Advances in recombinant DNA technology: corifollitropin alfa, a hybrid molecule with sustained follicle-stimulating activity and reduced injection frequency

B.C.J.M. Fauser¹, B.M.J.L. Mannaerts², P. Devroey³, A. Leader⁴, I. Boime⁵, and D.T. Baird⁶

¹Department of Reproductive Medicine and Gynecology, University Medical Center, Heidelbergs 100, 3584 CX Utrecht, The Netherlands ²Clinical Development Department, Schering-Plough, Oss, The Netherlands ³Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Brussels, Belgium ⁴The Ottawa Fertility Centre, Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada ⁵Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St Louis, MO, USA ⁶Division of Reproductive and Developmental Sciences, University of Edinburgh Centre for Reproductive Biology, The Queen’s Medical Research Institute, Edinburgh, UK

⁷Correspondence address. Tel: +31-88-7557524; Fax: +31-88-7555433; E-mail: b.c.fauser@umcutrecht.nl

Background: Recombinant DNA technologies have been used to develop longer-acting therapeutic proteins. One approach is to introduce sequences containing additional glycosylation sites. Using this technique, a new chimeric gene has been developed containing the coding sequences of the FSH β-subunit and the C-terminal peptide of the hCG β-subunit, which bears four O-linked oligosaccharide binding sites. Co-expression of the α-subunit and the chimeric FSH β-subunit produces a new recombinant molecule, named corifollitropin alfa, with a prolonged elimination half-life and enhanced in vivo bioactivity compared with wild-type FSH.

Methods: Medline searches by subject and additional searching by hand.

Results: Initial studies in pituitary-suppressed female volunteers confirmed the extended half-life of the compound. Phase II studies have shown that corifollitropin alfa is able to induce and sustain multi-follicular growth for an entire week in women undergoing ovarian stimulation using GnRH antagonist co-treatment for IVF. Corifollitropin alfa regimens have been developed with dosages of 100 and 150 μg, for patients with body weight ≤ 60 and > 60 kg, respectively.
CONCLUSIONS: Corifollitropin alfa is the first long-acting hybrid molecule with sustained follicle-stimulating activity developed for the induction of multi-follicular growth along with GnRH antagonist co-treatment for IVF. This new treatment option may be simpler and more convenient for patients compared with conventional long protocols of daily FSH injections in combination with GnRH agonist co-treatment. The safety and efficacy of such regimens is currently being evaluated in large comparative phase III clinical trials. The development of corifollitropin alfa is the first step towards a new generation of recombinant gonadotrophins.

Key words: corifollitropin alfa / ovarian stimulation / FSH / IVF

Introduction

Gonadotrophin therapy in various forms has been used to restore ovulation since the 1930s. Only after the introduction of IVF, gonadotrophins have been applied to stimulate multiple follicle development (Edwards and Steptoe, 1975; Lunenfeld, 2004). The latter treatment overrides the physiologic selection of a single dominant follicle by extending the time during which serum follicle-stimulating hormone (FSH) concentrations remain above the threshold level required for follicular recruitment and ongoing maturation (Brown, 1978; Baird, 1987; Fauser and van Heusden, 1997). The availability of a number of mature oocytes suitable for IVF procedures improves the likelihood of achieving fertilization, of generating good quality embryos, and of a successful pregnancy (Macklon et al., 2006).

The relatively short elimination half-life and rapid metabolic clearance of current FSH preparations requires that daily injections are administered to maintain steady state FSH levels above the threshold level during ovarian stimulation (Fauser and van Heusden, 1997). Frequent injections may increase stress, error rates and the treatment burden experienced by IVF patients. Therefore, it has previously been examined whether intermittent administration of recombinant FSH (rFSH) by increasing the loading dose could result in similar outcomes as daily FSH injections (Crooke et al., 1963; Balasch et al., 2001; Schoites et al., 2004). However, the development of FSH analogues with a longer terminal and a slower absorption to peak serum levels may be more helpful to render an extended injection-free period than to increase the initial dose of current FSH preparations.

Many therapeutic areas have seen a shift in drug delivery regimens in recent years, with a move towards reduced frequency of administration. For example, once-a-year zoledronate for the prevention and treatment of osteoporosis (Black et al., 2007), long-acting injectable risperidone for the treatment of schizophrenia (Möller, 2007) and contraceptive implants, such as etonogestrel, that provide sustained hormone release over a number of years (Croxatto et al., 1999). The main advantages of less frequent dosing are an increase in patient convenience, fewer chances for mistakes during drug administration and improved compliance (Richter et al., 2003), which is particularly relevant during long-term treatment. An additional benefit is that long-acting agents induce more stable serum levels of a drug compared with repeated dosing using a short-acting agent (Darney, 2000; Eerdekens et al., 2004).

This review addresses developments in gonadotrophin therapy for ovarian stimulation for IVF and discusses the latest advances in the production of longer-acting compounds with FSH bioactivity, including the development of corifollitropin alfa, a new hybrid molecule with sustained follicle-stimulating activity.

Methods

Searches were initially performed using the Medline database with the search terms ‘gonadotrophin therapy’, ‘ovarian stimulation’, ‘fertility’, ‘infertility treatment’, ‘follicle-stimulating hormone’. Where appropriate, further searches for references cited in the literature derived from the initial Medline search were performed manually or using Medline as appropriate. However, this was not a systematic review of the literature and inclusion of specific discussion topics was the subjective decision of the authors.

Native gonadotrophins: structure and function

FSH is a member of the glycoprotein hormone family, which also includes LH, hCG and thyroid-stimulating hormone. The glycoprotein hormones are cysteine-rich dimeric proteins made up of two non-identical, non-covalently linked α- and β-subunits. The α-subunit is common to all family members, whereas the β-subunit is unique to each hormone and confers biologic specificity (Ryan et al., 1988)(Fig. 1).

The glycoprotein hormones are biosynthesized as arrays of isoforms that differ from each other mainly in the structure of oligosaccharides covalently linked to the protein molecules. Post-translational modification of the primary protein structure results in differential glycosylation, which produces molecules with different isoelectric properties, molecular weights and bioactivity (Chappel, 1995). The main determinants of FSH polymorphism appear to be glycan complexity and sialic acid content (Crepus et al., 2001). Heavy sialylation produces acidic isoforms with longer half-lives in vivo than more basic isoforms. This effect is largely due to the pharmacokinetic (PK) properties of the acidic isoforms, as their in vitro potency tends to be similar to or lower than that of less sialylated isoforms (D’Antonio et al., 1999; Barrios-de-Tomasi et al., 2002).

The carbohydrate moieties on the FSH molecule serve a number of functions, including correct protein folding, assembly and secretion of the gonadotrophins and signal transduction (Stockell-Hartree and Renwick, 1992). Additional carbohydrate moieties may reduce metabolic clearance due to increases in molecular size and charge. Increased sialylation increases metabolic stability by decreasing glomerular filtration within the kidney (Wide, 1986; D’Antonio et al., 1999), and through protection against clearance by asialoglycoprotein receptors in the liver (Gottschalk et al., 1960; Morell et al., 1971; van Lenten and Ashwell, 1972). The most heavily glycosylated of the mammalian pituitary and placental glycoproteins is pregnant mares’ serum gonadotrophin (PMSG), which has multiple N- and O-linked glycosylation sites on both subunits (Murphy and Martinuk, 1991). PMSG glycosylation composition has a high sialic acid content that confers a significantly extended half-life compared with other glycoprotein hormones (Christakos and Bahl, 1979).
With the exception of hCG, the human glycoprotein hormones have relatively short terminal half-lives in vivo (Amin and Hunter, 1970; Sowers et al., 1979). HCG exhibits a nearly 10-fold increase in plasma half-life compared with LH (Kohler et al., 1968; Rizkallah et al., 1969), despite a high level of amino acid sequence homology between the two hormones (Stockell-Hartree and Renwick, 1992). The main structural difference between the two molecules is an additional 31 amino acids forming the so-called C-terminal peptide (CTP) of the hCG β-subunit. The hCG-CTP includes four additional O-linked carbohydrate side chains (Birken and Canfield, 1977), each of which has two terminal sialic acid residues (Kessler et al., 1979) (Fig. 1). Deletion of the CTP has been shown to decrease the in vivo activity of the hCG molecule 3-fold compared with wild-type in a rat ovulation assay (Matzuk et al., 1990).

**Ovarian stimulation for infertility treatment: how it all started**

It is nearly 100 years since ablation experiments in dogs provided the first empiric evidence of a role for the pituitary gland in the regulation of gonadal function (Crowe et al., 1910). Animal pituitary extracts with both FSH- and LH-like activities such as PMSG were used until the late 1960s for ovulation induction in women with gonadotrophin insufficiency. These products were eventually withdrawn from clinical use due to their antigenic potential, although PMSG is still used experimentally in laboratory animals and for ovulation induction in cattle (Lunenfeld, 2004). The first successful induction of ovulation with FSH derived from human pituitary glands was described by Gemzell et al. in 1958. Subsequent research focused on extracting and purifying gonadotrophins from human sources. Human pituitary gonadotrophins (hPG) were isolated at autopsy from lyophilized human pituitary glands (Gemzell et al., 1958), and were used successfully for over three decades for ovulation induction in anovulatory women. However, the supply of human pituitary glands was limited, and concerns over the risk of prion diseases from human brain tissue products led to the withdrawal of hPG in the early 1990s.

**Human urinary gonadotrophins**

An alternative human source of gonadotrophins was found in the urine of post-menopausal women (Lunenfeld, 2004). Human menopausal gonadotrophin (HMG) preparations were <5% pure FSH, contained much LH bioactivity and were contaminated with potentially immunogenic urinary proteins. HMG was successfully used to induce ovulation in hypogonadotrophic anovulatory women (Lunenfeld, 1963). The lack of purity and the limited batch-to-batch consistency may have negatively affected clinical results. It is now known that excessive LH levels during the early or late follicular phase (Hillier, 1994) may have a negative impact on subsequent fertilization, implantation and embryo survival rates (Kolibianakis et al., 2004).

Increasing efforts were made to separate and purify the individual components of urinary gonadotrophin preparations by various physical, chemical and immunologic means (Lunenfeld, 2004). In the early 1990s, monoclonal antibodies were used to produce highly purified urinary FSH (FSH-HP) from bulk HMG. The purity of FSH-HP was ~95%, compared with 1–2% for HMG (le Cotonnec et al., 1993). This increased purity reduced the total amount of injected protein and allowed for the first time for s.c. administration. Product consistency also enabled PK and pharmacodynamic (PD) analysis, and facilitated more patient-specific treatment plans adjusted to individual responses (Lunenfeld, 2004).

The world’s first IVF baby, Louise Brown, was born in 1978 following oocyte recovery in a natural cycle. However, it was subsequently shown that IVF success rates could be improved significantly by the use of exogenous gonadotrophins to stimulate multi-follicular development (Laufer et al., 1983; for review see Macklon et al., 2006). This new technology led to an exponential growth in the worldwide demand for gonadotrophin preparations and doubts regarding quality control, both in donor recruitment and in product purity and safety (Lunenfeld, 2004).

**Recombinant FSH**

The problem of the short supply of high-quality gonadotrophins was solved by the advent of recombinant DNA technology, which
permitted the large-scale production of pure recombinant human gonadotrophin preparations. Following the sequencing (Rathnam and Saxena, 1975; Saxena and Rathnam, 1976) and subsequent cloning (Keene et al., 1989) of the gene encoding the human FSH molecule, the first human rFSH preparations became commercially available in 1996 (De Leeuw et al., 1996; Houlus, 1996; Ollive et al., 1996). These recombinant molecules are structurally very similar to native pituitary FSH and the final product is highly purified (>99%), with a high specific in vivo bioactivity. The availability of the recombinant molecule allowed investigators to study the role of FSH in the complete absence of LH for the first time. Studies of rFSH in hypophysectomized animals (Mannaerts et al., 1994) and gonadotrophin-deficient women (Schoot et al., 1992, 1994) confirmed for the first time that growth of multiple follicles to pre-ovulatory sizes was possible in the complete absence of LH activity.

The FSH threshold/window concept and follicle development

At the same time as DNA technology was facilitating the development of recombinant gonadotrophins, the mechanism of action of FSH was further elucidated. In a normal menstrual cycle, the degeneration of the corpus luteum in the late luteal phase leads to a reduction in serum levels of estradiol (E2), progesterone and inhibin-A. These endocrine changes give rise to reduced inhibition of hypothalamic GnRH production. The subsequent increased frequency of pulsatile GnRH secretions stimulates a rise in FSH at the end of the luteal phase (Hall et al., 1992). FSH continues to rise gradually during the early follicular phase, exceeding the so-called threshold level required for continued follicular development (Brown, 1978; Baird, 1987; Fauser and van Heusden, 1997). This process is currently referred to as secondary or cyclic follicle recruitment.

FSH levels subsequently stabilize and plateau, before falling again due to negative feedback from inhibin-B and E2 produced by the growing follicles (Schipper et al., 1998a). Only follicles that happen to be at a more advanced stage of development during the inter-cycle FSH rise are able to respond to FSH (Zeleznik and Kubik, 1986). As serum FSH concentrations fall below the threshold level, all but the dominant follicle lose the stimulus to develop further and enter atresia (Zeleznik and Kubik, 1986; Van Santbrink et al., 1995).

Hence, the physiologic FSH window for follicle development is quite narrow and in normo-ovulatory cycles only a single (dominant) follicle escapes from atresia due to decreased dependency on FSH.

Ovarian stimulation prior to IVF or ICSI involves the application of relatively high doses of exogenous FSH in a timely manner to maintain serum FSH concentration above the threshold, necessary to support multi-follicular growth (Macklon et al., 2006) (Fig. 2). The prolonged period above this threshold (effectively extending the FSH window) facilitates the development of more than one follicle, thus increasing the number of oocytes available for subsequent IVF procedures (Lollis et al., 1995; Schipper et al., 1998b; Hohmann et al., 2001). Due to the relatively short t1/2 of rFSH of about 30 h (Mannaerts et al., 1993), daily FSH injections are needed during the stimulation period to prevent serum FSH levels from dropping below the threshold and subsequent follicular growth arrest. After each injection, peak serum FSH levels are reached within 10–12 h and then decline until the next injection. Steady state levels are reached only after 3–5 days of treatment, thus dose adjustments before stimulation Day 5 are not advised. The single dose and multiple dose PK properties of rFSH have been described for follitropin-alpha and follitropin-beta and are included in Table I (Mannaerts et al., 1993; Le Cotonnec et al., 1994a, b). Clearly, these properties are influenced by gender, route of administration, and dose and frequency of injections, rather than by the specific rFSH preparation injected (Mannaerts et al., 1996).

GnRH analogue co-treatment during ovarian stimulation prior to IVF or ICSI

Despite our understanding of the mechanism of FSH action during ovarian stimulation, one of the problems associated with stand-alone gonadotrophin therapy was the relatively high proportion of cycles exposed to a premature rise in LH concentrations (Pelincz et al., 2002). Developing follicles exposed to inappropriately high concentrations of LH (‘ceiling level’) may enter follicular growth arrest, premature luteinization and even ovulation prior to oocyte retrieval (Sanger and Yovich, 1985; Loumaye, 1990; Hillier, 1994).

The problem of premature luteinization could be overcome by the use of GnRH agonists causing suppression of pituitary gonadotrophins via GnRH receptor down-regulation and desensitization (Fleming et al., 1982; Porter et al., 1984). This discovery resulted in the widespread use of adjuvant GnRH agonist co-treatment during ovarian stimulation. However, due to the initial release of endogenous FSH and LH (‘flare-up’), the introduction of GnRH agonists required the development of the so-called ‘long protocol’, with agonist treatment beginning 2–3 weeks prior to the start of ovarian stimulation. One drawback of this approach is that pituitary suppression due to the...
administration of GnRH agonist results in an increased requirement for exogenous FSH. A further potential problem is that GnRH agonists are usually started in the mid-luteal phase of the pre-stimulation cycle aiming to reduce chances of ovarian cysts, which carries a small risk of agonist administration in the presence of an early pregnancy. GnRH antagonists circumvent many of the problems associated with GnRH agonist co-treatment. Unlike GnRH agonists, GnRH antagonists act by competitively blocking the receptor preventing the binding of endogenous GnRH (Klingmüller et al., 1995). Thus, inhibition of endogenous gonadotrophin release is induced within a few hours after GnRH antagonist injection and does not involve an initial ‘flare-up’ of gonadotrophins (Fauser and Devroey, 2005; Tarlatzis et al., 2006).

One benefit of the rapid action of GnRH antagonists is that administration is needed only when a premature LH rise may occur, usually during the mid- to late-follicular phase of the stimulation cycle (Diedrich et al., 1994). Another advantage is that higher levels of endogenous gonadotrophins are present at the start of stimulation, reducing the requirement for exogenous FSH in GnRH antagonist protocols compared with long agonist co-treatment regimens (Hohmann et al., 2003). Also, due to a shorter duration of stimulation with fewer intermediate-sized follicles and lower E₂ levels, there is a reduced risk of developing ovarian hyperstimulation syndrome (OHSS) (Kolibianakis et al., 2006). Fewer side effects, and less patient discomfort per cycle have been reported (Heijnen et al., 2004, 2007; Eijkemans et al., 2006). Moreover, improved tolerability of IVF treatment may well lead to a reduction in drop-out rates (Verberg et al., 2008). Therefore, the overall pregnancy rate per treatment started may be similar or increased compared with conventional GnRH agonist protocols.

Although GnRH antagonist regimens offer clear advantages compared with long GnRH agonist protocols, initial clinical uptake has been slower than expected (Fauser and Devroey, 2005; Tarlatzis et al., 2006), due mainly to concerns in regard to reduced pregnancy rates per cycle compared with conventional regimens. Some combined analyses suggested a small but significant reduction in pregnancy rates (Al-Inany et al., 2006). However, a recent systematic review has shown that the probability of a live birth after IVF does not depend on the type of GnRH analogue used (Kolibianakis et al., 2006). Outcomes from IVF cycles using GnRH antagonist protocols have no doubt benefited from increasing clinical experience with these products. As a result, the use of GnRH antagonists is now becoming more widespread in general IVF practice for ovarian stimulation in normal responders.

### Concepts of long-acting molecules with FSH bioactivity

A number of technologic approaches have been used to develop longer-acting FSH molecules, most of which have involved altering the structure of the FSH molecule itself. It was hypothesized that one way of extending the t₁/₂ of FSH would be to reduce glomerular filtration by increasing the molecular weight and charge of the molecule via the introduction of additional glycosylation. Increasing the number of N- or O-linked carbohydrate moieties extends t₁/₂ by as much as 100% compared with wild-type rFSH, but there appears to be a maximum plasma half-life beyond which further increases cannot be achieved by additional glycosylation (Weenen et al., 2004).

A unique approach was chosen by Boime and co-workers, who attached the CTP of the hCG β-subunit to the FSH β-subunit using site-directed mutagenesis and gene transfer techniques (Fares et al., 1992). They constructed a chimeric gene containing the sequence encoding the CTP of the hCG β-subunit fused to the translated sequence of the human FSH β-subunit (Fig. 1). The FSH β-CTP chimera was then transfected with the common glycoprotein α-subunit and expressed in Chinese hamster ovary (CHO) cells. It was found that the presence of the CTP sequence did not significantly affect assembly or secretion of the intact dimer by stable cell lines.

The chimeric recombinant molecule had similar in vitro receptor binding and steroidogenic activity compared with wild-type FSH but had significantly enhanced in vivo activity and plasma half-life (Fares et al., 1992). Further studies showed an ∼10-fold increase in biopotency for the chimeric molecule compared with wild-type FSH (LaPolt et al., 1992). It was also demonstrated that a single injection of this fusion molecule stimulated follicular maturation in rats sufficiently to facilitate ovulation induction 52 h later. In comparison, a single injection of the same dose of wild-type FSH was ineffective in increasing ovarian ovulatory potential. Interestingly, splitting the total dose of wild-type FSH into four injections given 12 h apart was as effective as chimeric FSH. These results indicate the importance of sustained plasma levels of FSH rather than total dose, for stimulating follicular maturation (LaPolt et al., 1992).

### Table I Pharmacokinetic properties of recombinant FSH in male and female gonadotrophin-deficient or pituitary-suppressed volunteers

<table>
<thead>
<tr>
<th>References</th>
<th>Route/subjects</th>
<th>Dose</th>
<th>Cₘₐₓ (ng/ml)</th>
<th>tₘₐₓ (h)</th>
<th>AUC₀→ₘ (ng.h/ml)</th>
<th>t½ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannaerts et al. (1993)</td>
<td>i.m.; 8 women</td>
<td>single; 300 IU</td>
<td>4.3 ± 1.7</td>
<td>27 ± 5</td>
<td>339 ± 105</td>
<td>44 ± 14</td>
</tr>
<tr>
<td></td>
<td>i.m.; 7 men</td>
<td>single; 300 IU</td>
<td>7.4 ± 2.8</td>
<td>14 ± 8</td>
<td>452 ± 183</td>
<td>32 ± 12</td>
</tr>
<tr>
<td>le Cotonnec et al. (1994a)</td>
<td>i.v.; 12 women</td>
<td>single; 300 IU</td>
<td>62 ± 18</td>
<td>–</td>
<td>575 ± 114</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>le Cotonnec et al. (1994b)</td>
<td>s.c.; 12 women</td>
<td>single; 150 IU</td>
<td>3 ± 1</td>
<td>16 ± 10</td>
<td>235 ± 144</td>
<td>37 ± 28</td>
</tr>
<tr>
<td></td>
<td>s.c.; 12 women</td>
<td>multiple; 150 IU/day</td>
<td>9 ± 3</td>
<td>8 ± 6</td>
<td>187 ± 61</td>
<td>24 ± 8</td>
</tr>
</tbody>
</table>

Cₘₐₓ, peak serum concentration at any time; tₘₐₓ, time at which serum peak FSH concentration is reached; t½, elimination (terminal) half-life; AUC₀→ₘ, area under the serum FSH concentration curve. Values are presented as means ± SD.
The generation of a new CHO cell line expressing the FSH hybrid molecule has led to the development of corifollitropin alfa, a new gonadotrophin preparation with increased in vivo FSH bioactivity. Interestingly, the linkage of CTP to recombinant hormones like erythropoietin has also led to the development of long-acting agents in other therapeutically relevant areas (Fares et al., 2007).

Other investigators have generated longer-acting FSH molecules by introducing additional sequences containing potential glycosylation sites at the N-terminus of the FSH α-subunit (Perlman et al., 2003), or by creating a contiguous, single-chain, covalently-bound fusion protein containing the common α- and FSH β-subunits separated by the hCG β-CTP (Ben-Menahem and Boime, 1996; Sugahara et al., 1996; Klein et al., 2002). Using the single-chain platform, an FSH analogue with additional N-linked carbohydrates has also been constructed (Klein et al., 2003). All these approaches have yielded similar results to corifollitropin alfa in terms of extending the in vivo half-life of the molecules.

An alternative approach to producing long-acting molecules involves fusion with the constant region fragment (Fc) domain of immunoglobin G1. A naturally occurring Fc receptor is expressed in the lungs and has been shown to transport high-molecular weight Fc fusion protein molecules non-invasively by the pulmonary route in non-human primates (Bitonti et al., 2004) and humans (Dumont et al., 2005). Two forms of FSH were created: Fc fusion protein (a single-chain configuration, with α- and FSH β-subunits fused sequentially to each arm of an Fc dimer, producing a molecule dimeric for both Fc and FSH) and a heterodimer format, with a single α-subunit fused to one arm of an Fc dimer and a single FSH β-subunit fused to the other arm (producing a structure monomeric for the FSH molecule) (Low et al., 2005). Both forms demonstrated increased stability in the blood with a t1/2 of 55–210 h after pulmonary delivery in non-human primates, compared with ~24–30 h for rFSH given intravenously in humans and intramuscularly in non-human primates (le Cotonnec et al., 1994; Weinbauer et al., 1994). Both Fc–FSH fusion protein forms were more effective than rFSH at stimulating ovarian response as measured by ovarian weight gain in rats and serum inhibin levels in cynomolgus monkeys, with the Fc heterodimer–FSH monomer being more potent than the single-chain (Low et al., 2005). This was possibly due to improved transmucosal transport in the lung with decreasing size and charge or to the increased stability conferred on the non-covalently linked heterodimeric FSH molecule by fusion of the α- and β-subunits to the dimeric Fc moiety (Dumont et al., 2006). This monomeric FSH:Fc molecule is the subject of further investigation, but is not currently in clinical development.

**Corifollitropin alfa: a new rFSH analogue**

Corifollitropin alfa is a new gonadotrophin preparation currently in development for the stimulation of multi-follicular development in women undergoing ovarian stimulation for IVF or ICSI. The active compound is a chimeric recombinant molecule composed of FSH and the CTP of the hCG β-subunit (Fig. 1). Like wild-type FSH, corifollitropin alfa interacts only with the FSH-receptor and lacks LH activity (Lapolt et al., 1992). However, corifollitropin alfa has a longer t1/2 and an extended time-interval (tmax) to peak serum levels (Cmax) (Duijkers et al., 2002).

In previous phase II trials, a single dose of corifollitropin alfa was able to induce and sustain multi-follicular growth during the first week of stimulation. Accordingly, the proposed class name for this new gonadotrophin is sustained follicular stimulants. Corifollitropin alfa has the same pharmacological activity as pure FSH preparations. However, a single dose of corifollitropin alfa is able to keep circulating FSH activity above the threshold necessary to support multi-follicular growth for an entire week (Fig. 2). As such, one injection of corifollitropin alfa replaces the first seven daily injections of rFSH. Thereafter, stimulation may be continued with daily FSH injections until the criteria for final oocyte maturation have been reached. In the context of improving treatment simplicity and reducing the burden of IVF treatment, corifollitropin alfa has been developed in combination with GnRH antagonist co-treatment.

To date, corifollitropin alfa has been tested in over 400 women in phase I and II clinical trials, in doses ranging from 7.5 to 240 μg. Phase I started with one trial in male hypogonadotropic hypogonadal volunteers (Bouloux et al., 2001), who received four repeated s.c. injections of 15 μg corifollitropin alfa to examine the safety and possible immunogenicity of corifollitropin alfa. In the next phase I study, the PK of a single dose of 30–120 μg corifollitropin alfa and the ovarian response to this dose were investigated in pituitary-suppressed female volunteers (Duijkers et al., 2002). Two phase II studies of corifollitropin alfa have been conducted in patients undergoing ovarian stimulation for IVF or ICSI using single doses of 120–240 μg (Devroey et al., 2004) and of 60–180 μg (The Corifollitropin Alfa Dose-finding Study Group, 2008).

To explore whether corifollitropin alfa could also be useful in classical ovulation induction in anovulatory patients diagnosed with polycystic ovary syndrome (PCOS), a first feasibility study of administration of low dosages (7.5–60 μg) of corifollitropin alfa was performed in anovulatory women (Balen et al., 2004). Distinct individual response differences regardless of dose were observed.

**Pharmacokinetics**

Exposure after injection of corifollitropin alfa can be measured most reliably by means of a specific corifollitropin alfa enzyme immunoassay, which does not cross-react with native or rFSH (Devroey et al., 2004). Although corifollitropin alfa may cross-react in a linear dose-related fashion in commercial FSH kits as demonstrated for the FSH DeltaIA assay (AutoDELFI, Wallac Oy, Finland), the immunoreactivity of corifollitropin alfa in such assays cannot be translated into international units, since the monoclonal antibodies raised against FSH have a different affinity for corifollitropin alfa.

The main calculated PK parameters for corifollitropin alfa in IVF patients are shown in Table II. The results of phase I and phase II trials in pituitary-suppressed volunteers and patients, respectively, show that the mean t1/2 of corifollitropin alfa is ~65 h for all doses tested between 60 and 240 μg, compared with ~35 h for rFSH (Duijkers et al., 2002; Balen et al., 2004; Devroey et al., 2004; The Corifollitropin Alfa Dose-finding Study Group, 2008). Dose-normalized (dn) area under the curve (AUC) and dn Cmax are similar across all doses, indicating that the PK parameters of corifollitropin alfa are dose-proportional over this range. Median Cmax of
corifollitropin alfa is reached between 25 and 45 h after injection. No differences were observed between the PK in volunteers pituitary-suppressed by oral contraceptives (Duijkers et al., 2002) and non-suppressed patients undergoing ovarian stimulation in a GnRH antagonist protocol. Elimination of corifollitropin alfa is not largely affected by body weight, but exposure is inversely correlated to body weight, exhibiting a linear relationship to both serum clearance and volume of distribution (The Corifollitropin Alfa Dose-finding Study Group, 2008).

In summary, the single-dose PK of corifollitropin alfa are characterized by a slow absorption resulting in peak levels within 2 days after injection. Thereafter, serum corifollitropin alfa levels decrease steadily, though the FSH activity may be retained above the FSH threshold for an entire week if the administered dose of corifollitropin alfa is sufficiently high.

### Efficacy

The PK profile of corifollitropin alfa after a single injection implies the highest FSH activity during the first 2 days of stimulation, followed by decreasing FSH activity until treatment with daily FSH is started. As such, the profile mimics rather the high FSH starting dose and if needed FSH step-down as practiced in North America instead of the low starting dose and if needed FSH step-up as practiced in Europe (Macklon et al., 2006). Injection of corifollitropin alfa in the early follicular phase of the menstrual cycle results in the ongoing stimulation of the recruited cohort of antral follicles. Therefore,

### Table II Pharmacokinetic properties of corifollitropin alfa in IVF patients

<table>
<thead>
<tr>
<th>Dose (µg)</th>
<th>n</th>
<th>$t_{1/2}$ (h)</th>
<th>$t_{\text{max}}$ (h)</th>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>$dn-C_{\text{max}}$ [(ng/ml)/µg]</th>
<th>$AUC_{0-\infty}$ (ng.h/ml)</th>
<th>$dn-AUC_{0-\infty}$ [(ng.h/ml)/µg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>75</td>
<td>65.7</td>
<td>41.9</td>
<td>1.90</td>
<td>0.0317</td>
<td>275</td>
<td>4.58</td>
</tr>
<tr>
<td>120</td>
<td>24</td>
<td>64.1</td>
<td>24.6</td>
<td>4.26</td>
<td>0.0355</td>
<td>511.7</td>
<td>4.26</td>
</tr>
<tr>
<td>180</td>
<td>75</td>
<td>65.3</td>
<td>41.2</td>
<td>3.71</td>
<td>0.0309</td>
<td>534</td>
<td>4.45</td>
</tr>
<tr>
<td>240</td>
<td>24</td>
<td>65.6</td>
<td>24.8</td>
<td>6.61</td>
<td>0.0367</td>
<td>815.2</td>
<td>4.53</td>
</tr>
<tr>
<td>240</td>
<td>76</td>
<td>66.0</td>
<td>44.1</td>
<td>5.53</td>
<td>0.0307</td>
<td>827</td>
<td>4.59</td>
</tr>
<tr>
<td>240</td>
<td>25</td>
<td>64.9</td>
<td>24.7</td>
<td>8.85</td>
<td>0.0369</td>
<td>1080</td>
<td>4.50</td>
</tr>
</tbody>
</table>

Data taken from †Devroey et al. (2004) and ‡The Corifollitropin Alfa Dose-finding Study Group, (2008). All values are means, unless otherwise stated. $AUC_{0-\infty}$, median area under the serum corifollitropin alfa concentration curve; $C_{\text{max}}$, peak serum concentration at any time; $dn$, dose-normalized; $t_{1/2}$, elimination (terminal) half-life; $t_{\text{max}}$, time at which serum peak corifollitropin alfa concentration is reached.

### Table III Efficacy of corifollitropin alfa for ovarian stimulation in two phase II IVF trials

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>120 µg (n = 25)</td>
<td>180 µg (n = 24)</td>
</tr>
<tr>
<td></td>
<td>240 µg (n = 25)</td>
<td>240 µg (n = 27)</td>
</tr>
<tr>
<td></td>
<td>60 µg (n = 77)</td>
<td>120 µg (n = 77)</td>
</tr>
<tr>
<td></td>
<td>180 µg (n = 79)</td>
<td></td>
</tr>
<tr>
<td>Median duration of additional stimulation from cycle Day 8 (days)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Median total dose rFSH From cycle Day 8 (IU)</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>No. of follicles on day of hCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;11 mm</td>
<td>12.7 ± 6.8</td>
<td>13.5 ± 7.1</td>
</tr>
<tr>
<td>&gt;15 mm</td>
<td>5.9 ± 2.5</td>
<td>6.6 ± 3.1</td>
</tr>
<tr>
<td>&gt;17 mm</td>
<td>3.3 ± 0.9</td>
<td>3.5 ± 1.2</td>
</tr>
<tr>
<td>No. of cumulus–oocyte complexes retrieved/started cycle</td>
<td>11.0 ± 7.1</td>
<td>11.1 ± 7.5</td>
</tr>
<tr>
<td>No. of metaphase II oocytes*</td>
<td>10.9 ± 6.9</td>
<td>8.5 ± 6.3</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>73 ± 27</td>
<td>68 ± 31</td>
</tr>
<tr>
<td>No. of embryos obtained</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8.5 ± 5.5</td>
<td>6.6 ± 4.9</td>
</tr>
<tr>
<td>Good-quality</td>
<td>4.8 ± 5.0</td>
<td>3.8 ± 3.3</td>
</tr>
<tr>
<td>Transferred</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.5</td>
</tr>
</tbody>
</table>

*Restricted to subjects with IVF or ICSI rFSH, recombinant follicle-stimulating hormone. All data represent mean ± SD, unless otherwise specified.
Corifollitropin alfa is effective in the stimulation of multi-follicular growth for IVF, but seems far less suitable for the induction of mono-follicular growth, as documented in a first feasibility study in anovulatory women (Balen et al., 2004). In this small trial, in several cases corifollitropin alfa induced multi-follicular growth even though much lower dosages were tested. To date, there are insufficient data to support the application of this compound for ovulation induction in anovulatory patients or in intrauterine insemination patients.

In IVF, the first case report of a pregnancy and live birth following corifollitropin alfa administration was reported 5 years ago (Beckers et al., 2003) in a patient participating in the first phase II clinical trial (Devroye et al., 2004). In this feasibility trial (see Table III), dosages of 120, 180 and 240 µg corifollitropin alfa were tested in 25 patients per dose group, but no significant dose–response relationship was observed either in terms of the total dose of FSH required from Day 8 onwards or in the number of oocytes retrieved. This suggested that the lowest effective dose might be lower than those tested. Nevertheless, the study confirmed for the first time the hypothesis that a single injection of corifollitropin alfa could induce and sustain multi-follicular development for an entire week and thus could replace the first seven injections of daily FSH.

Accordingly, the most recent dose-finding study investigated lower dosages of corifollitropin alfa, with initiation of GnRH antagonist fixed at stimulation Day 5 (The Corifollitropin Alfa Dose-finding Study Group, 2008). A total of 325 patients were randomized to receive corifollitropin alfa 60, 120 or 180 µg, or daily fixed 150 IU rFSH (see Table III). A statistically significant increase in the number of oocytes retrieved over this dose range was observed. However, the high cancellation rate in the 60 µg dose group (44%) indicated that this dose was too low to support the first 7 days of ovarian stimulation. When comparing the number of follicles ≥ 11 mm on stimulation Day 8 or on the day of hCG administration, a similar dose-related increase was noted (Fig. 3). Rises of serum inhibin-B and E2 levels were very similar between all treatment groups during the first 5 days of stimulation (Fig. 4). From Day 6 onwards, inhibin-B levels continued to increase in the 180 µg group and daily rFSH group, whereas inhibin-B reached a plateau from Day 6 to 8 in the 120 µg group and decreased in the 60 µg group. Median serum E2 levels declined from Day 6 onwards in the 60 µg group, reached a plateau in the 120 µg group, and continued to increase in the 180 µg group. For patients who received hCG, serum E2 levels were similar in the 60, 120 µg and rFSH groups, but ~1.5-fold higher in the 180 µg group (Fig. 4).

From these initial trials, it could be concluded that the optimum dose of corifollitropin alfa to sustain follicular development for one week was greater than 60 µg and lower than 180 µg. In order to select the final doses for phase III development, mathematical modelling was applied using historic PK and PD data from corifollitropin alfa, rFSH and GnRH antagonist (ganirelix) trials, which included more than 3000 subjects overall (De Greef et al., 2007). Using this approach, modelling revealed that, in the desired one week regimen, 150 µg is the most appropriate dose for achieving an optimal treatment outcome in terms of the number of oocytes or fertilized two-pronucleate oocytes for patients with a body weight > 60 kg. However, because of the inverse relationship between body weight and exposure, in women weighing ≤ 60 kg a lower dose of 100 µg was shown to result in a relevant decrease in exposure without compromising the follicular support during the first week of stimulation. A phase III programme using these single doses of corifollitropin alfa (100 and 150 µg) is ongoing, including follow-up studies of frozen-thawed embryos, pregnant women and their offspring.

Safety

Because of the extended action of long-acting FSH-like preparations, in theory safety could be of concern. Chances for OHSS could be augmented due to extended stimulation of the development of multiple follicles. Since the initial corifollitropin alfa dose has been given, it is not possible—as in daily injections—to reduce the dose in case signs of ovarian stimulation are observed during the mid-follicular phase. One week after the initial injection, daily FSH doses can be either reduced or withheld.

Corifollitropin alfa has proved to be well tolerated in all the studies conducted to date, with a safety profile comparable to that of daily rFSH. No antibody formation has been observed, even after repeated dosing (Bouloux et al., 2001). In addition,
Figure 4 Median serum hormone concentrations measured during stimulation and during the luteal phase in subjects who received hCG.

(A) FSH; (B) LH; (C) estradiol (E$_2$); (D) progesterone (P); (E) inhibin-B (The Confolitropin Alfa Dose-finding Study Group, 2008). OPU, oocyte pick-up; ET, embryo transfer; ET2, two weeks after embryo transfer.
measurement of local tolerance demonstrated that s.c. administration of corifollitropin alfa is well tolerated, as no moderate or severe local reactions occurred. Further, no increase in intensity of injection-site responses was observed after repeated exposure (Bouloux et al., 2001). The most commonly reported adverse events across all trials to date were headache and nausea, followed by abortion and pelvic pain. Signs and symptoms of OHSS were reported in 15 out of 307 patients treated for IVF or ICSI with 60–240 μg corifollitropin alfa, and 6 of these patients (2%) required hospitalization. This was similar to the incidence of OHSS reported in the rFSH group—four cases in 106 subjects, three of them (3%) requiring hospitalization (Devroye et al., 2004; The Corifollitropin Alfa Dose-finding Study Group, 2008).

Benefit-risk profile

One single s.c. injection of corifollitropin alfa is able to initiate and sustain multiple follicular growth for an entire week. Accordingly, the compound has been developed for the induction of multiple follicles in IVF patients. Corifollitropin alfa tested at low dosages has been shown to be less eligible for classical ovulation induction, since its PK profile with relatively high exposure during the first days after administration may not favour mono-follicular development.

In ovarian stimulation prior to assisted reproduction treatment, a single injection of corifollitropin alfa replaces the first seven injections of daily rFSH. As such, corifollitropin alfa may reduce the treatment burden during the first week of stimulation. Corifollitropin alfa may be injected at home by self-administration or at the IVF unit by the medical staff. The reduced frequency of dosing brings an obvious benefit to those that fear needles and also brings an anticipated increase in patient convenience, fewer chances for mistakes during treatment and improved compliance. From stimulation Day 8 onwards, patients may continue treatment with a fixed daily dose of rFSH, depending on their ovarian response. Patients who reach the criteria of triggering final oocyte maturation prior to Day 8 of stimulation do not need any daily FSH to be administered.

The benefits of the corifollitropin alfa regimen should be weighed against the potential risks, which will be finally determined in phase III trials. Corifollitropin alfa is being developed in two dosages, i.e. 100 and 150 μg which, based on simulations, will provide the same exposure and the same degree of ovarian stimulation in the recommended body weight groups. Overall, this may be slightly higher compared with daily rFSH. Although the ovarian response induced by corifollitropin alfa may decrease with the patient’s age and ovarian reserve, the dose of corifollitropin alfa cannot be reduced to obtain milder stimulation as the indicated doses are required to cover the one week treatment interval. After corifollitropin alfa injection, serum FSH activity declines from stimulation Day 3 (Cmax) onwards. Dose reductions during the first week of stimulation cannot be made in case of hyper-response. Therefore, corifollitropin alfa may be less suitable for patients with known risk factors for a hyper-response, such as patients with a history of hyper-response to medication, OHSS or patients with PCOS. Finally, for eligible patients, it remains to be confirmed in a large controlled phase III trial that the pregnancy rate/live-birth rate of this new treatment regimen is comparable to that of daily rFSH/GnRH antagonist protocols.

Future directions

Clinical research with gonadotrophin molecules that have been modified in their PK or PD properties from native or wild-type recombinant gonadotrophins may give further insight into factors which may affect ovarian response. The first compound which has been tested clinically is corifollitropin alfa. This compound introduces a new treatment regimen using a single injection in the early follicular phase followed by daily rFSH injections after 1 week of stimulation. A phase III trial programme is underway to study whether the relatively high exposure to FSH activity during the first days of stimulation affects follicular recruitment, steroid production, follicular and/or oocyte gene expression or endometrial receptivity.

The first phase III trials using corifollitropin alfa in combination with a fixed daily GnRH antagonist co-treatment protocol have recently been completed. This regimen will further simplify treatment and may reduce the treatment burden of IVF for patients. Future trials concerning corifollitropin alfa will need to compare clinical outcomes using GnRH antagonist co-treatment with those achieved using long GnRH agonist protocols.

Consistent with other therapeutic areas, novel drug development in the infertility field is likely to concentrate on less invasive delivery methods, such as the use of long-acting compounds or different routes of administration that may include transdermal, inhaled or oral agents. On the horizon is the development of orally active, low-molecular weight gonadotrophins, for which a first proof-of-concept study has been reported in female volunteers (Mannaerts, 2005).

Conflict of interest: B.C.J.M.F. has received fees and grants from the following companies (in alphabetic order); Andromed, Ardana, Ferring, Genovum, Merck Serono, Organon, Pantharei Bioscience, PregLem, Schering, Schering Plough, Serono and Wyeth. D.T.B. has received fees and/or grants from the following companies Organon, Schering Plough, Serono, Merck Serono, Ferring, Wyeth, Schering, Sofinnova, Exelgyn, Danco. He was a member of the DMEC of Phase 2 Trial of Corifollitropin alfa. P.D., A.L. and I.B. have received fees and grants from several pharmaceutical companies including Organon/Schering Plough. All authors have been involved as an investigator and/or an advisor in the research and development of corifollitropin alfa.

Funding

Medical writing support was provided by Angela Meadows at Prime Medica Ltd during the preparation of this paper, and supported by Schering-Plough. Responsibility for opinions, conclusions and interpretation of data lies with the authors.

References


StangerJD, Yovich JL. Reduced in vitro fertilization of human oocytes from patients with raised basal luteinizing hormone levels during the follicular phase. Br J Obstet Gynaecol 1985;92:385–393.


Wide L. The regulation of metabolic clearance rate of human FSH in mice by variation of the molecular structure of the hormone. Acta Endocrinol (Copenh) 1986;112:336–344.

Zeleznik AJ, Kubik CJ. Ovarian responses in macaques to pulsatile infusion of follicle-stimulating hormone (FSH) and luteinizing hormone: increased sensitivity of the maturing follicle to FSH. Endocrinology 1986;119:2025–2032.

Submitted on September 26, 2008; resubmitted on November 26, 2008; accepted on December 29, 2008.