Developments in human recombinant follicle stimulating hormone technology: are we going in the right direction?

Bart C.J.M. Fauser

Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Dijkzigt Academic Hospital and Erasmus University Medical School, Rotterdam, The Netherlands

Recent developments in recombinant DNA technology have enabled the large scale production of human recombinant follicle stimulating hormone (rFSH); and this compound has recently been introduced to the market. Understanding of the structure–function relationship of FSH isohormones is crucial in understanding discussions on the standardization procedures of gonadotrophin preparations, potential differences in clinical efficacy of the various gonadotrophin preparations and in comprehending future developments (long-acting and short-acting forms, and rFSH preparations with altered isohormone profiles). Differences between immunoreactive and bioactive serum FSH concentrations have been observed following the administration of rFSH. Accordingly, the isohormone distribution of rFSH is similar to, but not identical with, natural human FSH. Issues relevant to daily practice discussed in this review include: the total absence of urinary contaminants allowing for the safe s.c. administration of the compound. Production is independent from urine, and the capacity can be adjusted according to clinical needs. The relationship between serum oestradiol concentrations and number and size of follicles observed by ultrasound may change when rFSH is combined with gonadotrophin-releasing hormone (GnRH) agonist, due to low serum luteinizing hormone (LH) concentrations. In the case of endogenous serum LH concentrations within the normal range, exogenous administration of LH is redundant. In the near future, rFSH preparations with altered bioactivity will be available.

Key words: follicle stimulating hormone/ovarian stimulation/recombinant human gonadotrophins

Introduction

Follicle stimulating hormone (FSH) is part of the family of glycoprotein hormones and consists of two non-covalently linked protein subunits; the common α- and the hormone specific β-subunit. Within the pituitary gonadotroph cells, each subunit undergoes extensive post-translational glycosylation. Four asparagine-linked glycosylation sites on the protein backbone include two on the α-subunit...
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(position 52 and 78) and two on the β-subunit (positions 7 and 24). Carbohydrate side chains are branched and terminal oligosaccharides include sialic acid residues. Carbohydrates on gonadotrophins serve many important functions essential for bioactivity such as proper folding (important for three dimensional structure), receptor binding and signal transduction, and metabolic clearance. In contrast, protein subunits are the major determinant of immunoactivity (immunoassays apply antibodies directed towards specific epitopes) (Fauser and Hsueh, 1989). Minor differences in glycosylation causes heterogeneity of these glycoproteins. Therefore, pituitary (Stanton et al., 1992), serum (Ulloa-Aguirre et al., 1995), urinary (Harlin et al., 1986) and recombinant (Lambert et al., 1995) FSH should all be considered as a group of FSH isohormones. More acidic isoforms (high sialic acid content) have been shown to exhibit reduced receptor binding affinity and in-vitro bioactivity, whereas circulating half-life (and therefore in-vivo bioactivity) is extended (De Leeuw et al., 1996). Several investigators have shown that the isohormone distribution of FSH may vary dependant on changes in steroid concentrations, such as during the follicular phase of the menstrual cycle or in the post-menopause (Chappel, 1995; Ulloa-Aguirre et al., 1995). However, the physiological relevance of these observations has not yet been fully elucidated. Understanding the structure–function relationship of endogenous FSH isohormones is crucial for the comprehension of activity of gonadotrophin preparations. It should be realized that bioactivity may vary, despite similar immunoreactive FSH serum concentrations.

FSH preparations are largely used for the treatment of anovulatory infertile patients and for stimulating multiple follicle development in normo-ovulatory women for assisted reproduction. For 35 years, available preparations have been obtained from the urine of post-menopausal women, containing large quantities of both FSH and luteinizing hormone (LH). At present, recombinant DNA technology allows the production of human recombinant FSH (rFSH) by Chinese hamster ovary (CHO) cell lines (Keene et al., 1989; Howles, 1996; Olijve et al., 1996) with the rationale ‘to bring to clinics more consistent, better defined, safer, more user friendly mono-therapeutic preparations’ (Loumaye et al., 1995). Although the isohormone distribution of rFSH is similar to that of natural FSH (Keene et al., 1989), careful carbohydrate analysis has revealed minor differences such as differences in bisecting GlcNAc residues and the percentage of α 1–6 linked fucose residues (see also De Leeuw et al., 1996). Pharmacokinetic and pharmacodynamic characteristics of recFSH have been extensively studied in the human (Mannaerts et al., 1993; le Cotonnec et al., 1994a,b; Porchet et al., 1994; Mannaerts et al., 1996) and are comparable with urinary gonadotrophins. It should be realized that gonadotrophins can be quantified in various ways using immunoassays, receptor binding assays, in-vitro bioassays such as cultured rat Sertoli or granulosa cells, and in-vivo bioassays. Discrepancies in immunoreactive and bioactive FSH serum concentrations have been observed following the administration of rFSH in comparison with urinary gonadotrophin preparations (le Cotonnec et al., 1994b; Matikainen et al., 1994; Lambert et al., 1995), suggesting that minor differences exist in isohormone profile.
Table I. Overview of potential benefits of recombinant follicle stimulating hormone (rFSH) versus urinary gonadotrophin preparations

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<th>Characteristics of rFSH</th>
<th>Potential clinical implications</th>
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<td>Absence of urinary contaminants</td>
<td>Allows for safe s.c. administration</td>
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<tr>
<td>Not dependent on availability of urine</td>
<td>Availability guaranteed</td>
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<td>Potential for improved batch-to-batch consistency</td>
<td>More consistent treatment outcome?</td>
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<td>Totally devoid of LH</td>
<td><strong>Ovulation induction</strong>&lt;br&gt;LH/HCG should be added in WHO class I anovulation&lt;br&gt;Satisfactory for WHO class II (improved treatment outcome in PCOS?)&lt;br&gt;<strong>Ovarian hyperstimulation for IVF</strong>&lt;br&gt;Satisfactory in cases of combined stimulation with GnRH agonist or antagonist, although the relationship between serum oestradiol concentrations and follicle numbers may change</td>
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<td>Minor differences in isohormone distribution compared with urinary gonadotrophins</td>
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WHO class I = hypogonadotrophic, hypo-oestrogenic anovulation.<br>WHO class II = normogonadotrophic, normo-oestrogenic anovulation (including PCOS).<br>LH = luteinizing hormone; HCG = human chorionic gonadotrophin; PCOS = polycystic ovarian syndrome. GnRH = gonadotrophin-releasing hormone.

The present review will attempt to balance the potential clinical benefits of rFSH in comparison with urinary gonadotrophin preparations (see Table I). In addition, developments in the near future that may have clinical implications will also be discussed. Obviously, in everyday practice, financial issues (i.e. costs of preparations and coverage by insurance companies) should also be considered. These conditions, however, may vary from country to country and will not be addressed.

**Absence of urine contaminant**

Available human menopausal gonadotrophin (HMG) preparations are crude urinary extracts and contain only 1–2% of bioactive FSH. Local allergic reactions following repeated injections have been reported repeatedly (Biffoni et al., 1994). As much as 500 μg of non-specific, mainly unidentified proteins, are present in each ampoule. When tested by electrophoresis and Western blotting analysis, these urinary gonadotrophin preparations contain transferrin,
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immunoglobulins, urokinase, tumour necrosis factor and epidermal growth factor (Giudice et al., 1994). In highly purified urinary FSH preparations, the content of the non-FSH proteins has been reduced to <1% resulting in a >95% pure preparation (Loumaye et al., 1995).

rFSH is >99% pure. Its total absence of contaminating proteins allows for safe administration through the s.c. route, with improved tolerance for local injection. Various studies suggest that overall bioavailability is similar when it is administered via the i.m. and s.c. routes (Loumaye et al., 1995), and a recently published randomized trial in 195 in-vitro fertilization (IVF) patients showed similar local tolerance and clinical efficacy (Out et al., 1997). Patient convenience and possibly compliance will certainly be served by the easy and less painful s.c. self administration.

Availability and consistency

An extensive, labour-intensive, worldwide network is required to obtain large quantities of urine for the extraction of gonadotrophin preparations. Due to the ever-increasing demand for FSH preparations for fertility therapy, the amount of urine available may be insufficient. In addition, safety issues and proper quality control remains crucially important. Obviously, the production of recombinant gonadotrophins is independent from urine and it should be relatively easy to adjust production capacity according to clinical needs.

Several investigators have emphasized that the batch-to-batch consistency of urinary gonadotrophin preparations is limited (Harlin et al., 1986; Cook et al., 1988; Stone et al., 1989; Rodgers et al., 1995). In theory, consistency between different batches of rFSH may be improved, in comparison with consistency of urinary gonadotrophins. At present, however, data are lacking in the literature to support this notion. If this proved to be the case, it remains to be established whether this will result in improved treatment outcome. Administered doses are individualized, especially for the induction of ovulation (i.e. treatment of anovulatory patients). Consequently, the administration of ampoules with relatively low biopotency will result in a dose increase. Differences between patients are due to a disparity in ovarian response, rather than to differences in FSH serum concentrations (i.e. quantities of FSH administered). Circumstances may be different for IVF patients because, as a rule, ovarian function is normal in these women and fixed regimens are widely used.

Monotherapy

Patients suffering from hypogonadotrophic hypogonadism need exogenous LH in addition to FSH, for the stimulation of gonadal function. Extremely low LH concentrations lead to insufficient theca-derived androgen substrate for conversion to oestradiol by FSH-stimulated granulosa cell aromatase enzyme activity (Schoot
This concept is referred to as the 2-cell, two-gonadotrophin concept, emphasizing the need for both theca and granulosa cells and both LH and FSH for proper oestradiol biosynthesis (Fauser, 1997). However, follicles do grow despite low oestradiol production. The late follicular phase oestradiol concentrations identified in these patients reduce their chances for pregnancy due to absent cervical mucus production and poor endometrial receptivity. Hence Hull et al. (1994) and Kousta et al. (1996) suggested that, in these rare conditions, recombinant LH or human chorionic gonadotrophin (HCG) should be added to FSH for ovulation induction. On the basis of several studies it may be proposed that the lower threshold for serum LH concentrations allowing for adequate oestradiol biosynthesis is ~0.5 IU/l (Coelingh Bennink et al., 1994). It should be remembered that pulsatile gonadotrophin-releasing hormone (GnRH) administration should still be considered as the therapy of first choice in hypogonadotrophic patients with intact pituitary function.

It has been shown convincingly that the administration of FSH alone is sufficient for the treatment of patients presenting with serum LH concentrations within the normal range. This is true both for ovarian hyperstimulation before IVF and for treatment of anovulatory patients. Under these circumstances (representing the great majority of patients), the administration of exogenous LH is redundant. In fact, a meta-analysis of eight randomized trials comparing purified urinary FSH with HMG for IVF, disclosed improved clinical pregnancy rates in favour of FSH (Daya, 1995). Not surprisingly, improved clinical outcome following FSH was even more pronounced if the analysis was restricted to three trials where no additional GnRH agonist co-medication was used, suggesting that positive effects of FSH were particularly present in patients with intact endogenous LH secretion.

Moreover, several studies have shown that the rFSH preparations presently available are effective for ovarian stimulation before IVF and in the treatment of anovulatory patients (as will be discussed below). Interestingly in one study (Recombinant Human FSH Study Group, 1995), lower concentrations of late follicular-phase serum oestradiol concentrations were noted after rFSH treatment as compared with urinary FSH, despite similar follicle number and size. Another study (Out et al., 1995) observed higher oestradiol concentrations and increased follicle numbers in patients stimulated with rFSH. This notion may not have clinical implications because oestradiol concentrations were still in the supraphysiological range (~5000–6000 IU/l) in both studies. However, it should be recognized that the relationship between follicle number and oestradiol concentrations may change due to low LH concentrations when rFSH is used in combination with GnRH agonist (Fauser, 1997). This may have important implications for the monitoring of ovarian responses. A similar discrepancy may be observed in IVF patients when rFSH is combined with high doses of GnRH antagonist (F.H.de Jong and B.C.J.M.Fauser, unpublished observations).

Elevated LH concentrations are believed to be instrumental in a proportion of patients suffering from polycystic ovary syndrome (PCOS), characterized by hyperandrogenaemia, anovulation and polycystic ovaries. In addition, high LH
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values may be involved in increased chances for early pregnancy wastage as has been proposed by some investigators. It may be postulated that treatment outcome is impaired by the exogenous administration of LH with FSH in these patients. However, comparison of purified urinary FSH preparations versus HMG (containing similar quantities of LH and FSH activity) in these patients failed to show such an effect (Hughes et al., 1996) (for review, see Fauser and van Heusden, 1997).

Improved biopotency?

A thorough understanding of standardization procedures of gonadotrophin preparations is vital for proper interpretation of clinical studies regarding efficacy of gonadotrophin preparations. As required by regulatory agencies, gonadotrophin preparations are standardized by the classical in-vivo bioassay described 45 years ago (Steelman and Pohley, 1953). In immature rats (pretreated with a saturating dose of HCG), augmentation of ovarian weight is used as the end point of FSH bioactivity of the injected gonadotrophin preparation. This assay is cumbersome, costly and reproducibility is limited. Species differences in pharmacokinetic characteristics of various human gonadotrophin preparations have been recently demonstrated (De Leeuw et al., 1996) and questions have been raised regarding the validity of this assay for the recently-introduced rFSH preparations. There is a clear need for alternative assays with improved precision and accuracy. Alternatively, the FSH content of preparations could be quantified by means of actual amounts of protein, quantification of the isohormone profile by isoelectric focusing and densitometry (Mulders et al., 1998) or by the use of receptor binding and signal transduction, as assessed in vitro by CHO cells transfected with the human FSH receptor (Albanese et al., 1994; Schipper et al., 1996).

Two prospective randomized trials have been published recently comparing rFSH and urinary purified FSH (Metrodin®, Ares Serono, Geneva, Switzerland) in IVF patients. Both studies applied co-medication with GnRH agonist (long protocol) to prevent a premature rise in LH. One study involving the recFSH preparation Gonal-F® (Ares Serono) in 123 patients concluded that both preparations were equally effective (Recombinant Human FSH Study Group, 1995). In contrast, on the basis of a comparative trial on 981 subjects, it was concluded that the recFSH preparation Puregon® (NV Organon, Oss, The Netherlands) was more ‘effective’, because lower doses of exogenous FSH were needed and the treatment duration was reduced (Out et al., 1995). In addition, more oocytes were obtained in the latter study, and more embryos were available for cryopreservation due to the transfer of a restricted number only. It may be proposed that Puregon is more biopotent, but it remains speculative whether this will result in improved treatment outcome. The possibility that an increased amount of urinary FSH would give similar results cannot be ruled out at this point.

A randomized comparison between Puregon and Metrodin in 172 normogonadotrophic, clomiphene-resistant anovulatory patients showed the need for
lower doses and a shorter treatment duration in favour of recFSH but similar clinical outcome (Coelingh Bennink et al., 1998). So far, data from interim analyses on the use of Gonal-F for ovulation induction have been reported in abstract form only.

**Potential for changes in biopotency**

So far this review has focused on potential implications of rFSH. However, an even more exciting adventure may be the introduction of structurally-modified rFSH analogues. Several of these compounds will soon be available for human studies. This exciting new development represents a major challenge for clinical investigators and may allow for the development of new therapeutic strategies, improved efficacy and extended indications.

As mentioned previously, detailed analysis of rFSH isohormones has revealed a relationship between the isoelectric point and circulating half-life. In dogs, more acidic fractions presented with an extended circulating half-life (De Leeuw et al., 1996). However, receptor binding and in-vitro bioactivity of these fractions are reduced (Cerpa-Poljak et al., 1993; Flack et al., 1994). Effects of various separate FSH isohormones fractions on ovarian function as the ultimate end-point of in-vivo bioactivity can now be studied in the human.

Another possibility of extending the circulating half-life of recFSH has been created recently. A chimeric protein was constructed by fusing the carboxy-terminal peptide (CTP) of HCGβ (known to be responsible for the extended half-life of HCG in comparison with LH) with FSHβ. FSH–CTP shows similar receptor binding and in-vitro bioactivity when compared with wild-type FSH, although its circulating half-life is extended and its in-vivo biopotency is enhanced (Fares et al., 1992). A phase I clinical trial using this compound has started recently. Potential clinical benefits include less frequent administration, and reduced diurnal variability in serum FSH concentrations (Mizunuma et al., 1990). This may be helpful for fixed stimulation protocols for ovarian stimulation before IVF as well as for the induction of ovulation using the low-dose step-up protocol (Fauser and van Heusden, 1997). However, it should be realized that chances for ovarian hyperstimulation may also be increased due to accumulation of FSH in serum. Another exciting approach is the combination of the carboxy terminal part of HCGβ to the N-terminal part of the α-subunit. This single-chain HCG chimera has been shown to be biologically active and to have an extended circulating half-life (Sugahara et al., 1995; Boime et al. 1997). The single chain for FSH has also been constructed recently.

Finally, in-vitro animal studies have shown that partially deglycosylated recFSH molecules, designed by site-directed mutagenesis, selectively remove distinct carbohydrate residues and may act as FSH antagonists (Bishop et al., 1994; Keene et al., 1994; Fauser, 1996). These compounds may have a future use for contraceptive purposes.
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Are we going in the right direction?

rFSH preparations presently available on the market have been characterized extensively in terms of safety, pharmacokinetic and pharmacodynamic properties and clinical efficacy. Availability of these monotherapeutic agents for fertility therapy is guaranteed. rFSH can be safely administered s.c. and treatment outcome is at least as good as urinary FSH preparations. Modified recFSH molecules with altered biopotency and circulating half-life can be designed. The real challenge for clinical investigators will be to translate this fascinating new development into improved patient care, in terms of patient convenience and improved treatment outcome.

Several issues should be considered when working with rFSH preparations, now and in the near future: (i) exogenous LH or HCG should be added in cases of extremely low endogenous LH secretion, such as hypogonadotrophic hypogonadism. However, pulsatile GnRH remains the first-line therapy in case of intact pituitary function; (ii) the relationship between follicle number and size and serum oestradiol concentrations may change when rFSH is combined with GnRH agonists or antagonists for IVF. This may have implications for monitoring ovarian response; (iii) short-acting rFSH preparations may exhibit improved biopotency but may also dictate the need for more frequent twice daily injections. Long-acting rFSH preparations may be more patient-friendly because a less frequent administration may suffice. It should be understood that the chances for ovarian hyperstimulation may also be increased; (iv) it has yet to be established whether preparations with improved biopotency will result in better treatment outcome; and (v) clinical studies undertaken so far do not provide convincing evidence that monotherapy with FSH per se improved treatment outcomes versus FSH/LH preparations.

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B.C.J.M. Fauser


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B.C.J.M. Fauser

