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# Interference with follicle stimulating hormone regulation of human ovarian function*

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**This review summarizes observations on the background and potential clinical significance of interference with follicle stimulating hormone (FSH) regulation of human ovarian function. This interference may occur at the level of the pituitary by the secretion of FSH isoforms with reduced or absent bioactivity. In addition, interference with FSH may occur in the circulation, or within the ovarian follicular compartment. Although the full range of its significance remains to be elucidated, there are distinct indications that these mechanisms may be involved in normal ovarian physiology, as well as in abnormal response of the ovary to stimulation by endogenous FSH or by exogenously-administered gonadotrophin preparations. Moreover, recent advances in the determination of the structure–function relationship of FSH and FSH-receptor interaction, in combination with new developments in recombinant DNA technology, will allow the production of modified FSH- or FSH receptor-like molecules with altered bioactivity. The availability of FSH agonists and antagonists in the near future should provide a challenge for clinicians to improve treatment outcome and to find new indications for the use of these compounds.**

*Key words:* anti-FSH/bioactivity/FSH/follicle development/gonadotrophins

## Introduction

The classical endocrine concept teaches that gonadal function is ruled by pituitary gonadotrophins. Both follicle stimulating hormone (FSH) and luteinizing hormone (LH) represent a group of pituitary glycoprotein isohormones. FSH binds to a specific FSH glycoprotein transmembrane receptor, which is located exclusively on granulosa cells in ovarian follicles. Subsequent activation of the G-protein-coupled adenylate cyclase system results in cell proliferation and differentiated function. FSH regulates more advanced stages of follicle development, and may be involved in various forms of human ovarian dysfunction.

The present review focuses on the background and potential clinical relevance of substances (either present in the circulation or locally within the ovarian follicle) which interfere with normal FSH action (Figure 1). FSH action may be counteracted in various ways: (i) the wide spectrum of FSH isohormones may include forms which exhibit FSH antagonist activity by binding to the FSH receptor without eliciting a response; (ii) specific anti-FSH antibodies may be present in the circulation in some patients presenting with premature ovarian failure or with a reduced response to exogenously administered gonadotrophins; (iii) various proteins which inhibit FSH action, either by interfering with binding of FSH to the receptor or at the level of signal transduction, appear to be present both in serum and in follicular fluid. In particular, compounds present in the follicular compartment may be relevant for normal ovarian physiology. In addition, pure recombinant human (rh) FSH, produced by cell lines through recombinant DNA technology, has recently become available for clinical use. More insight into the structure–function relationship of glycoproteins may allow the production of modified hormones with specific FSH antagonist activity. This development may have significant clinical implications.

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## Structure–function relationship of FSH and FSH receptors

Gonadotrophic hormones are dimers composed of glycosylated protein chains. The common α-subunit [which is similar for all glycoproteins including LH, FSH, thyroid-stimulating hormone (TSH) and human chorionic gonadotrophin (HCG)] and the hormone-specific β-subunit are non-covalently linked by disulphide bonds. The α-chain consists of 92 amino acids, whereas human FSHβ contains 111 according to some authors (Combarnous, 1992) or 118 amino acids according to others (Catt and Dufau, 1991) (Figure 2, upper panel). The molecular weight of FSH is ~40 kDa, of which 30% consists of carbohydrates. Oligosaccharide groups are coupled through *N*-acetylglucosamine to certain asparagine (Asn) groups at specific locations of the protein. These glycosylation sites are located at position 52 and 78 in the α-, and 7 and 24 in the β-subunit. Carbohydrate additions to the FSH protein backbone are branched, consist of mannose, galactose, fucose, and may end with sialic acid. These side chains determine metabolic clearance and therefore affect in-vivo biopotency. Differences in the number of sialic acid residues determine changes in bioactivity of the isoforms, as will be discussed in the next

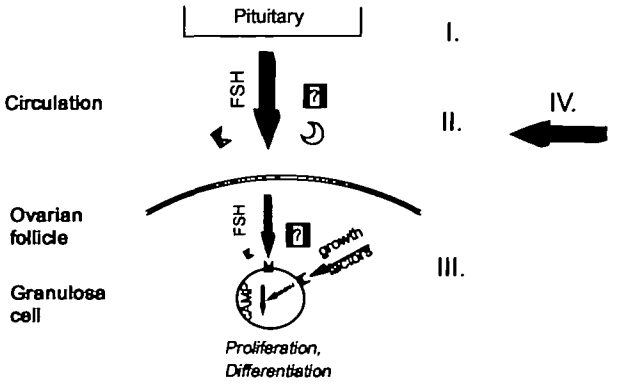


**Figure 1.** Schematic representation of different levels of potential interference with follicle stimulating hormone (FSH) in the human female. (I) pituitary FSH isohormones; (II) interference with circulating FSH (anti-FSH antibodies; FSH binding inhibitors); (III) intra-ovarian interference with FSH [FSH binding inhibitors; soluble extracellular part of FSH receptor; growth factors (epidermal growth factor, inhibin, insulin-like growth factor binding proteins)]; (IV) exogenous FSH antagonists.

section. Carbohydrate additions are not directly involved in receptor binding, but appear to be important for signal transduction. The three-dimensional conformation of the hormone (important for hormone–receptor interaction) is maintained by disulphide bonds. Specific regions in the polypeptide subunits (30–45 and 81–92 for α, and 33–53 and 81–95 for β) which are crucial for hormone–receptor interaction have recently been identified (Keutmann, 1992). Binding to the receptor is determined by both the α- and the β-subunit. Binding specificity is determined exclusively by the β-subunit, whereas the two binding regions on the subunit appear to be involved in binding affinity and post-receptor signal transduction (Combarnous, 1992).

Transmembrane receptors for glycoprotein hormones are each composed of a large (~700 amino acid residues) single polypeptide chain. This group of receptors is part of the G-protein-coupled gene family, and consists of: (i) a large (359 amino acids) hydrophilic *N*-terminal domain including several glycosylation sites important for ligand recognition and high affinity binding; (ii) a highly homologous region comprising seven transmembrane domains and three intracellular loops; (iii) a small intracellular *C*-terminal domain, important for signal transduction. Regions corresponding to residues 9–30 and 279–315 of the extracellular domain of the FSH receptor capable of specifically binding FSH, but not LH or TSH, have been identified (Dattatreyamurty and Reichert, 1992). A most interesting study has been published recently (Aittomaki *et al.*, 1995) concerning some Finnish families who presented with 'hypergonadotrophic ovarian dysgenesis'. Due to a mutation in exon 7 of the FSH receptor gene, alanine was substituted for valine at position 189 in the extracellular ligand-binding domain of the receptor. It was shown that this alteration in the FSH receptor (situated adjacent to a glycosylation site known to be essential for FSH ligand binding) resulted in a dramatic reduction of binding capacity and signal transduction, whereas ligand binding affinity remained unaltered. This loss of FSH

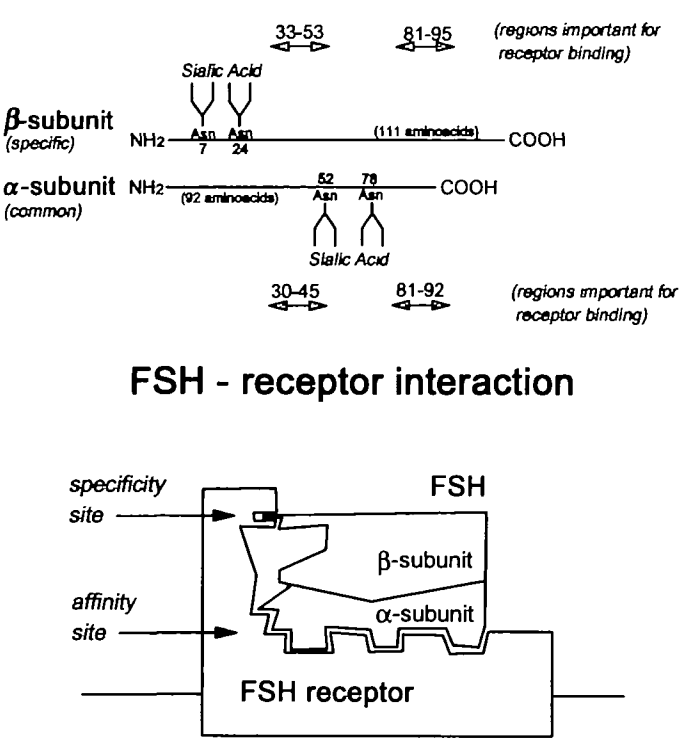


**Figure 2.** Schematic representation of the follicle stimulating hormone (FSH) glycoprotein structure (upper pannel) and FSH-receptor interaction (adapted from Combarnous, 1992; bottom pannel). Asn = asparagine.

receptor function fully explains the phenotype in females. However, the clinical picture of the male counterparts remains unclear at this moment. Other workers have failed to show FSH receptor deletions in a group of unselected women presenting with premature ovarian failure (Whitney *et al.*, 1995).

It has been postulated recently that FSH interaction with its receptor involves initial recognition of the amino terminal external portion of the receptor by certain regions located on FSHβ (specificity site), followed by three-dimensional conformational changes in both the hormone and the receptor allowing the binding of other regions (affinity sites) of the hormone to the external and transmembrane regions of the receptor (Figure 2; bottom panel) (Combarnous, 1992; Dias, 1992; Reichert, 1994). The LH receptor contains six potential glycosylation sites in the extracellular domain. Deglycosylation of this receptor [expressed in human embryonic kidney (HEK-293) cells] by site-directed mutagenesis using *N*-glycanase did not alter its ligand binding and signal transduction properties (Tapanainen *et al.*, 1993). However, intracellular synthesis and subsequent secretion of the hydrophilic portion may be affected by glycosylation of the human transmembrane receptor. Surprisingly, the α-subunit and HCGβ can be synthesized and secreted as a single chain, and still maintain biological activity (Sugahara *et al.*, 1995). This approach using genetically-engineered gonadotrophin chimera may help to further elucidate the significance of the three-dimensional gonadotrophin structure for hormone–receptor interaction.

## Pituitary FSH isohormones

- minor differences in carbohydrate moieties by changes in posttranslational glycosylation
- similar amino acid composition
  same immunoactivity
- 15 different isoforms
  chromatofocusing; (pH range 3.6-7.6)
- more acidic forms
  - decreased receptor binding, and *in-vitro* bioactivity
  - enhanced circulating half-life

*Stanton et al., 1992*
*Ulloa Aguirre et al., 1992*

**Figure 3.** Overview of major observations regarding pituitary follicle stimulating hormone (FSH) isohormones.

## FSH receptor binding inhibitors

- FSH receptor binding inhibitors present in human serum and follicle fluid
  *Reichert et al., 1979; Lee et al., 1990*
- Further characterization of binding inhibitors
  partial purification, *in vitro* bioactivity, immunoactivity
  *Lee et al., 1991; Lee et al., 1993*
- Binding inhibitors similar to IGF-BP3 in follicle fluid
  deduced from DNA structure and predicted amino acid sequence *Shimasaki et al., 1990*
- Proteins in follicular fluid that share an epitope with the extracellular domain of the FSH receptor
  *Dattatreyamurty et al., 1994*

**Figure 4.** Overview of major observations regarding follicle stimulating hormone (FSH) receptor binding inhibitors.

Northern blot analysis has resulted in the detection of several LH receptor mRNAs of different size. The number of recently-identified splice variants of the LH and FSH receptor encoding for a partially intact receptor is growing (Themmen and Grootegoed, 1994). Regulation of different forms of the transmembrane receptor may be of physiological relevance, as will be discussed later. Finally, mutations (resulting in amino acid substitutions in the transmembrane receptor region) causing a gain or loss of function of gonadotrophin receptor activity have been described in the human (for review see Fauser and Hsueh, 1995). These 'experiments of nature' may also help to further unravel the structure–function relationship of the gonadotrophin receptor.

### Pituitary FSH isohormones

Recent observations indicate that the anterior pituitary regulates not only the quantity of secreted hormones, but also its biopotency by regulating the enzymes involved in post-translational glycosylation of α- and β-protein subunits. Glycoprotein hormones can therefore be synthesized and secreted into the circulation in many forms. These forms are structurally related with regard to the protein backbone and therefore have similar immunoactivity. Using the technique of chromatofocusing (separating isohormones based on their differences in isoelectric characteristics) at least 15 (Stanton *et al.*, 1992) forms of immunoreactive FSH have been detected with isoelectric points (pI) ranging between 7.6 and 3.6 (Figure 3). Receptor binding affinity varied with pI value, with the more acidic forms exhibiting decreased receptor binding and diminished in-vitro bioactivity (Ulloa-Aguirre *et al.*, 1992). However, the circulating half-lives of these isoforms were longer.

Altered bioactivity may be detected by in-vitro bioassays, using secreted oestrogens as the end-point of FSH action following the addition of serum to cultured rat granulosa or Sertoli cells. Discrepancies between measured bioactivity and immunoreactivity (so called B/I ratios) may represent differences in biopotency due to changes in glycosylation (Chappel, 1995). Biopotency of secreted FSH may be regulated by steroid feedback, since different isohormone profiles have been detected during the normal menstrual cycle (Padzanabhan *et al.*, 1988; Zambrano *et al.*, 1995), in women lacking ovaries (Mason *et al.*, 1992a), and following administration of gonadotrophin-releasing hormone (GnRH) analogues (for review see Fauser *et al.*, 1989).

A clear decrease in FSH B/I ratio was observed following the administration of a GnRH antagonist in normal women (Dahl *et al.*, 1986). This medication resulted in a substantial increase in the percentage of basic isohormones. A distinct rise in FSH immunoreactivity, without a concomitant increase in bioactivity, was observed in the pH range 9.4–9.6. Moreover, this fraction clearly inhibited FSH-induced aromatase activity *in vitro*, suggesting the presence of naturally-occurring FSH antagonists in these subjects (Dahl *et al.*, 1988).

### Interference with circulating FSH

#### *Circulating FSH binding inhibitors*

The presence of a low molecular weight protein in serum specifically interacting with FSH-receptor binding has been proposed (Reichert *et al.*, 1979; Dias *et al.*, 1982; Sanzo and Reichert, 1982) (Figure 4). Indeed, reduction of serum inhibitory factors is needed for optimal in-vitro measurement of serum FSH bioactivity. Using the rat granulosa cell aromatase bioassay, serum samples should be pretreated with polyethylene glycol (Jia and Hsueh, 1986; Dahl *et al.*, 1989). However, so far these inhibitor proteins have not been fully characterized and their physiological significance remains to be determined. In the near future, the relevance of FSH binding inhibitors may be studied in more detail using eukaryotic cell lines [either Chinese hamster ovary (CHO), HEK-293, or COS-7 cells] stably transfected with human FSH receptor cDNA (Kelton *et al.*, 1992; Tilly *et al.*, 1992; Albanese *et al.*, 1994). A test system (using FSH-induced cAMP production as the end point) for specific detection of FSH binding inhibitors in clinical samples has recently been developed (Schipper *et al.*, 1995).

#### *Circulating anti-FSH antibodies*

The presence of circulating antibodies to human FSH in some patients receiving exogenous gonadotrophins [human

menopausal gonadotrophins (HMG)] has been known for quite some time (Spitz *et al.*, 1973; Tang and Faiman, 1983; Platia *et al.*, 1984). The presence of anti-FSH antibodies in subjects with a reduced response to gonadotrophin induction of ovulation have been reported on rare occasions. Interestingly, anti-FSH antibodies have been detected in 92% of individuals in a controlled study of 26 low responders of different ages undergoing ovarian stimulation for in-vitro fertilization (IVF) using gonadotrophin preparations, whereas none of the good responders exhibited these antibodies (Meyer *et al.*, 1990). This concept is appealing, although this study has not been confirmed by others, and anti-FSH antibodies have not been characterized in detail. These observations suggest that a low response to gonadotrophins does not necessarily indicate intra-ovarian abnormalities such as 'ovarian ageing', as widely believed at present. Fortunately, recently-published initial studies applying rhFSH to humans have shown the absence of anti-FSH antibody formation in these patients (Schoot *et al.*, 1994; Out *et al.*, 1995; Recombinant human FSH study group, 1995).

Premature ovarian failure results from an absence of ovarian oocytes (true early menopause). However, in a proportion of patients, ovaries may be unable to respond normally to endogenous FSH stimulation, due to the presence of substances which interfere with FSH. Circulating low molecular weight FSH receptor binding inhibitors (Chiauzzi *et al.*, 1982; Sluss and Schneyer, 1992) as well as immunoglobulins that can block FSH-induced in-vitro granulosa cell DNA synthesis (Van Weissenbruch *et al.*, 1991) have been described in these women. However, preliminary studies using human FSH receptor cell lines do not favour the presence of a high incidence of FSH blocking agents in women suffering from premature ovarian failure (Anasti *et al.*, 1995; B.C.J.M.Fauser and A.J.W.Hsueh, unpublished observations).

### *Potential for active immunization?*

Immunization against HCG is believed to be a viable approach for the prevention of unwanted reproduction (Aldhous, 1994; Griffin, 1994). HCGβ attached to a toxoid (modified bacterial toxin) induces the formation of antibodies against HCG, which in turn inhibits HCG support of corpus luteum function and implantation of the blastocyst. During the last decade several human studies have been undertaken by the World Health Organization, but it is still uncertain whether this approach can be used on a wide scale for female interception.

Monkeys immunized with sheep FSH exhibit an immune response and subsequent reversible infertility (Sheela Rani *et al.*, 1978; Murthy *et al.*, 1979; Wickings and Nieschlag, 1980; Srinath *et al.*, 1983; Moudgal *et al.*, 1992). The capacity of antibodies to neutralize endogenous FSH was more than adequate, since free FSH could not be detected by radio-immunoassay. Severe oligozoospermia was rapidly induced and mating experiments did not result in pregnancies. After stopping booster injections, fertility was restored in nine out of 10 monkeys (Moudgal *et al.*, 1992). Human experiments have not yet been performed, and the question as to whether this approach might be feasible for human contraceptive purposes remains to be answered.

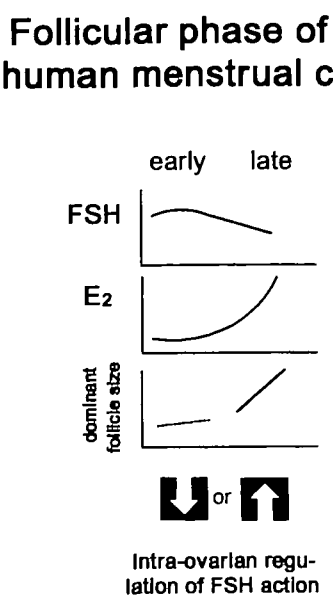


**Figure 5.** Schematic representation of potential intra-ovarian regulation of follicle stimulating hormone (FSH) action during the follicular phase of the human menstrual cycle.

## Intra-ovarian interference with FSH

### *FSH regulation of human follicle development*

The intercycle rise in circulating FSH concentrations is caused by demise of the corpus luteum and reduced negative steroid feedback actions. If these concentrations surpass a 'threshold' level, a group of follicles will acquire gonadotrophin dependence and continue their development. This process of follicle recruitment seems to be under the control of circulating FSH. Later during the follicular phase a single follicle gains dominance, reaches the mature Graafian stage and eventually ovulates (for review see Hodgen, 1982). Decremental serum FSH concentrations appear to be important for mono-follicle development (Fauser *et al.*, 1993). As indicated previously, the FSH isohormone profile might also change during the follicular phase, with a predominance of basic forms around mid-cycle (Padmanabhan *et al.*, 1988). Although there is evidence that basic forms are more biopotent *in vitro* (Ulloa-Aquirre, 1992), this may not be the case *in vivo* (Chappel, 1995).

Significant aromatase activity is only induced in the dominant follicle days after maximum serum FSH concentrations have been reached (van Santbrink *et al.*, 1995; van Dessel *et al.*, 1996a). Moreover, enhanced sensitivity of the dominant follicle to FSH ensures that this follicle continues its development despite decreased stimulation from lower FSH concentrations. These observations provide strong, though indirect, evidence that intra-ovarian modification of FSH action is crucial for normal ovarian function in the human. It may be hypothesized that, at the ovarian level, FSH action is either inhibited by local factors in the early follicular phase or enhanced in the late follicular phase (see Figure 5).

### *FSH binding inhibitors present in follicular fluid*

Polypeptide modulators may be present in follicular fluid which block the action of FSH either by inhibiting binding of

FSH to its receptor or by blocking postreceptor events induced by FSH (Ono *et al.*, 1986). A high molecular weight FSH receptor binding inhibitor has been shown to be present in human follicular fluid (Lee *et al.*, 1990, 1991, 1993). It has also been suggested that these proteins share epitopes with the FSH receptor (Dattatreyamurty *et al.*, 1994), and may therefore represent a secreted form of the hydrophilic extracellular domain of the transmembrane receptor. Indeed, recent studies indicate that alternative DNA splicing encodes for a shorter receptor protein with a variable length of the extracellular portion of the receptor (Tsai-Morris *et al.*, 1990; Gromoll *et al.*, 1992; Themmen and Grootegoed, 1994). Secreted forms of receptors acting as binding proteins have also been demonstrated for other hormone systems, including growth hormone (Leung *et al.*, 1987), insulin (Pezzino *et al.*, 1992) and TSH (Graves *et al.*, 1993).

Moreover, amino-terminal sequence analysis of the FSH inhibitor suggests structural similarity with insulin-like growth factor binding protein 3 (IGF-BP3) (Ling *et al.*, 1990; Shimasaki *et al.*, 1990). The concept that IGF-BP could directly inhibit FSH action is intriguing, since IGF-BP3 also binds IGF (which is produced by granulosa cells and strongly potentiates FSH action). Finally, purified α-inhibin (which is present in high concentrations in follicular fluid) also inhibited FSH binding to FSH receptors in tissue as well as those expressed in HEK-293 cells (Schneyer *et al.*, 1991).

### *Auto-paracrine regulation by growth factors*

During the last decade much attention has been focused on potential intra-ovarian regulation of FSH action by (locally-produced) growth factors (for review, see Fauser and Hsueh, 1988; McNatty *et al.*, 1992; Westergaard, 1992). The majority of growth factors were shown to enhance FSH-induced aromatase activity of cultured cells. However, epidermal growth factor (EGF) (Knecht and Catt, 1983) as well as inhibin (Miro and Hillier, 1992; Hillier and Miro, 1993) clearly inhibit FSH action *in vitro* in non-human primates. Among many others, IGFs are potent stimulators of FSH-induced differentiated granulosa cell function. Surprisingly, direct anti-FSH actions have been observed for IGF-BPs, both *in vitro* (Ui *et al.*, 1989) and *in vivo* (Bicsak *et al.*, 1991).

For obvious reasons, human ovarian material is hard to obtain, and therefore very few experiments have been carried out using non-IVF human granulosa or theca cell cultures. IGF-I has been shown to potently enhance FSH-induced oestrogen production by human granulosa cells *in vitro* (Mason *et al.*, 1993). However, it has been demonstrated that EGF has a strong (Mason *et al.*, 1990) and IGFBP-3 a minor (Mason *et al.*, 1992b) inhibitory effect on FSH action *in vitro*. Moreover, EGF was shown to inhibit, whereas IGF-1 enhanced, the activity of mRNA encoding the cytochrome P450 aromatase enzyme in human granulosa cells (Steinkampf *et al.*, 1988), which is in full agreement with the above-mentioned in-vitro observations. In the human, intra-follicular concentrations of EGF decrease with more advanced stages of maturation (Westergaard, 1992), which supports the concept of a decrease in intra-ovarian inhibition of FSH action during follicle development. The IGF-BP profile in follicular fluid has also been shown to vary during follicle development in the human, independent of changes in serum concentration (van Dessel *et al.*, 1996b).

## Potential methods of developing anti-FSH

### *rhFSH with modifed carbohydrate additions*

DNA encoding for human α- and FSH β-subunits has been transfected into CHO cells, and stable cell lines expressing the intact FSH dimer (rhFSH) have subsequently been selected (Keene *et al.*, 1989; Loumaye *et al.*, 1995). rhFSH has an isohormone profile comparable with that of pituitary or urinary FSH, with acidic isoforms again showing reduced receptor affinity and in-vitro bioactivity (Cerpa-Poljak *et al.*, 1993). Different cell lines may have different glycosylation patterns. *In vitro*, rhFSH produced by HEK-293 cells was 3–6 fold more biopotent due to an increased number of basic isoforms when compared with a pituitary FSH standard (Flack *et al.*, 1994). A more basic FSH preparation can also be obtained by selecting a specific pH range, from the entire rhFSH isohormone profile produced by CHO cells.

Improved knowledge regarding the structure–function relationship of these glycoproteins, together with novel developments in recombinant DNA technology, has already resulted in the development of FSH compounds with altered function. Chimeric genes were constructed by fusion of the sequence encoding for the *C*-terminal peptide (CTP) of the HCG β-subunit (known to be important for the prolonged half-life of HCG as opposed to LH) to the translation sequence of the FSH β-subunit (Fares *et al.*, 1992). The resulting FSH analogue (FSH-CTP) showed similar receptor binding and in-vitro bioactivity, but an enhanced circulating half-life and in-vivo biopotency (LaPolt *et al.*, 1992). This compound is likely to be clinically relevant, since less rapid clearance may allow less frequent administration as opposed to daily injections (Gast, 1995). In addition, single chain gonadotrophin chimera (where the α- and β-subunits are no longer dimerized) (Sugahara *et al.*, 1995) may represent a novel approach for further alteration of structure and function of human recombinant glycoprotein hormones.

Compounds which block FSH action may be important for the limitation of severe ovarian hyperstimulation syndrome (OHSS), if this can be recognized at an early stage. Moreover, the possibility that an FSH antagonist might prove to be an effective male or female contraceptive agent is extremely challenging. Indeed, induction of anti-FSH antibodies has proved to completely abolish fertility in male monkeys, as previously discussed. Multiple studies have shown that removal of all *N*-linked carbohydrates from pituitary FSH by enzymatic digestion results in a molecule which exhibits intact receptor binding, but strongly reduced signal transduction. This compound prevents normal FSH from binding to its receptor and therefore acts as an antagonist. Presently more refined methods are available for the modification of carbohydrate additions to proteins (Boime *et al.*, 1990, 1992), including: (i) glycosylation inhibitors; (ii) site directed mutagenesis (causing loss of glycosylation at the mutated sites); and (iii) production of recombinant glycoproteins by mutated cell lines resulting in

## Potential for the development of FSH antagonists

- Recombinant FSH with modified carbohydrate additions
  (produced by mutant cell lines or by site directed mutagenesis)
  *Galway et al., 1990, Bishop et al., 1994; Keene et al., 1994*
- Synthetic peptides corresponding with regions from FSH polypeptide subunits known to be important for receptor binding
  *Santo Coloma et al., 1992, Reichert et al., 1994*
- Soluble forms of the extra-cellular domain of the FSH receptor
  *Dattatreyamurty et al., 1994*

**Figure 6.** Overview of potential developments which may lead to the production of follicle stimulating hormone (FSH) antagonists.

selective deletions. This new technology may help in the production of modified rFSH compounds with altered biopotency (see also Figure 6).

Partially-deglycosylated forms of rhFSH produced by mutant CHO cells (lacking the glycosylation enzyme NAGT or with defective sialic acid transport systems) exhibit normal receptor binding, normal in-vitro bioactivity (which indicates normal signal transduction), but reduced in-vivo bioactivity (suggesting enhanced clearance from the circulation) in the rat model (Galway *et al.*, 1990). Two recent studies, using site-directed mutagenes, studied effects of selective deletions of various oligosaccharides. Deletion of Asn52 in the α-subunit results in a 10-fold decrease in-vitro bioactivity. Deletion of both α-subunit oligosaccharides (52 and 78) also results in a compound with strongly reduced in-vitro bioactivity, but which also blocks FSH action (Keene *et al.*, 1994). Furthermore, deletion of Asn78 on the α-subunit increases receptor binding affinity by 72%, but removal of other carbohydrates did not significantly affect receptor binding. Carbohydrates at position 52 on the α-subunit appeared to be important for signal transduction, whereas deletion of the Asn-linked oligosaccharides at location 7 and 24 of the β-subunit resulted in a distinctly (216%) increased biopotency (Bishop *et al.*, 1994).

### *Synthetic peptides*

It has been shown that certain regions of the FSH β-subunit (region 33–53 and 81–95) (Figure 2) are important for binding of FSH to the receptor (Santa Coloma and Reichert, 1990). Therefore, another experimental approach in the development of FSH antagonists is the production of synthetic peptides which prevent native FSH from binding to its receptor (Santa Coloma *et al.*, 1990, 1992; Reichert, 1994). Indeed, a synthetic peptide corresponding to amino acids 9–30 of the extracellular domain of the FSH receptor directly binds FSH (Dattatreyamurty and Reichert, 1992), and inhibits the activity of the native hormone. This seems to be a very attractive approach. However, additional studies are needed to decide whether this will be a viable approach for clinical use.

### *Extracellular domain of the FSH receptor*

The possibility that soluble forms of the extracellular portion of the FSH receptor may act as circulating ligand binding proteins suggests synthesis of these proteins as a potential option for the development of anti-FSH. More studies are needed to explore this area.

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