones [luteinizing hormone (LH), FSH, prolactin, testosterone, oestradiol], semen analysis and flow cytometry of sperm DNA. In addition, scrotal content was examined by ultrasonography (Sonoline Versa Pro, Siemens, Erlangen, Germany) at one pre-examination and at medication end (Behre *et al.*, 1995). Following the publication of Bartoov *et al.* (1994), electron microscopy (EM) was included in the analysis at the second pre-examination and was performed in 31 patients.

After fulfilment of all inclusion criteria in the two pre-examinations, 67 patients were allocated to the treated or placebo groups by third party true randomization. The code distinguishing the treatment groups was blinded for the examiners. Patients were treated for 12 weeks either with daily injections of 150 IU rhFSH (Gonal-F, Serono, Unterschleissheim, Germany) with 30 mg saccharose (*n* = 34) or placebo containing saccharose alone (*n* = 33). Treatment started 1.2 ± 0.2 months after the last pre-examination. Injections were always given s.c. into the abdominal wall at the same time of day. They were performed by the patients themselves after initial instruction by the examiners.

Further control examinations were performed 6 and 12 weeks after initiation of the treatment and 6 and 12 weeks after the end of treatment. All examinations included recent medical histories of the patient and female partner, detection of adverse events and side-effects, physical evaluation and clinical chemistry, red blood cell count, clotting factors, hormone (LH, FSH, inhibin B, testosterone, oestradiol) analysis, semen analysis and flow cytometry of sperm DNA. In patients for whom EM analysis had been performed at the pre-examination, it was repeated 12 weeks after initiation and 12

weeks after the end of treatment. An additional ultrasound examination of scrotal content was performed 12 weeks after initiation of the treatment. Pregnancies in female partners were recorded a further 3 months after the last control examination.

Patients

Primary efficacy end points were improvements of sperm parameters. Pregnancies were only secondary efficacy end points. However female partners had normal ovulatory cycles and biphasic basal body temperature was recorded. Untreatable ovarian dysfunction, known endometriosis or tubal blockage were female exclusion criteria. Patients (age >18 years) were allocated to the study if they fulfilled the following criteria: infertility for at least 1 year, no acute or history of varicocoele, undescended testis or testicular cancer, drug or alcohol abuse or any major systemic disease. Semen analysis revealed no azoospermia and at least two semen parameters (motility, concentration, morphology) below WHO criteria and no signs of genital tract infection or immunological infertility. Basal FSH concentrations were <12 IU/l and other reproductive hormones (LH, prolactin, testosterone, oestradiol) had to be normal.

A total of 211 patients was screened through the second pre-examination. Of these, 144 were then excluded because they did not want to participate or because of intercurrent illnesses, varicocele, undescended testis, abnormal hormone values or female causes. In all, 67 patients entered the study (34 treated group, 33 placebo group). Andrologically relevant illnesses or interventions in the medical case history were testicular biopsies (*n* = 10), circumcisions (*n* = 9), major infections of uretheral or efferent ducts (*n* = 4) and herniotomies (*n* = 2). Six patients had a history of allergy. Medication taken previously because of infertility were kallikrein (*n* = 26), anti-oestrogens (*n* = 12) and testosterone esters (*n* = 3). Previous failed assisted fertilization attempts were inseminations (*n* = 8), in-vitro fertilization (IVF) (*n* = 2) and intracytoplasmic sperm injections (ICSI) (*n* = 2). Ten patients had achieved a pregnancy with their partner in former years.

Sixty-six patients completed the study, including the 3 month observation period after the last examination. The mean duration of infertility was 4.6 ± 0.3 years. Mean age and body mass index were 32.89 ± 0.56 years and 25.63 ± 0.34 kg/m^2 respectively and were not different between the groups. One patient (placebo) dropped out before completing the study for personal reasons. Another patient (placebo) was excluded after completing the study, as the mixed agglutination reaction (MAR) test at the last three examinations revealed IgG and IgA titres between 50 and 100% as a sign of immunological infertility which had been masked in the previous examinations by low motility, making the MAR test non-analysable. Sixty-five patients were finally evaluated (34 treated group, 31 placebo group).

Hormone analysis

Blood samples were taken in the morning. Blood samples for hormone determinations were separated at 800 *g* and stored at –20°C until assayed. All hormone assays were assessed according to WHO guidelines (WHO, 1993a). Serum concentrations of LH and FSH were determined by highly specific time-resolved fluoroimmunoassays (DELFIA: Pharmacia, Freiburg, Germany). The intra-assay coefficients of variation were 1.7% for LH and 2.0% for FSH. The interassay coefficients of variation were 1.2% for LH and 0.7% for FSH. The normal range in our laboratory for LH is 2–10 IU/l and for FSH 1–7 IU/l. Serum testosterone was determined using a commercial radioimmunoassay (DSL-4100; Diagnostic Systems Laboratories, Sinsheim, Germany). Intra- and interassay coefficients of variation were 5.6 and 11.3% respectively. Oestradiol was measured

by radioimmunoassay by highly specific time-resolved fluoro-immunoassays (DELFIA). Intra- and interassay coefficients of variation were 3.8 and 3.8% respectively. The normal value for testosterone is >12 nmol/l; the upper normal limit for oestradiol is 250 pmol/l. Serum inhibin B concentrations were determined by solid phase enzyme-linked immunosorbent assay (MCA1312 KZZ, Serotec, Oxford, UK) with intra- and interassay coefficients of variation of 3.3 and 18% respectively.

Semen analysis

Semen light microscopy (LM) analysis was performed under internal (Cooper *et al.*, 1992) and external quality control (Neuwinger *et al.*, 1990) according to WHO guidelines (WHO, 1992a). Marker substances for determination of accessory gland function were measured for the epididymis (glucosidase), seminal vesicles (fructose) and prostate (zinc). In addition, the hypo-osmotic swelling test (HOS-Test) was performed. To exclude immunological infertility factors, the MAR test was performed. To exclude infections, a microbiological culture was performed if peroxidase positive cells exceeded 1×10^6/ml ejaculate and/or round cells exceeded 2×10^6/ml ejaculate.

Electron microscopy

Following the publication of Bartoov *et al.* (1994), electron microscopy (EM) was included in the analysis (*n* = 37 patients). EM cell preparation was performed as described previously (Neugebauer *et al.*, 1990). Transmission electron microscopy (EM 900; Carl Zeiss, Oberkochen, Germany) analysis was performed according to Bartoov *et al.* (1994). Three ultrathin sections were analysed from every sample. From these three sections, EM results were given only for patients where it was possible to analyse all three time points. This was possible for 18 treated patients and 13 placebo patients. The sperm quality index [sperm quality index = intact acrosome (EM)%×0.02 + intact head shape (EM)%×0.03 + sperm motility (LM)%×0.02 – tail defects (LM)×0.05 – 2.4] was performed as described by Bartoov *et al.* (1994).

Flow cytometry

Flow cytometric analysis of sperm head DNA condensation was performed from 200 µl ejaculate which was fixed in 50 ml 80% ethanol for 24 h. After centrifugation (3000 rpm) of 10 ml of the suspension, 9 ml of the suspension was gently removed. The remaining 1 ml and the pellet were thoroughly mixed with 1 ml 0.5% Pepsin A (Sigma P-7125, St Louis, Missouri, USA) and incubated for 5 min. Afterwards 8 ml of a 0.18 M Tris (Merck 8382, Darmstadt, Germany) buffer was added, containing 13.3 µg 4′,6-diamidino-2-phenylindole (DAPI, Sigma D-9542) and 80 µg sulphorhodamine 101 acid chloride (Sigma S-5138). After at least 30 min incubation, DAPI stained DNA condensation was measured by a Partec PAS III flow cytometer (Münster, Germany) as described previously (Behre *et al.*, 1989).

Statistics

Before commencement of the study statistical power analysis was performed on a level of significance of a = 5% and b = 80%. The minimum number of patients needed in each study group to detect a 50% difference in sperm concentration was determined to be 40. As 65 patients had been included in the analysis post-priori statistical power analysis revealed that the conducted study was able to detect a 75% difference in sperm concentration at the end of the treatment period.

All variables were checked for normal distribution in the Kolmogorov–Smirnov one-sample test for goodness-of-fit. When necessary, analysis was performed on logarithmically transformed data. Two-sided *P* values of 0.05 were considered significant. Differences to baseline values and between the study groups were tested by factorial ANOVA for repeated measurements. Covariances were tested by stepwise linear multiple regression analysis. All analyses were performed using the statistical software SPSS for Windows version 6.1 (SPSS, Chicago, IL, USA). In general, results are given as mean ± SEM.

Results

Patients

The treatment was well tolerated in both groups and in none of the cases was the study terminated because of side-effects. Routine analysis of clinical chemistry, red blood cell counts and clotting factors showed normal values overall and no significant changes during/after treatment for both study groups. Sonographic evaluation of testicular volume (Figure 1) showed a significant increase in the treated group (mean increase 5.1 ± 0.9 ml) compared to baseline values (*P* < 0.001) and to controls (mean increase 1.6 ± 0.7 ml, *P* < 0.05). All other clinical and sonographic parameters remained unchanged compared to baseline or placebo.

Hormone analysis

Mean hormone serum concentrations for LH, testosterone and oestradiol were in the normal range and did not show significant differences before and after treatment (Table I). Gonal-F treatment led to a significant increase of serum FSH in study weeks 6 (*P* < 0.001) and 12 (*P* < 0.001) compared to baseline concentrations and control group (Figure 2). The achieved FSH concentrations due to treatment (Figure 3) were only correlated with the baseline values (*r* = 0.83, *P* < 0.0001),
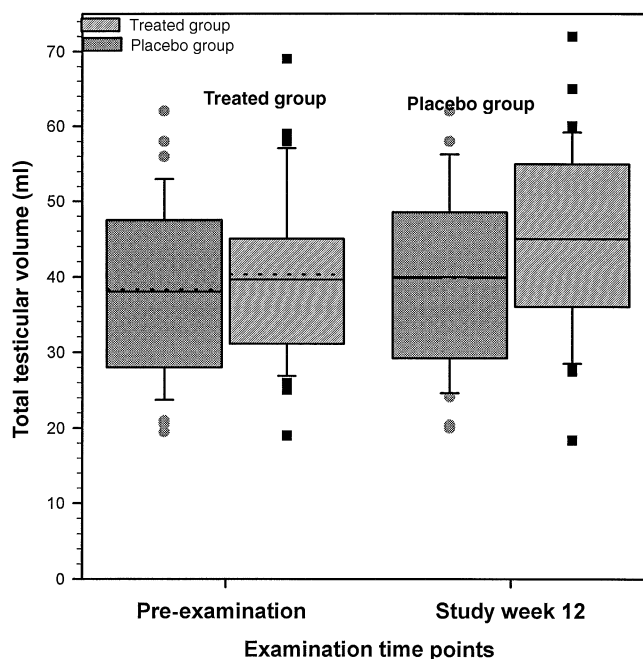

Figure 1. Total testicular volume (ml) measured by ultrasound before treatment initiation and at the end of treatment. The boxes represent 25th and 75th percentiles respectively. Inner solid line shows the 50th percentile, inner broken line the mean. Capped bars outside reflect the 10th and 90th percentile respectively and symbols mark all data outside.

Table I. Hormone and conventional semen parameters at the second pre-examination, at the end of treatment (study week 12) and after 12 week treatment (study week 24) with either 150 IU rhFSH/day or placebo. Data shown are means ± SEM

	Treated group ($n = 34$)			Placebo group ($n = 31$)		
	Second pre-examination	End of treatment	12 weeks after treatment	Second pre-examination	End of treatment	12 weeks after treatment
Body mass index (kg/m^2)	25.3 ± 0.5	25.5 ± 0.5	25.6 ± 0.5	25.9 ± 0.5	25.9 ± 0.5	25.3 ± 0.9
Sexual abstinence time (days)	3.5 ± 0.2	3.5 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.2 ± 0.2	3.3 ± 0.2
Ejaculate volume (ml)	3.9 ± 0.3	4.1 ± 0.3	3.8 ± 0.3	3.9 ± 0.2	3.8 ± 0.3	3.9 ± 0.3
Sperm motility (WHO grade a + b in %)	33.4 ± 3.0	30.5 ± 2.8	33.0 ± 2.9	29.6 ± 3.0	35.4 ± 3.0*	32.1 ± 2.9
Sperm concentration (×10^6/ml)	8.7 ± 1.5	9.9 ± 2.1	11.1 ± 2.1	8.7 ± 1.6	11.2 ± 2.1	10.3 ± 1.9
Sperm morphology (% normal forms)	9.6 ± 1.3	9.8 ± 1.1	9.4 ± 0.9	11.7 ± 1.2	11.5 ± 1.3	10.3 ± 1.0
Round cells (×10^6/ml)	3.4 ± 0.8	2.5 ± 0.4	3.0 ± 0.4	2.4 ± 0.5	2.0 ± 0.4	2.1 ± 0.4
Leukocytes (×10^6/ml)	0.25 ± 0.09	0.14 ± 0.05	0.15 ± 0.05	0.06 ± 0.03	0.08 ± 0.03	0.06 ± 0.03
FSH (IU/l)	5.0 ± 0.4	8.7 ± 0.5**	5.2 ± 0.5	5.6 ± 0.6	5.1 ± 0.5	5.1 ± 0.6
LH (IU/l)	3.8 ± 0.2	3.7 ± 0.2	4.0 ± 0.3	4.1 ± 0.2	3.9 ± 0.3	3.8 ± 0.3
Inhibin B (pg/ml)	165.4 ± 14.2	205.7 ± 16.3	175.2 ± 14.7	162.6 ± 18.3	183.3 ± 18.5	178.0 ± 17.5
Testosterone (nmol/l)	18.5 ± 0.7	19.7 ± 1.0	17.5 ± 0.8	15.8 ± 0.6	16.3 ± 0.7	14.9 ± 0.7
Oestradiol (pmol/l)	66.9 ± 2.5	59.6 ± 2.7	57.9 ± 2.2	61.0 ± 2.2	55.0 ± 2.4	51.6 ± 2.3

Significant difference ($P < 0.05$; **$P < 0.001$) between the treated and placebo group at the end of treatment.
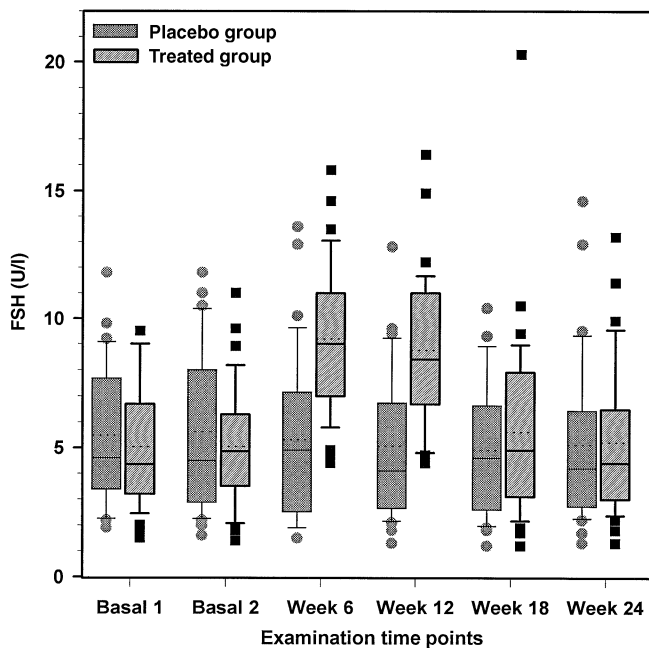LH = luteinizing hormone; FSH = follicle stimulating hormone, rhFSH = recombinant human FSH.

Figure 2. Follicle stimulating hormone (FSH) serum concentrations (IU/l) before (basal 1, basal 2), during treatment (week 6, week 12) and after treatment (week 18, week 24) with daily s.c. injections of 150 IU recombinant human FSH or placebo. Treatment led to a significant increase of serum FSH in study weeks 6 and 12 compared to baseline concentrations and control group ($P < 0.001$). The box represents 25th and 75th percentiles respectively. Inner solid line shows the 50th percentile, inner broken line the mean. Capped bars outside reflect the 10th and 90th percentile respectively and symbols mark all data outside.

Figure 3. Mean follicle stimulating hormone (FSH) values before treatment (pre-examinations 1 + 2) versus the mean FSH values achieved during (study weeks 6 + 12) treatment with 150 IU recombinant human FSH. Broken line represents the line of identity.

Semen analysis

Treatment with 150 IU recombinant FSH did not change any of the investigated conventional semen parameters compared to placebo or baseline values (Table I). However, in the placebo group there was a slight increase ($P > 0.05$; Figure 5) of progressive sperm motility (WHO grade a + b). The HOS-test as a marker of membrane integrity was analysable in 19 placebo and 18 treated patients and revealed no differences

while time of injection during the last 24 h or body mass index (data not shown) had no influence on FSH serum concentration increase. Inhibin B concentrations remained unchanged by treatment with rhFSH (Figure 4).
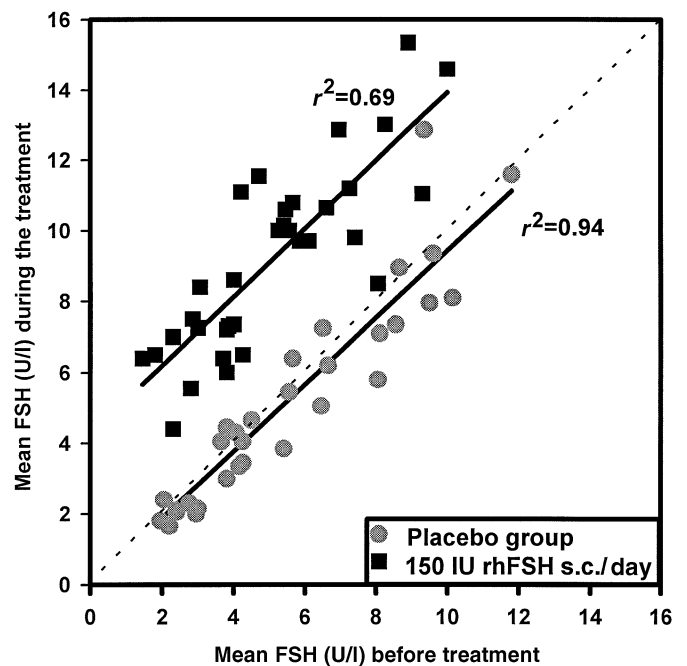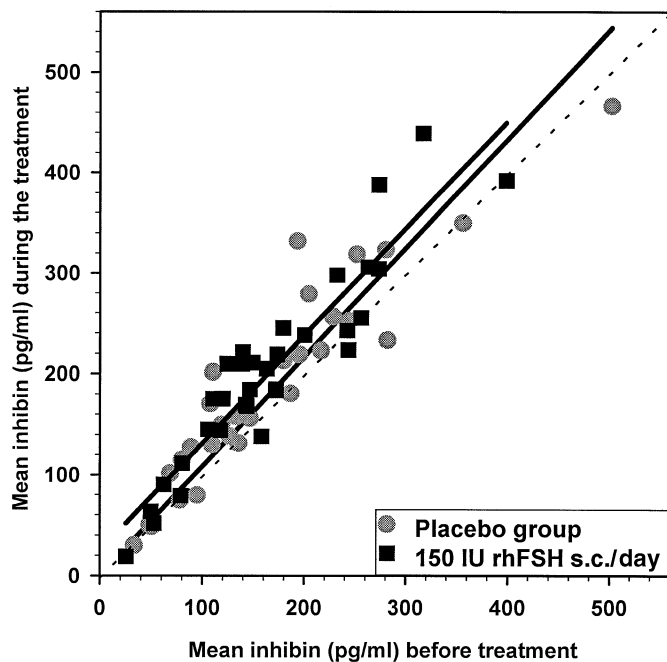
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Figure 4. Mean inhibin values before treatment (pre-examinations 1 + 2) versus the mean inhibin values achieved during (study weeks 6 + 12) treatment with 150 IU recombinant human follicle stimulating hormone (rhFSH). Broken line represents the line of identity.
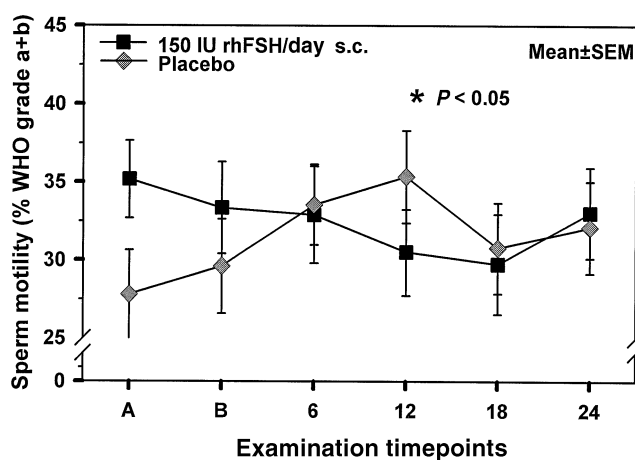

Figure 5. Progressive sperm motility (WHO grade a + b) before (A, B), during treatment (week 6, week 12) and after treatment (week 18, week 24) with daily s.c. injections of 150 IU recombinant human follicle stimulating hormone (rhFSH) or placebo. Treatment led to a significant increase of motility in the rhFSH-treated group compared to baseline concentrations and control group ($P < 0.05$) at study week 12.

between treatment groups at the end of the treatment period (treated 83.9 ± 1.2 versus placebo 85.4 ± 0.9). Ejaculate volume, seminal markers for the epididymis, prostate and seminal vesicles remained unchanged throughout all examinations (data not shown).

Electron microscopy

Treatment with 150 IU recombinant FSH did not change any of the investigated electron microscopy parameters or ratios

compared to placebo or baseline values (Table II). Nor were there significant differences in the sperm quality index (Bartoov, 1994) at any of the examined time points.

Flow cytometry

Flow cytometric analysis of sperm head DNA condensation revealed a significant improved condensation of the sperm head chromatin of the haploid cells in the treated group compared to baseline and placebo (Figure 6). DNA condensation and numbers of diploid cells remained unchanged in both groups.

Pregnancies

Of the 65 patients who completed the study, 61 (treated $n = 31$, placebo $n = 30$) could be evaluated for the induction of pregnancies in their partners. Four patients had to be excluded for pregnancy analysis because of later endoscopically confirmed tubal blockage ($n = 3$) or pregnancy ($n = 1$) in their partners, 4 days before initiation of medication in the male patients. Partners of four patients (treated $n = 2$, placebo $n = 2$) had induced pregnancies (determined by ultrasound and HCG concentration increase) within or 6 months after the treatment phase. The two pregnancies in the placebo group were achieved by insemination (study week 28) or ICSI (study week 23). The two pregnancies achieved in the treated group were spontaneous pregnancies (study weeks 5 and 1). Following the 6 month observation period after treatment further pregnancies of female partners occurred with the aid of ICSI (treated $n = 4$, placebo $n = 7$), IVF (treated $n = 1$), insemination (placebo $n = 1$) or spontaneously (treated $n = 1$).

Discussion

The availability of recombinant human FSH with comparable in-vivo immunological and biological characteristics comparable to natural FSH (Mannaerts *et al.*, 1991), along with uncontrolled studies with purified FSH has led to a reconsideration of FSH treatment in male infertility (Acosta *et al.*, 1992). After s.c. administration, the pharmacokinetics of rhFSH have been best described as a one-compartment model with an elimination half-life of approximately 1 day which reaches the maximal pharmacological effect 3–4 days after repeated administration (le Cotonnec *et al.*, 1994). Based on findings that the half-life of bioactive FSH is considerably shorter than the half-life of immunologically detectable FSH, it was concluded that most of the dosing regimes currently used are too low (Jockenhövel *et al.*, 1990). Therefore we used a dose of 150 IU rhFSH s.c./day which was at least twice as high as the doses (75–150 IU FSH three times a week) used by others so far (Acosta *et al.*, 1992; Bartoov *et al.*, 1994; Ranieri *et al.*, 1994; Sigg and Baciu, 1994; Ballesca *et al.*, 1995; Ben-Rafael *et al.*, 1995; Montag *et al.*, 1995; Merino *et al.*, 1996; Glander and Kratzsch, 1997; Matorras *et al.*, 1997).

We were able to increase serum FSH concentrations significantly in the treated group with negligible side effects. No other reproductive hormone concentrations were affected. As time of injection during the last 24 h had no influence on FSH serum concentrations and as the FSH concentrations achieved

Table II. Electron microscopy parameters at the second pre-examination, at the end of treatment (study week 12) and 12 weeks after treatment (study week 24) with either 150 IU rhFSH/day or placebo. Data shown are means ± SEM

	Treated group (n = 18)			Placebo group (n = 13)		
	Second pre-examination	End of treatment	12 weeks after treatment	Second pre-examination	End of treatment	12 weeks after treatment
% Normal sperm head	6.9 ± 1.1	7.8 ± 1.5	8.7 ± 1.8	8.0 ± 2.2	9.5 ± 2.3	7.6 ± 1.7
% Normal nucleus	20.9 ± 3.0	22.4 ± 3.4	21.8 ± 2.7	18.5 ± 4.3	22.2 ± 2.9	24.6 ± 3.9
% Normal acrosome	13.3 ± 2.2	13.2 ± 2.8	11.7 ± 2.8	17.3 ± 4.1	15.0 ± 3.4	18.1 ± 5.0
% Normal axonem	44.7 ± 6.6	37.1 ± 6.9	38.9 ± 5.1	34.4 ± 8.1	39.7 ± 7.3	39.1 ± 8.3
Sperm quality index	–1.91 ± 0.18	–1.74 ± 0.13	–1.92 ± 0.15	–1.66 ± 0.2	–1.43 ± 0.16	–1.77 ± 0.13
Normal sperm head/ abnormal nucleus	0.17 ± 0.03	0.20 ± 0.05	0.21 ± 0.05	0.20 ± 0.08	0.25 ± 0.08	0.21 ± 0.07
Normal sperm head/ abnormal acrosome	0.22 ± 0.03	0.26 ± 0.05	0.34 ± 0.10	0.29 ± 0.08	0.32 ± 0.07	0.21 ± 0.05

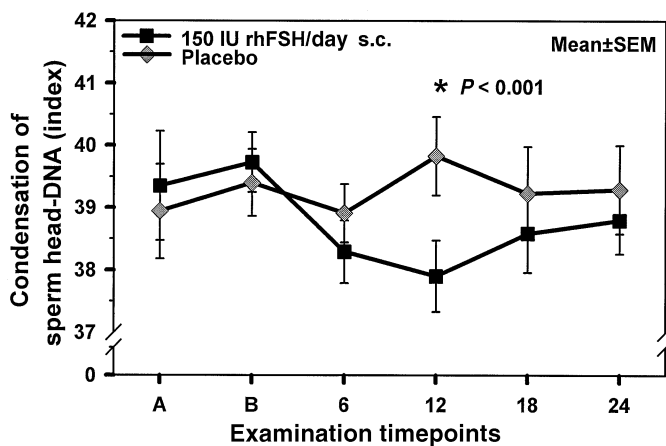rhFSH = recombinant human follicle stimulating hormone

Figure 6. Condensation of sperm head DNA (index) of haploid cells before (A, B), during treatment (week 6, week 12) and after treatment (week 18, week 24) with daily s.c. injections of 150 IU recombinant human follicle stimulating hormone (rhFSH) or placebo. Treatment led to a significant increase of chromatin condensation in the rhFSH-treated group compared to baseline concentrations and control group ($P < 0.001$) at study week 12.

were only correlated with the baseline values, we conclude that FSH concentrations in each treated patient were continuously increased during the whole treatment phase after 3–4 days equilibration time. Despite the high rhFSH dose used and the resulting constant elevation of FSH serum concentrations, we were not able to stimulate an endocrine response to the treatment in terms of inhibin B concentrations.

However, the sonographic testicular volume increased significantly compared to baseline values and to controls as a possible consequence of rhFSH treatment. In contrast to the placebo group, the increase of testicular volume in the treated group could be explained only in part by methodological variation, which has been shown to be 7.0 ± 0.7% in humans in our clinic (Behre *et al.*, 1990). In monkeys, administration of FSH stimulated spermatogenesis and increased Sertoli cell secretion (Van Alphen *et al.*, 1988; Weinbauer *et al.*, 1992). In monkeys also, probably as a result of the proliferation of early spermatogenetic cells or Sertoli cell secretion, an increase of testicular volume to 132 ± 9.5% of baseline could be observed after 6 weeks of treatment. As the number of Sertoli cells is thought to be constant after puberty, it therefore seems

likely that the increase in testicular volume seen in the treated group in our study (113.2 ± 2.3% of baseline) is probably due to a stimulation of early spermatogenetic stages in FSH-treated patients. This speculation is in agreement with the testicular enlargement seen in men with pituitary FSH-secreting adenomas, where the enlargement is partly reversible after reducing FSH concentrations to the normal range (Heseltine *et al.*, 1989). In addition, the flow cytometric analysis of the spermatozoa reveals a higher rate of DNA condensation in the FSH-treated patients, indicating an influence on late phases of sperm maturation.

We were not able to achieve any improvement of conventional semen parameters compared to placebo or baseline values, which is in agreement with others (Acosta *et al.*, 1992; Bartoov *et al.*, 1994; Ranieri *et al.*, 1994; Ballesca *et al.*, 1995; Ben-Rafael *et al.*, 1995; Montag *et al.*, 1995; Comodo *et al.*, 1996; Matorras *et al.*, 1997). Why we were not able to increase conventional semen parameters despite a significant increase in testicular volume, and also probably spermatogenetic cells, remains unclear. It seems possible that, despite the highest dose used so far, the FSH dose was still too low, as unchanged inhibin B concentrations throughout the study may indicate. Another reason for this failure might be that the treatment was not long enough, although rhFSH medication in our study encompasses the duration of a whole spermatogenetic cycle. In addition, the group of patients with idiopathic infertility may represent a highly heterogeneous group of patients in which not all patients are likely to respond to FSH treatment.

In contrast to uncontrolled studies, we could not detect any significant improvement in the treated group of any electron microscopical parameter, including the sperm quality index (Bartoov *et al.*, 1994; Ben-Rafael *et al.*, 1995) in the treated group. It remains unclear if changes would have been detected using another method of submicroscopical evaluation (Baccetti *et al.*, 1995; Strehler *et al.*, 1997) as this method might offer possibilities for more accurate submicroscopical sperm evaluations. However the non-significant increase for the sperm quality index and the normal heads during the treatment detected in the treated group was stronger in the placebo group. On the other hand, baseline conventional and electron microscope parameters of our patients were much lower than those seen by Bartoov *et al.* (1994). Apart from inter-centre

variance, this could also be due to different kinds of patients which were evaluated in the studies and could explain the differences in this outcome parameter.

In contrast to the majority of uncontrolled studies and in agreement with the controlled studies published (Comodo *et al.*, 1996; Matorras *et al.*, 1997), we could not detect a significant increase in pregnancy rates in the partners of the treated group. The two spontaneous pregnancies in the treated group cannot unequivocally be attributed to the treatment as the number is too small to reach statistical significance.

In conclusion, at the chosen dose and duration of treatment, rhFSH did not lead to an improvement of conventional, biochemical or EM sperm parameters. The increased testicular volumes and higher sperm DNA condensation in the treated group, however, indicate that FSH is exerting an effect on spermatogenesis and sperm maturation. It therefore appears worthwhile to try to find parameters that might identify patients who may benefit from FSH treatment. More research will be required before FSH can be generally recommended for the treatment of idiopathic male infertility.

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