13 Follicle-Stimulating Hormone

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INTRODUCTION

About 15 % of all couples experience infertility at some time during their reproductive lives. Nowadays, infertility can be treated by the use of assisted reproductive technologies (ART), such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). A common element of these programs is the treatment with follicle-stimulating hormone (FSH) to increase the number of oocytes retrievable for the IVF or ICSI procedure (multifollicular development). Patients suffering from female infertility because of chronic anovulation may also be treated with FSH, then with the aim to achieve monofollicular development.

Natural FSH is produced and secreted by the anterior lobe of the pituitary, a gland at the base of the brain. Its target is the FSH receptor at the surface of the granulosa cells that surround the oocyte. FSH acts synergistically with estrogens and luteinizing hormone (LH) to stimulate proliferation of these granulosa cells, which leads to follicular growth. As the primary function of FSH in the female is the regulation of follicle growth and development, this process explains why deficient endogenous production of FSH may cause infertility. In males, FSH plays a pivotal role in spermatogenesis.

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FSH preparations for infertility treatment are traditionally derived from urine from (post) menopausal women. As over 100,000 l of urine may be required for a single batch, many thousands of donors are needed. Hence, the source of urinary FSH is heterogeneous and the sourcing cumbersome. Moreover, in addition to FSH, these urinary preparations contain impurities including pharmaceutically active proteins such as LH. Recombinant DNA technology allows the reproducible manufacturing of FSH preparations of high purity and specific activity, devoid of urinary contaminants. Recombinant FSH is produced using a Chinese hamster ovary (CHO) cell line, transfected with the genes encoding for the two human FSH subunits (Van Wezenbeek et al. 1990; Howles 1996). The isolation procedures render a product of high purity (at least 99 %), devoid of LH activity and very similar to natural FSH.

Currently, there are several clinically approved recombinant FSH-containing drug products on the various markets. The most widely approved products are Gonal-F®, manufactured by Merck Serono S.A., and Puregon®, with the brand name of Follistim® in the USA and Japan, manufactured by N.V. Organon, now part of Merck Sharp and Dohme. Regulatory authorities have issued two distinct International Nonproprietary Names (INN) for the two corresponding recombinant FSH drug substances, i.e., follitropin-α (Gonal-F®) and follitropin-β (Puregon®/Follistim®). In addition, a few other recombinant FSH preparations (claimed to be "biosimilar" to follitropin-α or follitropin-β) are under development or available in a very limited number of countries.

FSH IS A GLYCOPROTEIN HORMONE

Follicle-stimulating hormone belongs to a family of structurally related glycoproteins which also includes luteinizing hormone (LH); chorionic gonadotropin (CG), collectively called the gonadotropins; and thyroid-stimulating hormone (TSH, also named thyrotropin). Each hormone is a heterodimeric protein

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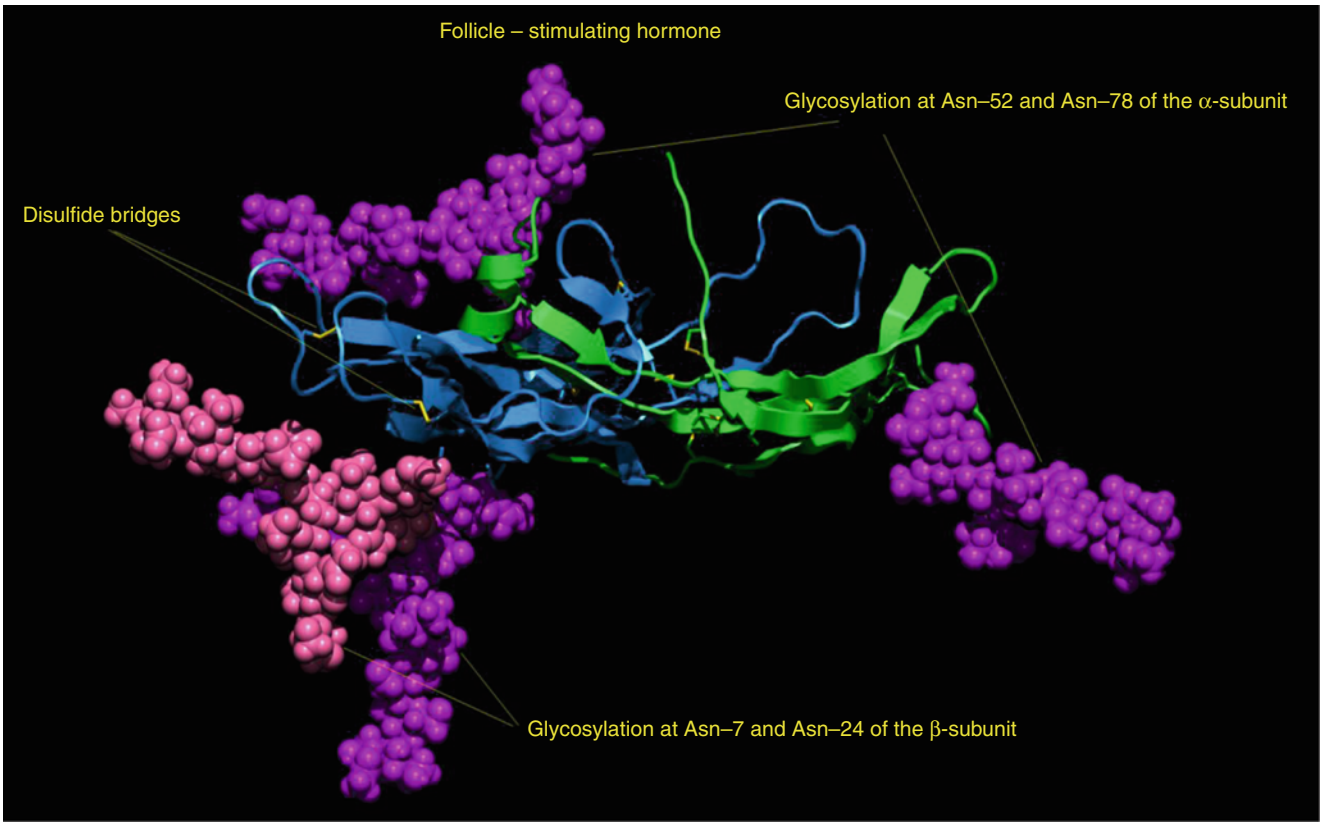

Figure 13.1 ■ A three-dimensional model of FSH. The ribbons represent the polypeptide backbones of the α-subunit (*green ribbon*) and the β-subunit (*blue ribbon*). The carbohydrate side chains (*violet and pink space-filled globules*) cover large areas of the surface of the polypeptide subunits.

consisting of two non-covalently associated glycoprotein subunits, denoted α and β. The α-subunit contains five intra-subunit disulfide bonds and is identical for all these glycoproteins, and it is the β-subunit (having six intra-subunit disulfide bonds) that provides each hormone with its specific biological function.

The glycoprotein subunits of FSH consist of two polypeptide backbones with carbohydrate side chains attached to the two asparagine (Asn) amino acid residues on each subunit. The oligosaccharides are attached to Asn-52 and Asn-78 on the α-subunit (92 amino acids) and to Asn-7 and Asn-24 on the β-subunit (111 amino acids). The glycoprotein FSH has a molecular mass of approximately 35 kDa. For the FSH preparation to be biologically active, the two subunits must be correctly assembled into their three-dimensional heterodimeric protein structure and posttranslationally modified (Fig. 13.1).

Assembly and glycosylation are intracellular processes that take place in the endoplasmic reticulum and in the Golgi apparatus. This glycosylation process leads to the formation of a population of hormone isoforms differing in their carbohydrate side-chain composition. The carbohydrate side chains of FSH are essential for its biological activity since they (1) influence FSH receptor binding, (2) play an important role in the signal transduction into the FSH target cell, and (3) affect the plasma residence time of the hormone.

Recombinant FSH contains approximately one third carbohydrate on a mass per mass basis. The carbohydrate side chains are composed of mannose, fucose, N-acetyl-glucosamine, galactose, and sialic acid. Structure analysis by ^{1}H-NMR spectroscopy on oligosaccharides enzymatically cleaved from follitropin-β reveals minor differences with natural FSH. For instance, the bisecting GlcNAc residues are lacking in the recombinant molecule, simply because the FSH-producing CHO cells do not possess the enzymes to incorporate these residues. Furthermore, the carbohydrate side chains of recombinant FSH exclusively contain α2-3-linked sialic acid, whereas in the natural hormone α1-6-linked sialic acid occurs as well. However, all carbohydrate side chains identified in recombinant FSH are moieties normally found in other natural human glycoproteins.

Whereas FSH only contains N-linked carbohydrates, human chorionic gonadotropin (hCG) also carries 4 O-linked (at serine or threonine residues)

carbohydrates, all located at the carboxy-terminal peptide (CTP) of its beta subunit. This glycosylated CTP is the major difference with the beta subunit of LH and is demonstrated to be responsible for the much longer plasma residence time of hCG compared to natural LH (Matzuk et al. 1990).

PRODUCTION OF RECOMBINANT FSH

The genes coding for the human FSH α-subunit and β-subunit were inserted in cloning vectors (plasmids) to enable efficient transfer into recipient cells. These vectors also contained promoters that could direct transcription of foreign genes in recipient cells. CHO cells were selected as recipient cells since they were easily transfected with foreign DNA and are capable of synthesizing complex glycoproteins. Furthermore, they could be grown in cell cultures on a large scale. To construct an FSH-producing cell line, N.V. Organon, the manufacturer of Puregon®/Follistim®, used one single vector containing the coding sequences for both subunit genes (Olijve et al. 1996). Merck Serono S.A., the manufacturer of Gonal-F®, used two separate vectors, one for each subunit gene (Howles 1996). Following transfection, a genetically stable transformant producing biologically active recombinant FSH was isolated. For the CHO cell line used for manufacturing Puregon®/Follistim®, it was shown that approximately 150–450 gene copies were present.

To establish a master cell bank (MCB), identical homogeneous cell preparations of the selected clone are stored in individual vials and cryopreserved until needed. Subsequently, a working cell bank (WCB) is established by the expansion of cells derived from a single vial of the MCB, and aliquots are put in vials and cryopreserved as well. Each time a production run is started, cells from one or more vials of the WCB are cultured.

Both recombinant FSH products are isolated from cell culture supernatants. These supernatants are collected from a perfusion-type bioreactor containing recombinant FSH-producing CHO cells grown on microcarriers. This is because the CHO cell lines used are anchorage-dependent cells, which implies that a proper surface must be provided for cell growth. The reactor is perfused with growth-promoting medium during a period that may continue for up to three months (see also Chap. 3). The downstream purification processes for the isolation of the two recombinant FSH products are different. For Puregon®/Follistim®, a series of chromatographic steps, including anion and cation exchange chromatography, hydrophobic chromatography, and size-exclusion chromatography, is used. Recombinant FSH in Gonal-F® is obtained by a similar process of five chromatographic steps but also includes an immunoaffinity step using a murine FSH-specific monoclonal antibody (European Public Assessment Report, Gonal-F 2011). In both production processes, each purification step is rigorously controlled in order to ensure the batch-to-batch consistency of the purified product.

DESCRIPTION OF RECOMBINANT FSH

■ Structural Characteristics

Like urinary-sourced (natural) FSH, the recombinant versions exist in several distinct molecular forms (isohormones), with identical polypeptide backbones but with differences in oligosaccharide structure, in particular in the degree of terminal sialylation. These isohormones can be separated by chromatofocusing or isoelectric focusing on the basis of their different isoelectric points (pI, as has been demonstrated for follitropin-β (De Leeuw et al. 1996) (Fig. 13.2)). The typical pattern for FSH indicates an isohormone distribution between pI values of 6 and 4. To obtain structural information at the subunit level, the two subunits were separated by RP-HPLC and treated to release the N-linked carbohydrate side chains. Fractions with low pI values (acidic fractions) displayed a high content of tri- and tetrasialo oligosaccharides and a low content of neutral and monosialo oligosaccharides. For fractions with a high pI (basic fractions) value, the reverse was found. The β-subunit carbohydrate side chains appeared to be more heavily sialylated and branched than the α-subunit carbohydrate side chains. The low pI value isohormones of follitropin-β have a high sialic acid/galactose ratio and are rich in tri- and tetra-antennary N-linked carbohydrate side chains, as compared with the side chains of the high pI value isohormones.

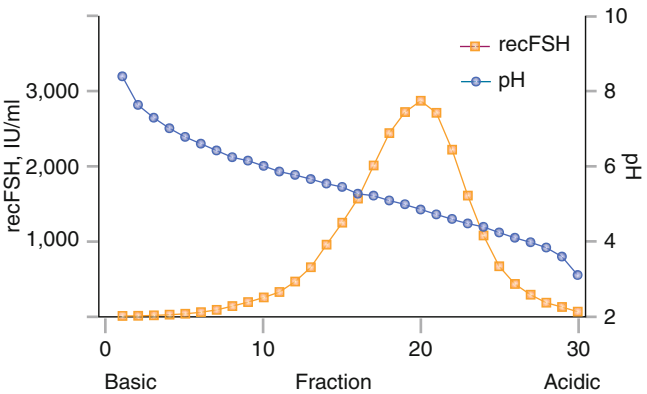

Figure 13.2 ■ Isohormone profile of recombinant follicle-stimulating hormone (follitropin-β) after preparative free-flow focusing (De Leeuw et al. 1996). The FSH concentration was determined by a two-site immunoassay that is capable of quantifying the various isohormones equally well.

One of the tools for further characterization is the immunoassay. Due to the specific recognition characteristics of the antibodies used, this assay determines FSH-specific structural features and provides a relative measure for the quantity of FSH, as it is not sensitive to the differences in glycosylation.

■ Biological Properties of Recombinant FSH Isohormones

An FSH preparation can be biologically characterized with several essentially different assays, each having its own specific merits (Mannaerts et al. 1992). The receptor-binding assay provides information on the proper conformation for interaction with the FSH receptor. Receptor-binding studies with calf testis membranes have shown that FSH isoform activity in follitropin-β decreases when going from high to low pI isoforms. The in vitro bioassay measures the capability of FSH to transduce signals into target cells (the intrinsic bioactivity). The in vitro bioactivity, assessed in the rat Sertoli cell bioassay, also decreases when going from high to low pI isoforms. The in vivo bioassay provides the overall bioactivity of an FSH preparation. It is determined by the number of molecules, the plasma residence time, the receptor-binding activity, and the signal transduction. Interestingly, in contrast to the receptor-binding and in vitro bioassays, the in vivo biological activity determined in rats shows an approximate 20-fold increase between isoforms with a pI value of 5.49, as compared to those with a pI of 4.27. These results indicate that the basic isohormones exhibit the highest receptor-binding and signal transduction activity, whereas the acidic isohormones are the more active forms under in vivo conditions. This notion also warrants pharmacokinetic studies to further characterize the biological properties of FSH preparations.

■ Pharmacokinetic Behavior of Recombinant FSH Isohormones

The pharmacokinetic behavior of follitropin-β and its isohormones was investigated in Beagle dogs that were given an intramuscular bolus injection of a number of FSH isohormone fractions, each with a specific pI value. With a decrease in pI value from 5.49 (basic) to 4.27 (acidic), the AUC increased and the clearance decreased, each more than tenfold (Fig. 13.3). A more than twofold difference in elimination half-life between the most acidic and the most basic FSH isohormone fraction was calculated. The absorption rate of the two most acidic isoforms was higher than the absorption rates of all other isoforms. The AUC and the clearance for the follitropin-β preparation, being a mixture of all isohormone fractions, corresponded with the center of the isohormone profile (Fig. 13.3). In contrast, the elimination of the follitropin-β preparation occurred at a rate similar to that of the most acidic fractions, indicating that the elimination rate is largely determined by the removal of the most acidic isoforms from the plasma.

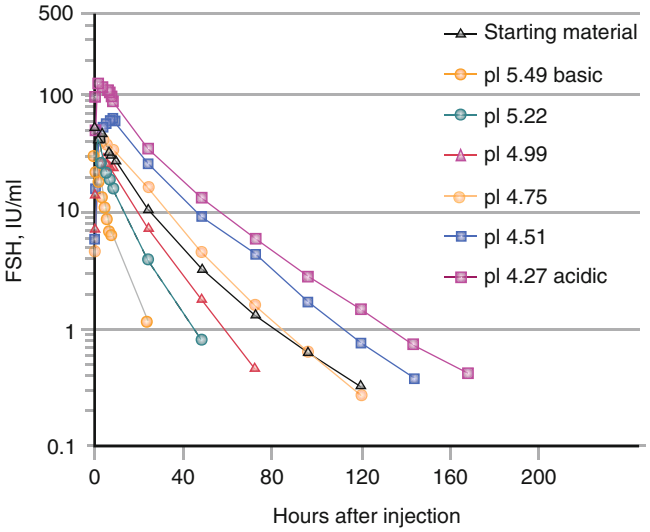

Figure 13.3 ■ Kinetic behavior of FSH isoforms after a single intramuscular injection (20 IU/kg) in Beagle dogs.

Thus, for follitropin-β isohormone fractions, a clear correlation exists between pI value and pharmacokinetic behavior. Increasing acidity leads to an increase in the extent of absorption and elimination half-life and to a decrease in clearance.

PHARMACEUTICAL FORMULATIONS

Recombinant FSH preparations distinguish themselves from the earlier urinary FSH preparations by their high purity (at least 99 %). However, pure proteins are relatively unstable and are therefore often lyophilized, unless some specific stabilizing measures can be taken. FSH preparations are available in different strengths and presentation forms, both as freeze-dried products (powder, cake) and as solution for injection (see Table 13.1). Follitropin-α was originally formulated with sucrose (bulking agent, lyoprotectant), sodium dihydrogen phosphate/disodium hydrogen phosphate, phosphoric acid, and sodium hydroxide (for pH adjustment). In 2002, L-methionine (antioxidant) and polysorbate 20 (to prevent adsorption losses) were added to the single-dose formulation. Follitropin-β is formulated with sucrose, sodium citrate (stabilizer), polysorbate 20 (lyoprotectant and agent to prevent adsorption losses), and hydrochloride/sodium hydroxide (for pH adjustment). The lyophilized preparations are to be reconstituted before use to obtain a ready-for-use solution for injection. In addition to the

Market preparation	Presentation	Container	Strength
Gonal-F® (follitropin-α)	Powder	Ampoule	75 and 150 IU
Merck Serono	Powder	Vial	37.5 IU (2.8 μg), 75 IU (5.5 μg), 150 IU (11 μg), 300 IU, and 600 IU
	Powder	Multidose vial	300 IU (22 μg), 450 IU (33 μg), 1,050 IU (77 μg)
	Solution for injection	Cartridge	300 IU (22 μg), 450 IU(33 μg), 900 IU (66 μg)
Puregon®/Follistim® (follitropin-β)	Solution for injection	Vial	50, 75, 100, 150, 200, and 225 IU
Merck/Merck Sharp and Dohme	Solution for injection	Cartridge/pen injector	150, 300, 600, and 900 IU
Elonva® (corifollitropin alfa) Merck/Merck Sharp and Dohme	Solution for injection	Prefilled syringe equipped with an automatic safety system	100 and 150 μg

Table 13.1 ■ The presentation forms of recombinant FSH products.

freeze-dried presentation form, a solution for injection with several strengths of follitropin-β could be developed. To stabilize the solutions, 0.25 mg of L-methionine had to be added. Furthermore, the solution in the cartridge contains benzyl alcohol as preservative. For follitropin-α, a multidose solution for injection in a prefilled pen became available in 2004. This solution contains poloxamer 188 instead of polysorbate 20, and m-cresol has been added as preservative.

The Puregon®/Follistim® solution for injection is available in vials and is very suitable for titration because of the large range of available strengths as expressed in IU's. Pen injectors have been developed with multidose cartridges containing solution for injection, giving the patient improved convenience.

The solutions for injection should be stored in the refrigerator for a maximum of 3 years with the container kept in the outer carton to protect the solution from light. The patient can keep the solutions at room temperature for a maximum of 3 months. The multidose solution of follitropin-α has a shelf life of 2 years and can be stored for 1 month at room temperature.

CLINICAL ASPECTS

Recombinant FSH products on the market have been approved for two female indications. The first indication is anovulation (including polycystic ovarian disease) in women who are unresponsive to clomiphene citrate (an estrogen receptor modulator). The second indication is controlled ovarian hyperstimulation to induce the development of multiple follicles in medically assisted reproduction programs, such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). In addition, recombinant FSH may be used in men with congenital or required hypogonadotropic hypogonadism to stimulate spermatogenesis.

For the treatment of anovulatory patients (aiming at monofollicular growth), it is recommended to start Puregon®/Follistim® treatment with 50 IU per day for 7–14 days and gradually increase dosing with steps of 50 IU if no sufficient response is seen. This gradual dose-increasing schedule is followed in order to prevent multifollicular development and the induction of ovarian hyperstimulation syndrome (a serious condition of unwanted hyperstimulation). In the most commonly applied treatment regimens in IVF, endogenous gonadotropin levels are suppressed by a GnRH agonist or by the more recently approved GnRH antagonists (Cetrotide® and Orgalutran®/Ganirelix acetate injection®). It is recommended to start Puregon® treatment with 100–200 IU of recombinant FSH followed by maintenance doses of 50–350 IU. The availability of a surplus of collected oocytes allows the vitrification of embryos for replacement in frozen-thawn embryo transfer (FTET) cycles. Similar treatment regimens are recommended for Gonal-F®.

After subcutaneous administration, follitropin-β has an elimination half-life of approximately 33 h (Voortman et al. 1999). Steady-state levels of follitropin-β are therefore obtained after 4–5 daily doses reaching therapeutically effective plasma concentrations of FSH. Follitropin-β is administered via the subcutaneous route with good local tolerance. Bioavailability is approximately 77 %. In a large fraction of patients treated with follitropin-β, no formation of antibodies against recombinant FSH- or CHO-cell-derived proteins was observed. Injections of the follitropin-β preparations can be given by the patient herself or her partner.

A NEWLY DEVELOPED FSH ANALOG

The need for daily injections of FSH, especially in combination with GnRH agonists, is a burden for the women treated in an ART regimen. Therefore, several

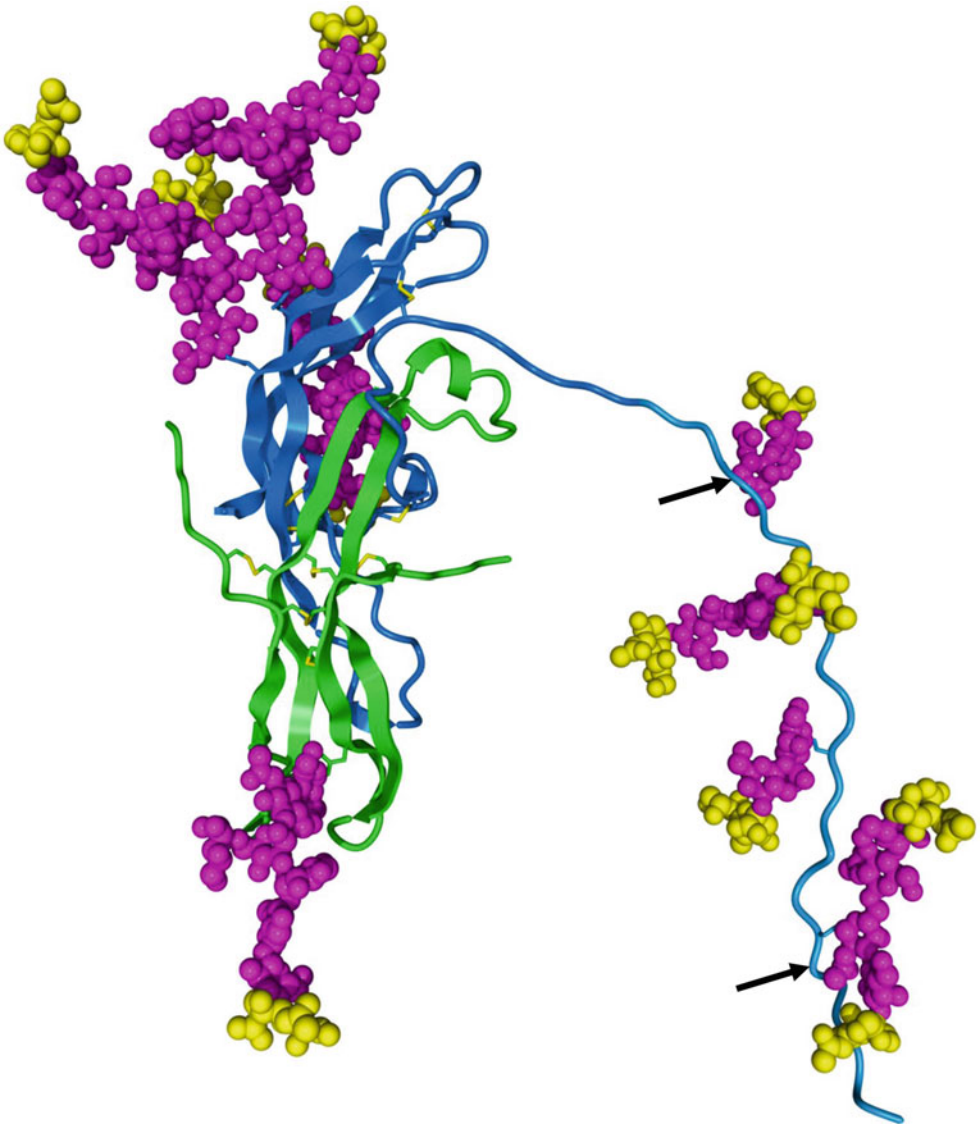

Figure 13.4 ■ A three-dimensional model of corifollitropin alfa. The ribbons represent the polypeptide backbones of the α-subunit (*green ribbon*) and the β-subunit (*blue ribbon*). The carbohydrate side chains (*pink and yellow space-filled globules*) represent N-linked and O-linked carbohydrates. The sialic acid carbohydrates are depicted in yellow. The *arrows* indicate additional O-linked carbohydrate sites (Courtesy MLCE Kouwijzer and R Bosch).

different approaches have been undertaken to arrive at FSH preparations that need fewer injections, such as slow-release formulations, addition of N-linked carbohydrates, and other chemical modifications including pegylation (Fauser et al. 2009). An elegant approach pioneered by Irving Boime and collaborators is based on the longer in vivo half-life of hCG compared to LH. Using genetic engineering, the beta subunit of FSH was extended by one or two CTPs of hCG. It was demonstrated that fewer injections with preparations containing such molecules were needed to induce similar pharmacodynamic effects in laboratory animals. Subsequently, a new cell line was generated by Organon (now part of Merck Sharp & Dohme) that produced corifollitropin alfa (the INN of this molecule), an FSH analog in which the beta subunit was extended by a single CTP (28 amino acids). Thorough biochemical analysis demonstrated the expected amino acid sequence of the alpha subunit and the extended beta subunit but revealed two additional O-linked glycosylation sites in corifollitropin alfa (Henno van den Hooven, Ton Swolfs, personal communication) compared to the 4–5 sites reported in hCG (Fig. 13.4). Nonclinical evaluation demonstrated that the receptor-binding and transactivation profile of this new molecular entity was specific and comparable to that of rec-FSH without intrinsic TSH-receptor or LH-receptor activation. However, the in vivo half-life was increased 1.5–2-fold in the species tested, and a 2–4-fold increase of bioactivity was found across all in vivo pharmacodynamic parameters tested (Verbost et al. 2011). These observations were corroborated by a very extensive data set obtained in a broad panel of clinical trials (phase I, II, and III), including the largest comparator controlled trial of its kind in fertility (the comparator being rec-FSH) (Devroey et al. 2009; Fauser et al. 2010). A single subcutaneous dose of corifollitropin alfa (Elonva®) can be used to initiate and sustain multifollicular growth for 7 days while the efficacy and safety of this novel biopharmaceutical were

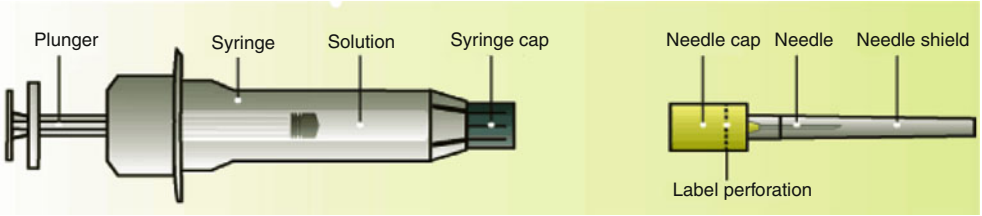

Figure 13.5 ■ Prefilled syringe with corifollitropin alfa solution to be assembled with a needle assembly. The syringe is equipped with an automatic safety system to prevent needle sticking.

similar to that of daily injections with recombinant FSH. Whereas normally more than 7 days of FSH treatment has to be given after the first injection, in about one third of the women treated with FSH-CTP, no additional FSH treatment was needed.

Dedicated clinical research revealed no clinically relevant immunogenicity against the FSH analog (Norman et al. 2011), despite being a fusion protein. Hence, by virtue of its ~2-fold increased in vivo half-life, corifollitropin alfa has demonstrated to provide a valuable alternative for FSH by acting as a sustained follicle stimulant. Elonva® is approved (EU) for controlled ovarian stimulation (COS) in combination with a GnRH (gonadotropin-releasing hormone) antagonist for the development of multiple follicles in women participating in an assisted reproductive technology (ART) program. It is supplied in prefilled syringes equipped with an automatic safety system to prevent needle stick injuries after use and is packed together with a sterile injection needle. Each prefilled syringe contains 0.5 ml solution for injection (Fig. 13.5).

FSH provides a great example of the evolution of biopharmaceuticals, starting from the natural form (urine derived), via close imitations thereof (recombinant FSH), towards further improved biopharmaceuticals (FSH analogs, corifollitropin alfa being the only CTP form that made it to the market). Such developments in pharmaceutical biotechnology are clearly to the benefit of the patients in need for effective, safe, and convenient treatment options.

REFERENCES

De Leeuw R, Mulders J, Voortman G, Rombout F, Damm J, Kloosterboer L (1996) Structure-function relationship of recombinant follicle stimulating hormone (Puregon®). Mol Hum Reprod 2:361–369

Devroey P, Boostanfar R, Koper NP, Jzerman PI, Mannaerts BMJL, Fauser BC, for the ENGAGE Investigators (2009) A double-blind, non-inferiority RCT comparing corifollitropin alfa and recombinant FSH during the first seven days of ovarian stimulation using a GnRH antagonist protocol. Hum Reprod 24:3063–3072

European Public Assessment Report Gonal-F (Follitropin alpha), CPMP/415/95, revision 18, 11-April 2011. European Agency for the Evaluation of Medicinal Products

Fauser BCJM, Mannaerts BMJL, Devroey P, Leader A, Boime I, Baird DT (2009) Advances in recombinant DNA technology: corifollitropin alfa, a hybrid molecule with sustained follicle-stimulating activity and reduced injection frequency. Hum Reprod Update 15:309–321

Fauser BCJM, Alper MM, Ledger W, Schoolcraft WB, Zandvliet A, Mannaerts BMJL (2010) Pharmacokinetics and follicular dynamics of corifollitropin alfa versus recombinant FSH during controlled ovarian stimulation for in vitro fertilisation. Reprod Biomed Online 21:593–601

Howles CM (1996) Genetic engineering of human FSH (Gonal-F®). Hum Reprod Update 2:172–191

Mannaerts BMJL, De Leeuw R, Geelen J, Van Ravenstein A, Van Wezenbeek P, Schuurs A, Kloosterboer L (1992) Comparative in vitro and in vivo studies on the biological properties of recombinant human follicle stimulating hormone. Endocrinology 129:2623–2630

Matzuk MM, Hsueh AJ, Lapolt P, Tsafriri A, Keene JL, Boime I (1990) The biological role of the carboxy-terminal extension of human chorionic gonadotropin (corrected) beta-subunit. Endocrinology 126:376–383

Norman RJ, Zegers-Hochschild F, Salle BS, Elbers J, Heijnen E, Marintcheva-Petrova M, Mannaerts B (2011) Repeated ovarian stimulation with corifollitropin alfa in patients in a GnRH antagonist protocol: no concern for immunogenicity. Hum Reprod 26(8):2200–2208

Olijve W, de Boer W, Mulders JWM, van Wezenbeek PMGF (1996) Molecular biology and biochemistry of human recombinant follicle stimulating hormone (PuregonR). Mol Hum Reprod 2:371–382

Van Wezenbeek P, Draaier J, Van Meel F, Olyve W (1990) Recombinant follicle stimulating hormone. I. Construction, selection and characterization of a cell line. In: Crommelin DJA, Schellekens H (eds) From clone to clinic developments in biotherapy, vol I. Kluwer, Dordrecht, pp 245–251

Verbost P, Sloot WN, Rose UM, de Leeuw R, Hanssen RGJM, Verheijden GFM (2011) Pharmacologic profiling of corifollitropin alfa, the first developed sustained follicle stimulant. Eur J Pharmacol 651:227–233

Voortman G, van de Post J, Schoemaker RC, van Gerwen J (1999) Bioequivalence of subcutaneous injections of recombinant human follicle stimulating hormone (Puregon®) by Pen-injector and syringe. Hum Reprod 14:1698–1702

FURTHER READING

Mannaerts BMJL (2013) Innovative drug development for infertility therapy, Ph.D. thesis, Utrecht, The Netherlands, ISBN 978-90-393-5939-6

Seyhan A, Ata B (2011) The role of corifollitropin alfa in controlled ovarian stimulation for IVF in combination with GnRH antagonist. Int J Womens Health 3:243–255