Efficacy and safety of recombinant human follicle stimulating hormone (Gonal-F) with urinary human chorionic gonadotrophin for induction of spermatogenesis and fertility in gonadotrophin-deficient men

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In order to evaluate the efficacy and safety of recombinant human follicle stimulating hormone (r-hFSH) in combination with urinary human chorionic gonadotrophin (HCG) to induce spermatogenesis and fertility in gonadotrophin-deficient men, we conducted a prospective, open, non-comparative multicentre study in two Australian academic medical centres. Ten men with gonadotrophin deficiency requiring induction of spermatogenesis and fertility were treated with HCG for 3–6 months followed by the s.c. self-administration of injections of r-hFSH in combination with HCG for 18 months. Among the eight men who commenced r-hFSH treatment, seven demonstrated sperm output at a median of 6 months and five achieved the target sperm output of $1.5 \times 10^6$ per ml at a median of 9 months of FSH treatment. Mean testicular volume increased by 4.2 ml during FSH treatment. Three men produced pregnancies in their partners, two of which resulted in the birth of healthy babies and a third patient’s partner had a miscarriage. We conclude that r-hFSH is well tolerated and effective in inducing testes growth, spermatogenesis and fertility in gonadotrophin-deficient men. The efficacy of r-hFSH seems comparable with urinary FSH at restoring normal fertility in gonadotrophin-deficient men.

Key words: gonadotrophin deficiency/HCG/recombinant FSH/spermatogenesis/testis

Introduction

Gonadotrophin deficiency due to structural or functional disorders of the pituitary and/or hypothalamus is among the few disorders of male fertility amenable to specific treatment (Baker, 1994). Gonadotrophin therapy has been available for over 3 decades (Gemzell and Kjessler, 1964; MacLeod et al., 1964) with hormones originally purified from extracts of human pituitaries or urine. Pituitary extracts have a known risk of transmissible prion disease (Healy and Evans, 1993) and the advent of recombinant follicle stimulating hormone (r-hFSH) has made it feasible to replace urinary menotropin preparations. Gonal F (Ares-Serono, Geneva, Switzerland), is a recombinant form of human FSH obtained from genetically engineered Chinese hamster ovary cells in which the genes encoding the alpha and beta chains of human FSH have been introduced through recombinant DNA technology. r-hFSH has advantages over urinary FSH preparations in formulation due to its greater purity, higher specific activity, more consistent composition and theoretically unlimited supply. In addition, s.c. administration of r-hFSH has improved clinical utility due to its favourable pharmacokinetics (le Cotonne et al., 1994; Porchet et al., 1994; Handelsman et al., 1995) as well as being more suited for convenient self-injection. However, no systematic studies of the efficacy and tolerability of r-hFSH for the induction of spermatogenesis and fertility have been reported.

Materials and methods

Study design

This phase III study (GF 6410) had a prospective, open, non-comparative design. It was conducted in two Australian centres according to a protocol approved by the local institutional ethical review committees in accordance with National Health and Medical Research Council guidelines for human experimentation. The goal of the study was to establish the efficacy of r-hFSH administered by the s.c. route in association with human chorionic gonadotrophin (HCG) for induction of spermatogenesis and fertility in gonadotrophin-deficient infertile men with potentially fertile wives. The study design had a pre-entry phase of 6 months during which men were treated with HCG alone to re-establish testosterone secretion and extragonadal androgenic effects as well as to determine if FSH was required to initiate spermatogenesis. The primary efficacy endpoint was a sperm concentration of >$1.5 \times 10^6$/ml with secondary endpoints including effects on testicular volume, plasma inhibin B and pregnancy. In addition, the safety of r-hFSH administered s.c. for 18 months was also monitored by conventional clinical toxicological monitoring as well as local tolerance of injections.

Gonadotrophin deficiency in men is relatively uncommon and the low proportion of gonadotrophin-deficient men requiring fertility treatment at any one time makes recruitment for this type of study difficult. The study was non-comparative because: (i) the existence of a well-established treatment (urinary FSH) made placebo unethical; and (ii) a comparative design against standard treatment (urinary FSH) was not feasible in a timely fashion. The use of historical control data is possible because: (i) azoospermia due to gonadotrophin deficiency does not improve spontaneously; (ii) the study end-points (plasma testosterone, sperm output, pregnancy) are objective; and (iii) the response to urinary FSH is well documented in the literature (Gayral et al., 1975; Mattei and Roulier, 1978; Gattuccio et al., 1984; Finkel et al., 1985; Ley and Leonard, 1985; Okuyama et al., 1986; Burris et al., 1988; Liu et al., 1988; Mastrogiacomo et al., 1991;
Saal et al., 1991; Schopohl et al., 1991; Okada et al., 1992; Jones and Darne, 1993; Kirk et al., 1994; Kliesch et al., 1994; Kung et al., 1994; Burgues et al., 1997; European Metrodin HP Study Group, 1998).

Healthy, gonadotrophin-deficient men aged 17–56 years (with bone age >15 years and/or anosmia) living in a stable relationship and desiring fertility were eligible for the study. They had to be androgen deficient (serum testosterone <10 nmol/l), azoospermic (or aspermic) and have had no androgen therapy for 5 weeks (or HCG for 2 weeks) and willing to provide informed consent. They were also required to be free of any significant medical disease, drug abuse, regular medication that impairs testicular function. Men were excluded if they were unable to collect semen, had known testicular pathology (orchitis, torsion, uncorrected bilateral cryptorchidism, major varicocele, Klinefelter’s syndrome) or vasal obstruction or if previous HCG treatment required a dose of >10 000 IU/week to normalize blood testosterone concentration. Female partners were investigated and managed according to the judgement of their own infertility specialist prior to entry into the study.

**Treatment**

After screening and establishing eligibility, men were treated in the pretreatment phase with HCG (Profasi; Ares-Serono, Geneva, Switzerland), 2000 IU s.c. twice weekly for 3 months. If serum testosterone had not reached normal concentrations by the second month, HCG dose was increased to 2000 IU three times weekly or higher as required to achieve this objective. If men had normal concentrations of plasma testosterone but remained azoospermic at the end of 6 months HCG treatment, they were eligible to commence treatment with recombinant FSH (Gonal F; Ares-Serono, Geneva, Switzerland) 150 IU s.c. three times each week for a further 18 months. Throughout r-hFSH treatment, HCG was continued at the dose required during pretreatment phase to promote normal concentrations of serum testosterone. The r-hFSH dose was increased in some patients because of prolonged azoospermia. Injection sites were rotated around the anterior abdominal wall. Recombinant FSH was reconstituted in 0.5–1 ml of diluent and HCG in 1 ml of diluent and then immediately administered by the subject or by a family member.

**Monitoring**

Weight, pulse, blood pressure and testicular volume (by Prader orchidometer) were recorded at baseline, at the end of pretreatment and then 3 monthly during r-hFSH treatment for 18 months. At baseline, height and pubertal stage were also recorded. Blood was taken for measurement of plasma testosterone, oestradiol, luteinizing hormone (LH), FSH and inhibin B at baseline, at the end of pretreatment and at 3 month intervals thereafter. During treatment, blood samples were taken at 2–3 days after the last HCG injection. Semen analysis was performed at baseline and at the end of pretreatment to confirm azoospermia, and then at 3 monthly intervals throughout the study. All adverse events and intercurrent illnesses and their management were recorded. Local tolerance to each r-hFSH injection was monitored and any evidence of itch, swelling, redness, bruising or pain recorded.

**Assays**

Semen analysis was performed according to the World Health Organization (1992). Hormones [LH, FSH, testosterone, oestradiol, inhibin B, cortisol, prolactin, thyroid stimulating hormone (TSH), thyroxine] and biochemical variables were measured by standard immunoassays as described previously (McDonald et al., 1993; Handelsman et al., 1996; Zhengwei et al., 1998). FSH antibodies were measured by immunoprecipitation assay in which a mixture of 100 µl radio-iodinated FSH tracer and a 1:10 final dilution of plasma samples in a total volume of 300 µl were incubated overnight at room temperature followed by addition of 100 µl carrier protein solution and then precipitation with 1 ml 20% polyethylene glycol and centrifugation (15 min, 4°C, 3000 g) and washing with 8% polyethylene glycol. Any sample with precipitated radioactivity higher than twice non-specific background was declared positive.

**Data analysis**

Data were expressed as mean and standard error of the mean or median and range with P values <0.05 being considered significant unless otherwise specified. Computations were performed with SAS, SPSS and StatXact version 3 for Windows.

The fertility of gonadotrophin-deficient men treated with r-hFSH was evaluated quantitatively by comparison of the number of pregnancies observed in this study with those expected from healthy fertile men with equivalent sperm output. This was achieved by using unique quantitative fertility data arising from the two WHO male contraceptive efficacy studies (WHO Task Force on Methods for the Regulation of Male Fertility, 1990, 1996). In these WHO studies, healthy men requiring contraception had their sperm output reduced by administration of exogenous testosterone and their fertility was estimated by prospective observations (WHO, 1994). Statistical confidence intervals were calculated by regarding observed pregnancies in this study as discrete, infrequent stochastic events for which a Poisson distribution can be assumed and Poisson confidence intervals were calculated accordingly. For each time interval between semen samples observed in this study, the sperm concentration at the midpoint of each month was calculated by linear interpolation. An expected pregnancy rate for that time interval was then estimated by calculating the time the sperm concentration spent in each of the specified sperm output ranges. By summing intervals over each patient and over all patients, an expected pregnancy rate for each patient and for the whole study could be determined.

**Results**

**Patients**

The study was completed between March 1994 and July 1997, during which time 10 men (six in Sydney, four in Melbourne) were recruited. All men had hypogonadotrophic hypogonadism due to pituitary or hypothalamic disease confirmed by imaging of the pituitary fossa (by computed tomography or skull X-ray), hormone assays (including pituitary stimulation tests) and karyotype. Two men had anosmia.

At entry, men had a median age 37 (range 26–48) years, height 180 (155–193) cm, weight 86.4 (57–104) kg and body mass index 26.1 (22.9–31.1) kg/m². Mean testis volume was ≤4 ml in seven and >4 ml in three men, with an overall median of 3.5 (range 1.5–11) ml. Self-reported alcohol consumption was nil or light in nine men while three men were smokers (six never, one ex-smoker). The age at diagnosis was a median of 23 (11–39) years and all had received prior treatment for a median of 14 (0–31) years with androgens (n = 3), gonadotrophins (n = 4) or both (n = 3). Their partner’s ages ranged from 23 to 39 years with six having conceived previously in the same relationship and only one having significant adverse female fertility factors (endometriosis requiring diathermy).

Two men were treated with thyroxine and cortisone acetate.
Figure 1. Time course of semen volume (ml, top panel), plasma testosterone (nmol/l, middle panel) and plasma oestradiol (pmol/l, lower panel) at entry to study (before any treatment), and the end of pretreatment phase (up to 6 months of HCG treatment alone) and at 3 month intervals during treatment with HCG plus r-hFSH. Data are presented as mean and SEM. After the break on the x-axis, the mean of the individual peak values for each variable is illustrated. The horizontal dashed lines indicate the reference range for healthy eugonadal young men. See text for more details.

Figure 2. Time course of sperm concentration (top panel), total sperm output (middle panel) and motility (WHO grade a + b%, lower panel) at entry to study (before any treatment), and the end of the pretreatment phase (up to 6 months of HCG treatment alone) and at 3 month intervals during treatment with HCG plus r-hFSH. Data presented as mean and SEM. After the break on the x-axis, the mean of the individual peak values for each variable is illustrated. See text for more details.

Figure 3. Time course of mean testis volume (ml, upper panel), plasma FSH (IU/l, middle panel) and plasma inhibin B (pg/ml, lower panel) at entry to study (before any treatment), and the end of pretreatment phase (up to 6 months of HCG treatment alone) and at 3 month intervals during treatment with HCG plus r-hFSH. Data presented as SEM. After the break on the x-axis, the mean of the individual peak values for each variable is illustrated. See text for more details.

Efficacy

Pretreatment

During the pretreatment period when men were treated with HCG alone, the median dose of HCG was 4800 (3286–7474) IU/week and median duration of treatment was 17 (12–24) weeks. The median total HCG dose was 40 (32–80) 2000 IU ampoules. HCG treatment was associated with significant increase in testosterone, oestradiol, semen volume (Figure 1) and testicular volume (Figure 2), whereas weight was not significantly changed.

Treatment

Two men were withdrawn before entry to the r-hFSH treatment phase due to inability to normalize plasma testosterone concentration within 6 months in one case or being non-azoospermic after HCG treatment in the other. One man was withdrawn at 12 months due to difficulty in maintaining testosterone concentrations and the need for alternative treatment. During the combined treatment period of 78 (56–79) weeks, the dose of HCG [median 233 (188–462) 2000 IU ampoules] and r-hFSH [median 243 (171–310) 150 IU ampoules] administered varied between individuals and tended to increase with time. The addition of r-hFSH to HCG did not further change testosterone or oestradiol but FSH and inhibin B rose significantly (Figure 3).

Testicular volume, sperm concentration and total sperm output increased progressively during r-hFSH treatment (Figures 2 and 4). Mean testicular volume grew from a mean of 4.8 ml at entry to 9.6 ml at the end of the study. Spermatozoa were eventually present in the ejaculate of seven out of eight men after a median time of 6 months. Target sperm concentration (≥1.5×10^6/ml) was achieved by five out of eight men after a median of 9 months treatment. Sperm motility and morphology (data not shown) also progressively improved with r-hFSH treatment. A history of cryptorchidism was associated with a reduced maximum testicular volume (5 ± 1.1 versus 11.7 ± 1.4, P = 0.008) but no signi-
significant difference in either pretreatment testicular volume or maximum sperm concentration. When considering the maximal response for each individual, there were marked variations between men for semen volume (range of individual peak responses 2.0–6.0 ml), sperm concentration (0–40 × 10⁶/ml), total sperm output (0–124 × 10⁶ spermatozoa/ejaculate), mean testis volume (5–13.3 ml) and inhibin B (39.5–159 pg/ml).

Throughout treatment, all 10 couples reported regular intercourse ranging from 2 to 4 times per week. Two pregnancies were recorded during the study resulting in the normal vaginal delivery of healthy boys, and another after the completion of the study resulted in a miscarriage after frozen embryo transfer following intracytoplasmic injection of cryostored sperm. Based on the fertility data from the WHO contraceptive studies, the expected number of pregnancies was 5.1 (95% confidence interval 0.5–14) for the sperm output observed in this study.

Safety

The only unexpected adverse effects reported were a single subject who reported an upper respiratory tract illness with retro-sternal pain, which was regarded as unrelated to r-hFSH treatment.

The haematological and clinical chemistry variables were normal at entry and no significant changes were observed during treatment. There were no significant changes in weight, blood pressure, pulse rate or urinalysis during r-hFSH treatment. Anti-FSH antibodies were negative for all subjects who received r-hFSH at each time-point.

Local tolerance was evaluated after each injection. Among eight men having a total of 1930 r-hFSH injections, information was available on local reactions for 1928 (99.9%) injections. No cases of local reaction led to modification or interruption of treatment. The assessment of local reactions per patient indicated marked inter-subject variability, with six out of eight men reporting no or only mild local reactions to injections while two out of eight men reported at least one severe local reaction (pain). The assessment of local reactions per injection indicated that after 98.1% of injections there was no or only a mild reaction; only four injections were reported to cause severe local reactions (all injection site pain). Among all local reactions, the subjects rated 96% as mild with only 21 (3.7% of all reactions, 1.1% of all injections) regarded as moderately severe (bruising, pain). Considering any degree of severity, the local reactions reported comprised pain (after 23.6% of r-hFSH injections), redness (9.6%), swelling (6.9%), bruising (6.4%) and itch (1.2%).

Discussion

This is the first systematic study of the efficacy and safety of r-hFSH for the induction of spermatogenesis and fertility in gonadotrophin-deficient men. Previous reports are restricted to short-term biochemical studies (Mannaerts et al., 1996; Raivio et al., 1997) or single case reports (Quinton et al., 1994; Kliesch et al., 1995; Quinton et al., 1996).

In this study, r-hFSH induced testicular growth, spermatogenesis and fertility in gonadotrophin-deficient men with minimal adverse effects. The magnitude of these effects of r-hFSH was similar to historical data on the use of urinary FSH suggesting that the genetically engineered glycoprotein has similar biological efficacy to urinary FSH. For example, the magnitude of testicular growth induced by HCG combined with r-hFSH in this study (~5 ml) is comparable with that from other studies (3–8 ml) using urinary-derived FSH (Ley and Leonard, 1985; Okuyama et al., 1986; Burris et al., 1988; Schopohl et al., 1991; Jones and Darne, 1993; Kliesch et al., 1994; Kung et al., 1994; Burgues et al., 1997; European Metrodin HP Study Group, 1998).

In the present study, one man out of the eight (13%) failed to produce any spermatozoa despite treatment with HCG and r-hFSH. This proportion of failed treatment is comparable with that reported in previous larger studies (weighted mean 21%, range 9–53%) using urinary-derived FSH (Burris et al., 1988; Schopohl et al., 1991; Kliesch et al., 1994; Kung et al., 1994; Burgues et al., 1997; European Metrodin HP Study Group, 1998). Similarly, the median time to appearance of spermatozoa (6 months) is very similar to previous studies (5–6 months) with urinary FSH (Burris et al., 1988; Schopohl et al., 1991; Jones and Darne, 1993; Burgues et al., 1997; European Metrodin HP Study Group, 1998). For time-dependent biological variables where the end-point may not be achieved, the mean time to end-point is a biased estimate (since it neglects the contribution of men who never achieve sperm output) and the median time is a preferable estimator of time to end-point. Where the mean time to appearance of spermatozoa during urinary FSH treatment has been reported the times seem (surprisingly) longer [e.g. 6.7 and 8.7 months (Kliesch et al., 1994), 14 months (Kung et al., 1994)], which may reflect the unreliability of this estimator.

This study involved gonadotrophin-deficient infertile men all seeking paternity unlike many previous studies of gonadotrophin replacement therapy in which few, if any, men were seeking fertility (Gattuccio et al., 1984; Okuyama et al., 1986; Liu et al., 1988; Mastrogiacomo et al., 1991; Saal et al., 1991; Schopohl et al., 1991; Kirk et al., 1994; Burgues et al., 1997; European Metrodin HP Study Group, 1998). Our findings are comparable with those studies where fertility was an important...
end-point (Finkel et al., 1985; Ley and Leonard, 1985; Burris et al., 1988; Jones and Darne, 1993; Kliesch et al., 1994).

It is well known that among gonadotrophin-deficient men undergoing gonadotrophin therapy, conception usually occurs at low sperm output (Burger and Baker, 1984) leading to suggestions that such men may have relatively high fertility. Alternatively, the prolonged duration of treatment (typically >12 months) required to induce such conceptions may be interpreted as demonstrating relatively low fertility. These conflicting interpretations about the overall fertility of gonadotrophin-treated men are difficult to reconcile. To evaluate this issue, we estimated quantitatively the fertility of gonadotrophin-deficient men having combined HCG/r-hFSH treatment by comparison with healthy fertile men who have their sperm output temporarily suppressed by the administration of testosterone. This comparison demonstrated that gonadotrophin-treated men had marginally, but not significantly, lower fertility than healthy fertile men with matching sperm output. While more powerful statistical comparisons are required, this finding suggests minimal or no intrinsic defect in spermatozoa produced during treatment of gonadotrophin deficiency with gonadotrophin therapy.

The r-hFSH was well tolerated locally and systemically. In particular, local reactions were infrequent and, when present, of minimal significance. There was no evidence of any adverse effects on routine toxicological evaluations including weight, blood pressure, clinical chemistry and haematological tests. Furthermore, there was no evidence of allergy or antibodies to FSH. These findings confirm the safety of r-hFSH as demonstrated in clinical trials in women (Anonymous, 1995). In contrast to women, however, men exhibit no manifestations of FSH overdose equivalent to ovarian hyperstimulation.

The use of gonadotrophin therapy to induce spermatogenesis and fertility in gonadotrophin-deficient men is among the few specific treatments of male infertility (Baker, 1994). Traditionally, spermatogenesis is regarded as dependent upon pituitary gonadotrophin secretion. Pituitary secretion of LH stimulates Leydig cell testosterone secretion while FSH has its specific receptors located exclusively on Sertoli cells. Classically, FSH is regarded as necessary for quantitative restoration of spermatogenesis (Matsumoto et al., 1986; Schaison et al., 1993). This view is supported by the recent observations of a man with an inactivating mutation of the FSH beta subunit who had small testes and azoospermia (Phillip et al., 1998) and another man with complete gonadotrophin deficiency in whom an activating mutation of the FSH receptor led to remarkable preservation of testicular volume, spermatogenesis and fertility (Gromoll et al., 1996). Nevertheless, there remains controversy about the need for FSH in human spermatogenesis. Clinical studies suggest that prolonged HCG administration may initiate (De Sanctis et al., 1988; Vicari et al., 1992), maintain (Johnsen, 1978; Burger and Baker, 1984) or reinitiate (Matsumoto et al., 1986) spermatogenesis in some gonadotrophin-deficient men. Recent experimental studies also raise doubts about the requirement for FSH. Spermatogenesis and fertility are induced and maintained by testosterone alone in mice with complete congenital gonadotrophin deficiency (Singh et al., 1995) and a similar phenotype is also evident in mice with selective FSH deficiency due to inactivation of the FSH β-subunit gene (Kumar et al., 1997) or FSH receptor (Dierich et al., 1998). These latter findings are supported by evidence that men with an inactivating mutation of the FSH receptor are also fertile despite having small testes and reduced sperm output (Tapanainen et al., 1997). In the present study, only one out of 10 men who started HCG treatment developed spermatozoa in the ejaculate within the 6 months allowed by the protocol; however, this must be considered a minimal estimate since it is unknown how many more would have developed sperm during more prolonged treatment with HCG alone. Further fundamental studies of the role of FSH in human spermatogenesis are needed. In the interim, the clinical use of FSH to stimulate spermatogenesis after an inadequate response to HCG alone in gonadotrophin-deficient men is appropriate treatment on the available evidence.

In conclusion, this study confirms the efficacy and safety of r-hFSH in treatment of gonadotrophin-deficient men. The efficacy of r-hFSH seems comparable with urinary FSH and its safety is at least comparable with its satisfactory application in women. The availability of a novel form of a theoretically unlimited supply of FSH with consistently high purity and biological activity which is well suited to self-administration by s.c. injection provides improved opportunities for effective and safe treatment of gonadotrophin-deficient men.

References


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