

Induction of Spermatogenesis by Recombinant Follicle-Stimulating Hormone (Puregon) in Hypogonadotropic Azoospermic Men Who Failed to Respond to Human Chorionic Gonadotropin Alone

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ABSTRACT: A multicenter, open-label, randomized efficacy and safety study was performed with combined human chorionic gonadotropin (hCG) and recombinant follicle-stimulating hormone (recFSH) (Puregon®) treatment to induce spermatogenesis in hypogonadotropic hypogonadal male patients. Patients were pretreated for 16 weeks with hCG to normalize testosterone levels. A total of 30 of 49 (61%) subjects had normalized testosterone levels but were still azoospermic after the hCG-alone phase. These patients were randomized into 2 treatment schemes with recFSH (2 × 225 IU recFSH per week [group A] or 3 × 150 IU recFSH per week [group B]), in combination with hCG for a period of 48 weeks. Total testosterone increased during the hCG-alone period from 1.08 and 1.22 ng/mL to 6.26 and 4.52 ng/mL for groups A and B, respectively. Combined gonadotropin treatment was effective in inducing spermatogenesis (sperm count $\geq 1 \times 10^6$ /mL) in 14 of 30 subjects (47%) and this was achieved after a median duration of treatment of approximately 5.5 months. Treatment time necessary for first sperm cells to appear in the ejaculate was related to the initial testicular

volume. Subjects with a history of maldescended testes (11 of 30 subjects, 37%) showed a lower mean response to treatment as indicated by the relatively lower number of subjects reaching levels of at least 1×10^6 sperm cells per milliliter. Combined testicular volume increased during combined gonadotropin treatment from 11.4 to 24.0 mL. Although subjects with a history of maldescended testes had a lower starting testicular volume, subjects with and without a history of maldescended testes showed approximately the same relative increase in testicular volume. Total testosterone levels showed only a minor further increase during the combined gonadotropin treatment period. In conclusion, a weekly dose of 450 IU (3 × 150 IU or 2 × 225 IU) recFSH, in addition to hCG, was able to induce spermatogenesis in many hypogonadotropic azoospermic men who failed to respond to treatment with hCG alone.

Keywords: Follistim, gonadotrophin deficiency, male infertility, testis.

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Among the available therapies to improve spermatogenesis in men with functional disorders of the pituitary or hypothalamus or both, gonadotropin therapy is most widely used (Whitcomb et al, 1990; Crowley et al, 1992; Zitzmann and Nieschlag, 2000). This treatment consists of the administration of either human chorionic

gonadotropin (hCG) alone or in combination with a follicle-stimulating hormone (FSH)-containing preparation such as human menopausal gonadotropin. In hypogonadotropic men, FSH is given to stimulate the Sertoli cells. Concomitantly, high doses of hCG are given to supply the necessary luteinizing hormone (LH) bioactivity to stimulate the Leydig cells. Upon combined treatment with FSH and hCG, it takes at least 3 to 4 months before spermatogenesis is restored.

Treatment in this study occurred in 2 phases. Since hCG alone is sufficient to induce spermatogenesis in some patients, hCG was initially given to normalize tes-

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tosterone levels. Subjects who were still azoospermic after this first phase were treated with a combination of both hCG and recombinant FSH (recFSH). Both gonadotropins were injected subcutaneously, providing a more convenient method than the intramuscular route of administration, which was common practice in the past. The efficacy and tolerability of recFSH, in combination with hCG, for the induction of spermatogenesis was studied using 2 treatment regimens (225 IU 2 times a week and 150 IU 3 times a week), resulting in an equal weekly dose of 450 IU.

The objective of this study was to investigate the efficacy and safety of recFSH in combination with hCG for initiation or improvement (or both) of spermatogenesis in hypogonadotropic hypogonadal men.

Materials and Methods

Subject Population and Study Design

The study was designed as a multicenter, open-label, phase III, randomized study in 6 centers in Australia, Denmark, Germany, and the United Kingdom. The study protocol was approved by local ethics committees of the study centers. Before participation all subjects gave their written informed consent.

Subjects were first treated with subcutaneous (SC) hCG (Pregnyl, Organon, Oss, The Netherlands) twice a week for a period of 16 weeks. Treatment was started with 1500 IU hCG twice weekly. In case of insufficient response after 8 weeks, as assessed by serum testosterone levels, the dose was increased to 3000 IU twice weekly. Eligible subjects (ie, those with normalized testosterone levels and still azoospermic) were randomized into 2 different treatment schedules of SC-injected recFSH (Purigon, Organon, Oss, The Netherlands), either 225 IU twice weekly (group A) or 150 IU recFSH 3 times a week (group B) for 48 weeks. In the phase of combined gonadotropin treatment, hCG injections continued twice weekly at the same dose level as was used during the hCG-alone phase. recFSH and hCG were always given as separate injections. At screening, after the hCG-only phase and at fixed time points during the study, testicular volumes were assessed and blood and semen samples were taken for hormonal measurements and determinations of semen characteristics, respectively. Injection sites were monitored to assess the local tolerance of injection of recFSH and hCG. For safety reasons, standard blood chemistry and urinalysis were performed. Since recFSH is produced by a CHO cell line transfected with the human genes for FSH, the potential presence of antibodies against FSH or CHO cell-derived proteins in serum was determined.

Inclusion criteria were subjects between the ages of 18 and 60, body mass index less than or equal to 35 kg/m², azoospermia, low circulating levels of gonadotropins (FSH and LH \leq 2 IU/L and testosterone \leq 6 nmol/L), presence of scrotal testes, adequate replacement of other pituitary hormones (if applicable), good general physical and mental health, and ability and willingness to comply with the protocol and to give written informed con-

sent. Exclusion criteria were testicular pathology of clinical importance, including vasectomy; any serious disease affecting testicular function; and presence of clinically relevant abnormalities of blood chemistry, hematology, or urinalysis. Furthermore, previous treatment within various time periods preceding the start of the study with FSH (6 months), gonadotropin-releasing hormone (GnRH) (6 months), hCG (3 months), or testosterone undecanoate (2 weeks), or treatment with injectable testosterone preparations (6 weeks) was prohibited. Other exclusion criteria were hypophysectomy within a period of 6 months before the start of this study, past or present oncological treatment, diabetes mellitus, untreated hyperprolactinemia, use of drugs known to impair testicular function, history of alcohol and drug abuse, and administration of investigational drugs within 90 days before study.

Hormonal Measurements

The hCG-alone phase and combined gonadotropin treatment period comprised a total of 64 weeks. On the first day of weeks 1, 8, and 16 of the hCG-alone phase (before each hCG injection), and on the first day of weeks 17, 22, and every 6 weeks thereafter in the combined gonadotropin treatment period (before the first recFSH injection of that particular week) and at the final visit (scheduled in week 65), blood samples were collected for the assessment of FSH, hCG, testosterone (T), estradiol (E₂), sex hormone-binding globulin (SHBG), and inhibin B. Inhibin B serum concentrations were analyzed centrally by NV Organon's Department of Drug Metabolism and Kinetics, Oss, The Netherlands, using a validated enzyme immunoassay (EIA) kit (InstruChemie, Hilversum, The Netherlands). Serum FSH, LH, hCG, T, E₂, and SHBG concentrations were determined centrally by ABL, Assen, The Netherlands, using kits for time-resolved fluoroimmunoassays (Delfia, Wallac Oy, Turku, Finland).

Blood Chemistry/Hematology, Urinalysis, Physical and Andrological Examination, and Vital Signs

At screening, in week 16 and every 12 weeks during the combined gonadotropin treatment period, routine blood chemistry, hematology, urinalysis, and physical and andrological examinations were performed, as well as measurements of body weight, height, blood pressure, and heart rate.

Determination of Antibodies Against FSH or CHO Cell-Derived Proteins

All antibody determinations were performed centrally by NV Organon's Department of Drug Metabolism and Kinetics. The presence of anti-FSH antibodies was investigated in blood samples collected at screening at week 22 and every 6 weeks thereafter up to and including week 64 and at the final visit scheduled at week 65 with a validated radio-immunoassay using purified FSH as a standard. Anti-CHO IgG and anti-CHO IgE antibodies in human serum were determined with a validated EIA.

Semen Analysis and Determination of Testicular Volume

Semen was collected for analysis of sperm concentration, motility, and morphology on the first day of weeks 8, 16, 22, and every 6 weeks thereafter up to and including week 64 after a minimum of 3 days of abstinence. Sperm concentrations were

Table 1. Demographic and andrological characteristics (\pm SD) of patients included in the treatment phase

	Group A: 2 \times 225 IU/wk			Group B: 3 \times 150 IU/wk		
	n	Mean	SD	n	Mean	SD
Age (y)	15	30.1	5.6	15	30.7	8.0
Weight (kg)*	13	86.1	16.0	15	87.7	13.1
BMI (kg/m ²)*†	12	26.5	4.16	14	27.2	4.42
Testicular volume (mL)						
Left	14	5.38	2.25	13	4.34	3.18
Right	14	5.44	2.38	13	4.35	3.58
Serum hormones						
LH (IU/L)	10	0.6	0.0	10	0.6	0.0
E ₂ (pg/mL)*	14	37.7	11.5	14	23.6	6.3
Inhibin B (pg/mL)*	13	69.2	61.3	13	66.0	32.6
FSH (IU/L)*	14	1.1	0.5	14	1.1	0.4
hCG (IU/L)*	14	47.0	39.7	14	35.7	16.7
SHBG (nmol/L)*	14	26.7	12.1	14	30.2	11.1
Total T (ng/mL)*	14	6.26	2.34	14	4.52	2.51

* Parameter determined at baseline (ie, last assessment during hCG-alone phase), otherwise at screening.

† BMI indicates body mass index; LH, luteinizing hormone; E₂, estradiol; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; SHBG, sex hormone-binding globulin; and T, testosterone.

measured locally at each study center according to the WHO manual (1992). For exploratory reasons, mean sperm concentrations were statistically compared between the treatment groups using the two-sample Student's *t* test, assuming unequal variances.

Testicular volume was assessed according to a similar time schedule with an additional assessment at the final visit in week 65 using orchidometry or ultrasonography.

Local Tolerance

The local tolerance of the subcutaneous route of administration was assessed by filling in diary cards. The maximal intensity of redness, itching, swelling, pain, and bruising experienced during the preceding period after the last injection was scored as none, mild, moderate, or severe. The hCG and recFSH injection sites were monitored separately.

Results

Disposition of Subjects

A total of 49 subjects were included in the trial. Nineteen subjects discontinued during the hCG-alone phase for various reasons: 7 subjects in whom testosterone levels did not normalize, 9 subjects in whom spermatogenesis occurred on hCG alone, and 3 subjects for other reasons. The remaining 30 subjects were randomized over the 2 recFSH treatment groups. Relevant characteristics of the treatment groups are given in Tables 1 and 2. Parameters of all randomized subjects obtained at the various assessments were analyzed, if available. At screening and at

Table 2. Medical history of patients included in the treatment phase

	n/N	
	Group A	Group B
Cause of hypogonadism		
Hypothalamic	12/15	14/15
Pituitary	2/15	0/15
Hypothalamic + pituitary	1/15	1/15
Type of previous treatment		
GnRH†	4/14*	3/15
Gonadotropins	8/14*	11/15

* For one subject information is missing.

† GnRH indicates gonadotropin-releasing hormone.

baseline (start of recFSH treatment), no relevant differences with respect to age, height, weight, body mass index, heart rate, diastolic and systolic blood pressure, and andrological history existed between the 2 treatment groups. The mean age at screening was 30.1 years in group A and 30.7 years in group B. A difference of approximately 1 mL existed in mean testicular volume between the treatment groups.

A total of 11 subjects were diagnosed as idiopathic hypogonadotropic hypogonadal, 15 subjects presented with Kallmann syndrome, and in 4 subjects other causes for their hypogonadotropic hypogonadal condition were found. Eleven subjects (7 in group A and 4 in group B) presented with a history of either unilateral or bilateral maldescended testes, often also referred to as cryptorchidism. For all subjects without maldescended testes the baseline (ie, before starting combination therapy) FSH serum concentration was 1.1 (SD 0.3) and for subjects with maldescended testes baseline FSH serum concentration was 1.2 (SD 0.3) IU/L.

Several men received previous treatment with GnRH or gonadotropins or both. A total of 8 men were treatment naïve (5 in group A and 3 in group B). For one man in group A, no data were available (see Table 2).

Induction of Spermatogenesis

Fourteen (47%) subjects reached a sperm concentration of at least 1×10^6 /mL at one of their assessments after a median treatment time of 165 days (approximately 5.5 months), ranging from 25 to 327 days.

In Figure 1, the mean sperm concentrations are given for the treatment groups. In this figure the standard deviations are not given because they are very large and, in combination with the logarithmic ordinate, make the figure rather confusing. Although the response in group B seems slightly lower than in group A, the onset of spermatogenesis and the results at the end of treatment are comparable and no statistically significant differences ($P > .05$) were found between the treatment groups. The

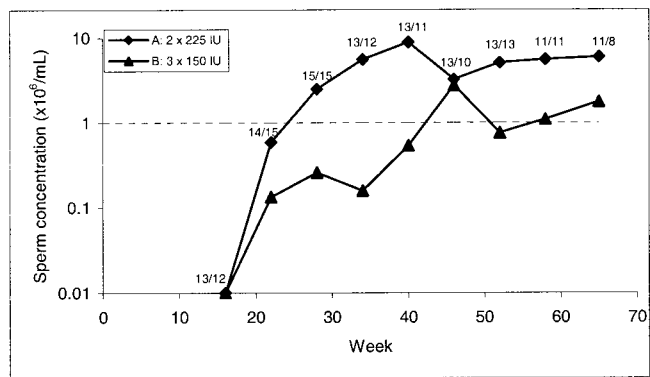


Figure 1. Mean sperm concentrations per treatment group. Numbers represent the number of observations included at each assessment (order: group A/group B). Note semilog scale.

abstinence time was recorded; the median abstinence time calculated over all samples was 4 and 3 days for groups A and B, respectively.

Sperm concentrations of $20 \times 10^6/\text{mL}$ or higher, regarded by the WHO as the lower limit of the normal range (WHO, 1992), were reached by 3 subjects. The mean sperm count, over all subjects, exceeded a value of $1 \times 10^6/\text{mL}$ after combined gonadotropin treatment for 28 weeks. A total of 10 (33%) men remained completely azoospermic, 5 (33%) in group A and 5 (33%) in group B.

The subject population was comprised of a relatively large subgroup of men with a history of unilateral or bilateral undescended testes. Therefore, in Figure 2 the development of spermatogenesis is depicted for men with and without undescended testes. In men without a history of undescended testes, the mean sperm count exceeded $1 \times 10^6/\text{mL}$ between weeks 22 and 28. Thirteen of the 19 men without a history of undescended testes (68%) showed an increase in sperm concentration.

Except for one individual (subject ID 17), the induction of spermatogenesis in the group with a history of undescended testes was much slower. Although subject 17 was diagnosed as having bilateral undescended testes, this subject reached sperm concentrations of $39.5 \times 10^6/\text{mL}$. To show the influence of this high value on the total group of men with undescended testes, a third curve is given in Figure 2, representing all men with undescended testes with the exception of subject 17. In the group of men without a history of undescended testes, 7 subjects showed an increase in sperm concentration to $\geq 1 \times 10^6/\text{mL}$ over at least 4 assessments.

At the end of the combined gonadotropin treatment period, an overall mean fraction of 35% (SD 25%) of progressive sperm cells (total of rapid and slow progressive cells) was reached and the overall mean percentage of morphologically normal sperm cells was 24% (SD 16%).

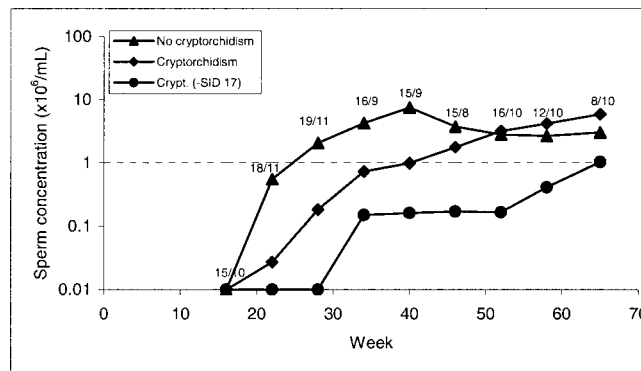


Figure 2. Mean sperm concentrations in subjects with or without a history of undescended testes or cryptorchidism. Numbers represent the number of observations included at each assessment (order: without/with cryptorchidism). The curve designated as “Crypt (-SID 17)” represents all 10 men with a history of undescended testes with the exception of subject ID 17 (see text). Note semilog scale.

Testicular Size

Over the whole combined gonadotropin treatment period consisting of 48 weeks, for both treatment groups mean testicular volume increased from 5.5 (SD 2.8) and 5.9 (SD 3.2) mL to 11.7 (SD 6.5) and 12.3 (SD 6.5) mL, for left and right testicle, respectively. Although group A started with a higher testicular volume, the relative increase was the same in both groups. Subjects with a history of undescended testes had a lower starting testicular volume, but subjects with or without a history of undescended testes showed the same relative increase in testicular volume.

There was a clear relation between the initial testicular volume and the time needed for first sperm cells to appear in the ejaculate. The relation was identical for both treatment groups (Figure 3).

Hormonal Responses

During the hCG-alone phase of 16 weeks, mean serum T levels increased from 1.08 and 1.22 ng/mL to 6.26 and 4.52 ng/mL in groups A and B, respectively. During the treatment period with combined hCG and recFSH, mean serum T levels showed a minor increase (Figure 4). Although the variability in T levels was high, the mean T levels in group A were consistently higher than in group B. This difference was already present before combined gonadotropin treatment and is therefore not related to the different treatment regimens with recFSH.

The mean inhibin B levels remained fairly constant during the hCG-alone phase but showed a gradual increase during combined gonadotropin treatment (Figure 5A). After an initial increase, the mean serum FSH concentration reached a steady state. At the end of treatment, group A had slightly higher FSH levels but variability was also high (Figure 5B). Overall mean serum levels of SHBG remained fairly constant and E_2 levels showed a

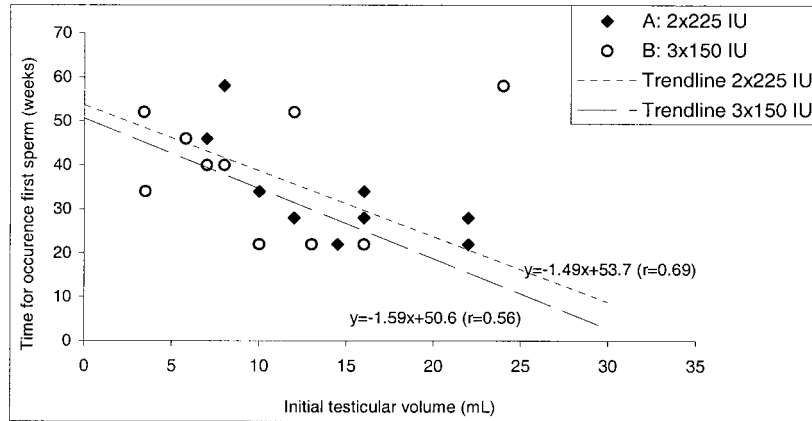


Figure 3. Time (wk) until first sperm cells appear in the ejaculate, for all individuals for whom results are available, in relation to the initial testicular volume. In calculation of the regression line for 3×150 IU, 1 outlier, with the highest initial testicular volume, was deleted.

trend toward higher levels over the treatment period (Figure 5C and D).

Safety

Two subjects (4.1%) in the hCG-alone phase and 2 subjects (6.7%) in the combined gonadotropin treatment period reported one serious adverse event (SAE) each. The SAEs included one case each of hyperglycemia, gynecomastia, pilonidal cyst, and hemorrhoids. All 4 subjects recovered. Nine subjects experienced at least one adverse event (AE) classified as “related to treatment” (3 [6.1%] in the hCG-alone phase, six [20%] during combined gonadotropin treatment). In the hCG-alone phase, 1 subject discontinued because of an aggravated depression, whereas no subjects discontinued because of AEs in the period of combined gonadotropin treatment. Among the drug-related AEs, there were 2 cases of acne, injection site reaction, and injection site pain, and single cases of varicose veins, gynecomastia, and a dermoid cyst. With regard to laboratory values and vital signs, no clinically relevant findings were observed. The local tolerance data were comparable between the 2 treatment groups. The

mean percentage of days without pain for the Puregon injection calculated for all subjects in the combined gonadotropin treatment period was 76% for subjects in group A and 91% for subjects in group B. Mostly mild pain was reported. For itching, bruising, swelling, and redness, some mild cases were reported and a few moderate and severe cases. Itching was only experienced in group A on an average of 8% of all treatment days. Bruising was seen in both treatment groups, on an average of 13% and 14.4% of all treatment days in groups A and B, respectively. Swelling of the injection site occurred on an average of 11.8% and 0.3% of the treatment days. Redness occurred approximately 9% of the treatment days in both groups.

Antibodies against FSH or CHO cell-derived proteins were not present.

Discussion

In the present study, a weekly dose of 450 IU (2×225 IU or 3×150 IU) recFSH in addition to hCG was able to induce spermatogenesis in hypogonadotropic azoospermic men who failed to respond to treatment with hCG alone. The pharmacodynamic effect of recFSH was further documented by the increase of testicular volume and the gradual increase of serum inhibin-B. Outcomes on pregnancy were not recorded in this study, as the study population was deemed too small to draw any pertinent conclusion about the chances to establish a pregnancy.

Treatment of hypogonadotropic hypogonadism may be initiated for 2 purposes: induction of fertility or androgenization (Burger et al, 1981). Combinations of hCG and FSH have been successful in the treatment of hypogonadotropic hypogonadism. In the past, combinations of hCG and hMG were used (Gayral et al, 1975; Kuhnenuri, 1976, Finkel et al, 1985; Ley and Leonard, 1985; Saal et

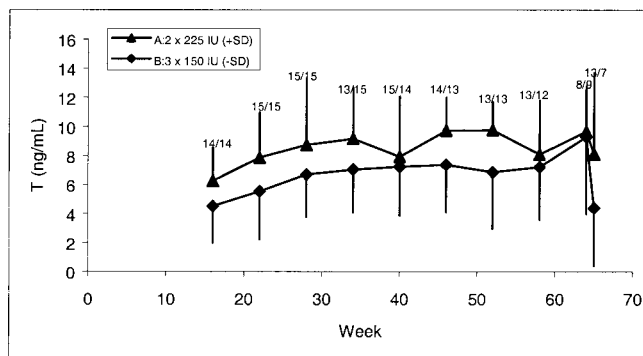


Figure 4. Mean (\pm SD) testosterone levels (ng/L). Numbers represent the number of subjects included at each assessment (order: group A/group B).

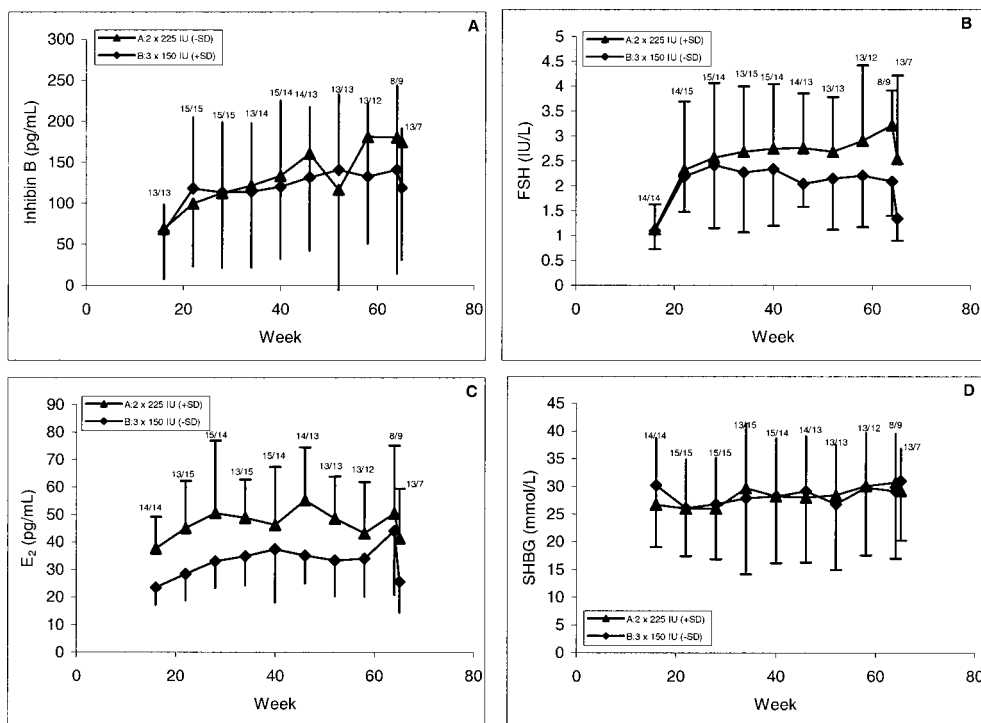


Figure 5. Mean hormone levels. Numbers represent number of subjects included at each assessment (order: group A/group B). A, inhibin B; B, follicle-stimulating hormone; C, estradiol; and D, sex hormone-binding globulin.

al, 1991; Jones and Darne, 1993; Kirk et al, 1994; Kung et al, 1994; Büchter et al, 1998; Bouvattier et al, 1999). More recently, studies with highly purified and recombinant FSH have been performed (Burgués et al, 1997; European Metrodin HP study group, 1998; Liu et al, 1999).

Recombinant products such as Puregon have an improved purity compared with urinary preparations. A pharmacokinetic-pharmacodynamic study in men with hypogonadotropic hypogonadism showed that Puregon is well tolerated and shows dose-linear serum FSH levels (Mannaerts et al, 1996). In addition, a case report supported that recFSH in combination with hCG was effective in stimulation of testicular function in a hypogonadotropic hypogonadal man (Kliesch et al, 1995).

In the present study, 14 of 30 men reached sperm concentrations $\geq 1 \times 10^6/\text{mL}$. This is slightly lower than reported in some other studies (Burgués et al, 1997; European Metrodin HP Study Group, 1998; Liu et al, 1999). This may be explained by difference in duration of treatment. In this study, treatment duration was 48 weeks (11 months), whereas 18 months or more were reported in other studies. Also, different dosage schemes comprising daily injections were described (Jones and Darne, 1993). Furthermore, our subject population included a relatively large number of men with a history of maldescended testes (37%), which is regarded as a less favorable condition for positive therapeutic outcome (Finkel et al, 1985; Kirk et al, 1994; Bouvattier et al, 1999). Subjects with a his-

tory of maldescended testes may also require longer treatment in comparison with subjects without this preexisting condition. All subjects, except one, reaching normal or nearly normal values for sperm concentration were in the group of men without a history of maldescended testes. The mean time of 22–28 weeks required for sperm concentrations to reach levels of at least $1 \times 10^6/\text{mL}$ is comparable with or even shorter than that in other studies (Kliesch et al, 1994; Burgués et al, 1997; Büchter et al, 1998; European Metrodin HP Study Group, 1998; Liu et al, 1999). By the end of the study, most patients did not reach a normal sperm concentration (lower limit $20 \times 10^6/\text{mL}$, according to WHO limits), although this is not a prerequisite for fertility in this population (Finkel et al, 1985; Ley and Leonard, 1985; Burris et al, 1988; Kliesch et al, 1994).

It should be noted that this study was not designed as a dose-finding study. The treatment regimens applied have been used for decades already in clinical practice. Only very few studies have applied higher weekly doses than 450 IU of FSH (Gayral et al, 1975; Saal et al, 1991; Jones and Darnes, 1993; Kirk et al, 1994). Since these studies included different populations concerning hypothalamic or pituitary disorders and maldescended testes, different treatment regimens for hCG, different treatment durations, and different definitions of efficacy, it is difficult to assess the effect of higher FSH doses. Therefore the applied doses and regimens from past studies and the

present study do not necessarily have to be at the top of the dose response curve.

Apart from the concentration of sperm cells, motility and morphology are regarded as critical factors for fertility (Rogers et al, 1983; Zamboni, 1992; Bonde et al, 1998). The mean values for sperm motility were within the range normally obtained after gonadotropin therapy (Burris et al, 1988). In the present study, an overall mean fraction of 35% (SD 25%) of progressive sperm cells (total of rapid and slow progressive cells) was reached at the end of treatment. According to WHO criteria (WHO, 1992), the lower limit of normal motility is 50%. Several studies involving intrauterine insemination, however, report a value of 30% of progressive motility as a good prognostic factor for positive outcome (Dickey et al, 1999). The overall mean percentage of normal morphology at the end of treatment in this study was 24% (SD 16%), which is slightly lower than the value of 30% that is regarded as the lower limit for normal sperm morphology by the WHO.

Consistent with data in the literature, a clear relation between the initial testicular volume and the treatment time required for sperm to appear in the ejaculate was found (Burris et al, 1988; Büchter et al, 1998; Liu et al, 2002).

Testicular volume progressively increased during treatment. As expected, mean T levels showed a sharp increase during the hCG-alone phase. In the combined gonadotropin treatment period, only minor or no changes in T levels were found. The fact that group A showed consistently higher T levels than group B may be explained by the higher initial testicular volume in this group.

For inhibin B, a strong increase to values seen in normozoospermic men was observed (Andersson et al, 1998; Mahmoud et al, 1998; Foresta et al, 1999; Kamischke et al, 2001). Inhibin B is produced by the testis and is a measure for Sertoli cell function (Anderson et al, 1997). Since it is known that FSH is the principal stimulus for the secretion of testicular inhibin B, this increase also indicates the efficacy of the recFSH treatment.

Since no differences were seen in local tolerance scores between the 2 treatment schemes, it can be concluded that both regimens are equally well tolerated by patients. As for convenience, patients may have a preference for a treatment scheme with 2 injections per week instead of 3 injections per week.

In conclusion, recFSH induced spermatogenesis effectively in many hypogonadotropic hypogonadal men by weekly administration of 3×150 IU or 2×225 IU, the latter being most convenient for the patient. Long-term treatment with recFSH was safe and well tolerated and antibodies against FSH or CHO cell-derived proteins were not detected.

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