Title: IMPROVED RECOMBINANT HUMAN FOLLICLE-STIMULATING HORMONE

Abstract: The present invention pertains to improved FSH preparations which are capable of stimulating sex steroid release at much lower concentration than the commonly used urinary FSH or recombinant FSH obtained from CHO cells and which act independent of cAMP signaling. These improved FSH preparations can be used in infertility treatment.
FIELD OF THE INVENTION

The present invention pertains to the field of gonadotropins. In particular, improved recombinant human follicle-stimulating hormone (rhFSH) is provided. This improved rhFSH is useful in the treatment of infertility, in particular in human patients.

BACKGROUND OF THE INVENTION

Gonadotropins are a group of protein hormones which regulate gonadal function in the male and female and thereby play an important role in human fertility. They are secreted by gonadotrope cells of the pituitary gland of vertebrates after stimulation by the gonadotropin-releasing hormone (GnRH). Gonadotropins are heterodimeric glycoproteins including follicle stimulating hormone (FSH), luteinizing hormone (LH) and chorionic gonadotropin (CG). The gonadotropins share identical alpha-subunits but comprise different beta-subunits which ensure receptor binding specificity.

FSH comprises a 92 amino acid alpha-subunit and a 111 amino acid beta-subunit which confers specific binding to the FSH receptor. Both subunits of the natural protein are modified by glycosylation. The alpha-subunit is naturally glycosylated at Asn52 and Asn78 and the beta-subunit at Asn7 and Asn24. Both subunits are produced in the cells as precursor proteins and then processed and secreted. FSH regulates the development, growth, pubertal maturation, and reproductive processes of the body. In particular, it stimulates the maturation of germ cells and thus is involved in spermatogenesis and folliculogenesis.
Folliculogenesis is induced by FSH, for example, by binding of FSH to FSH receptors on the surface of granulosa cells. FSH receptors are G protein-coupled receptors which activate the coupled G protein upon binding of FSH. The G protein in turn activates adenyl cyclase, resulting in the production of cAMP, a second messenger molecule. The increasing cAMP concentration in the cell activates several downstream targets, in particular cAMP dependent protein kinases, which then lead to the synthesis of progesterone. The progesterone is secreted by the granulosa cells, inducing folliculogenesis.

FSH is widely used in the treatment of infertility, either alone or in combination with other agents, in particular LH. In the art, generally FSH purified from post-menopausal human urine (urinary FSH) or FSH recombinantly produced by Chinese hamster ovary (CHO) cells has been used for human treatment. However, there is considerable heterogeneity associated with FSH preparations due to different isoforms present. Individual FSH isoforms exhibit identical amino acid sequences but differ in the extent and nature of their glycosylation. Particular isoforms are characterized by heterogeneity of the carbohydrate branch structures and differing amounts of sialic acid (a negatively charged terminal monosaccharide unit) incorporation, both of which influence the specific bioactivity of the isoform. Thus, the glycosylation pattern of the FSH has a significant influence on its biological activity.

However, urinary FSH from different donors and different preparations can significantly vary in its carbohydrate structures. Furthermore, FSH obtained from CHO cells exhibits a glycosylation pattern specific for these hamster cells which is not identical to human glycosylation patterns. These differences result in varying biological activities and adverse effects of the obtained FSH and thus, of the pharmaceutical preparations which are to be administered to the patient.

In view of this, it is one object of the present invention to provide improved FSH preparations.

Furthermore, it is an object of the present invention to provide FSH preparations with novel therapeutic or pharmacological characteristics.

Furthermore, it is an object of the present invention to provide FSH preparations which have an improved glycosylation pattern.

SUMMARY OF THE INVENTION

The present inventors have found that improved FSH preparations obtained from human cells which preferably have been selected for an optimized glycosylation are
able to induce the secretion of sex steroids such as estradiol and progesterone at lower concentrations than corresponding FSH preparations obtained from human urine or CHO cells. Additionally, it has been surprisingly found that this low concentration effects are independent of cAMP signaling in the target cells. Thus, the improved FSH preparations according to the present invention induce the secretion of sex steroids such as progesterone at concentrations which do not result in an increase of cAMP secretion. Therefore, it is believed that the improved FSH preparations according to the present invention are capable of inducing a signal transduction pathway leading to sex steroid secretion which is different from the known signal transduction pathway using cAMP as second messenger described for the commonly used FSH preparations. Therefore, the FSH preparations according to the present invention have surprising characteristics which are useful in therapy.

The present invention provides, in a first aspect, a recombinant FSH having a glycosylation pattern comprising one or more of the following characteristics:

(i) a relative amount of glycans carrying bisecting N-acetylglucosamine (bisGlcNAc) of at least 35%;

(ii) a relative amount of glycans carrying fucose of at least 60%; and/or

(iii) a relative amount of 2,6-coupled sialic acid of at least 30%.

In a second aspect, the present invention provides recombinant FSH obtainable by production in human cells or a human cell line, preferably in the cell line GT-5s (deposited on July 28, 2010). It was found that FSH produced in a respective cell line results in an improved glycosylation profile as is described above and below.

Also provided is a pharmaceutical composition, comprising the recombinant FSH according to the present invention.

Furthermore, the present invention pertains to the recombinant FSH or the pharmaceutical composition according to the present invention for use in infertility treatment.

Furthermore, the present invention pertains to the recombinant FSH or the pharmaceutical composition according to the present invention for inducing and/or stimulating the secretion of sex steroids also independent of cAMP.

Furthermore, the present invention pertains to the recombinant FSH or the pharmaceutical composition according to the present invention for stimulating or co-stimulating germ cell maturation by a biological process which is independent of cAMP signaling.
Furthermore, the present invention pertains to the recombinant FSH or the pharmaceutical composition according to the present invention for inducing and/or stimulating the secretion of sex steroids at FSH concentrations at which no significant cAMP release is induced.

Other objects, features, advantages and aspects of the present invention will become apparent to those skilled in the art from the following description and appended claims. It should be understood, however, that the following description, appended claims, and specific examples, which indicate preferred embodiments of the application, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following.

DETAILED DESCRIPTION OF THE INVENTION

Definitions:

As used herein, the following expressions are generally intended to preferably have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise.

The expression "comprise", as used herein, besides its regular meaning also includes and specifically refers to the expressions "consist essentially of" and "consist of. Thus, the expression "comprise" refers to embodiments wherein the subject-matter which "comprises" specifically listed elements does not comprise further elements as well as embodiments wherein the subject-matter which "comprises" specifically listed elements may and/or indeed does encompass further elements.

The term "FSH" refers to follicle-stimulating hormone, a gonadotropin. FSH is a glycoprotein comprised of two subunits, labeled alpha and beta subunits. Preferably, the FSH is human FSH, in particular human FSH composed of an alpha subunit having the amino acid sequence of SEQ ID NO: 1 and an beta subunit having the amino acid sequence of SEQ ID NO: 2. However, one or more, such as 1, 1 or 2, up to 3, up to 5, up to 10 or up to 20, amino acid substitution, addition and/or deletions may be present in one or both subunits. Preferably, the amino acid sequence of the alpha subunit shares an overall homology or identity of at least 80%, more preferably at least 85%, at least 90%, at least 95% or at least 98% with the amino acid sequence according to SEQ ID NO: 1. Furthermore, the amino acid sequence of the beta subunit preferably shares an overall homology or identity of at least 80%, more preferably at least 85%, at least 90%, at least 95% or at least 98% with the amino acid sequence according to
SEQ ID NO: 2. The subunits of the FSH are preferably two separate polypeptide chains, however, the term "FSH" as used herein also encompasses embodiments wherein the two subunits are covalently attached to each other, e.g. by cross-linking agents or a linking polypeptide chain, and embodiments, wherein one or both subunits are further divided into several polypeptide chains. Preferably, the FSH according to the invention is capable of binding to and/or activating the FSH receptor, preferably the human FSH receptor.

Preferably, both subunits comprise one or more carbohydrate structures attached to the polypeptide chain. More preferably, the carbohydrate structures are attached to asparagine residues of the subunits. In particularly preferred embodiments, the alpha subunit comprises two carbohydrate structures attached to Asn52 and Asn78 and/or the beta-subunit comprises two carbohydrate structures attached to Asn7 and Asn24. The amino acid residues carrying the carbohydrate structures are designated with respect to the human amino acid sequences of the alpha and beta subunits according to SEQ ID NOs: 1 and 2, respectively. The sugar part of human FSH is preferably composed of fucose, galactose, mannose, galactosamine, glucosamine, and/or sialic acid.

FSH as used according to the present invention preferably is recombinant FSH, more preferably recombinant human FSH. The term "recombinant FSH" refers to FSH which is not naturally produced by a living human or animal body and then obtained from a sample derived therefrom, such as urine, blood or other body liquid, feces or tissue of the human or animal body. Preferably, recombinant FSH is obtained from cells which have been biotechnologically engineered, in particular cells which have been transformed or transfected with a nucleic acid encoding FSH or the alpha or beta subunits of FSH. According to preferred embodiments, recombinant FSH is obtained from human cells comprising an exogenous nucleic acid encoding FSH. Respective exogenous nucleic acids can be introduced e.g. by using one or more expression vectors, which can be introduced into the host cell e.g. via transfection. Respective methods for recombinantly producing proteins and FSH are well known in the prior art and thus, need no further description.

The FSH according to the invention preferably is FSH, more preferably human FSH, obtainable by production in a human cell, preferably a human cell line such as the cell line GT-5s, deposited on July 28, 2010 under the accession number DSM ACC according to the requirements of the Budapest Treaty at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), InhoffenstraSe 7B, 38124 Braunschweig (DE) by the Glycotope GmbH, Robert-Rossle-Str. 10, 13125 Berlin (DE), or a cell line derived therefrom. According to the present invention, the terms "GT-5s" and "GT-5s cell line" also include cells or cell lines derived from GT-5s. Furthermore, the FSH
according to the invention preferably is FSH, more preferably human FSH, having one or more specific glycosylation characteristics as disclosed herein.

The FSH according to the present invention is glycosylated, i.e. it is modified by one or more oligosaccharides attached to the polypeptides chains. These oligosaccharides, also named glycans or carbohydrates, may be linear or branched saccharide chains. Depending on the number of branches the oligosaccharide is termed mono-, bi-, tri- or tetraantennary (or even pentaantennary). A monoantennary oligosaccharide is unbranched while a bi-, tri- or tetraantennary oligosaccharide has one, two or three branches, respectively. A glycoprotein with a higher antennarity thus has more oligosaccharide endpoints and can carry more functional terminal saccharide units such as, for example, sialic acids. "At least triantennary" as used herein refers to oligosaccharides having an antennarity of at least 3, including triantennary, tetraantennary and pentaantennary oligosaccharides. "At least tetraantennary" as used herein refers to oligosaccharides having an antennarity of at least 4, including tetraantennary and pentaantennary oligosaccharides.

The degree of sialylation of FSH is normally expressed as Z-number. The Z-number indicates the relative negative charge of the glycan structures of a glycoprotein. The Z-number is calculated by the formula:

\[ Z = A_1 \% \times 1 + A_2 \% \times 2 + A_3 \% \times 3 + A_4 \% \times 4 \]

wherein A1\% is the percentage of glycans with a charge of -1, A2\% is the percentage of glycans with a charge of -2, A3\% is the percentage of glycans with a charge of -3, and A4\% is the percentage of glycans with a charge of -4. These percentages are calculated with respect to all glycans attached to the FSH, including charged as well as uncharged glycans. Since the charge of the glycans of FSH is generally only determined by their sialic acid residues and FSH generally has four glycan structures, the Z-number is an indication for the amount of sialic acids on the FSH or the acidity of the FSH.

A "relative amount of glycans" according to the invention refers to a specific percentage or percentage range of the glycans attached to the FSH glycoproteins of a FSH preparation, wherein 100 % of the glycans refers to all glycans attached to the FSH glycoproteins of the FSH preparation.

The term "nucleic acid" includes single-stranded and double-stranded nucleic acids and ribonucleic acids as well as deoxyribonucleic acids. It may comprise naturally occurring as well as synthetic nucleotides and can be naturally or synthetically modified, for example by methylation, 5'- and/or 3'-capping.
The term "vector" is used herein in its most general meaning and comprises any intermediary vehicle for a nucleic acid which enables said nucleic acid, for example, to be introduced into prokaryotic and/or eukaryotic host cells and, where appropriate, to be integrated into a genome of the host cell. Vectors of this kind are preferably replicated and/or expressed in the host cells. A vector preferably comprises one or more selection marker for selecting host cells comprising the vector. Suitable selection markers are resistance genes which provide the host cell with a resistance e.g. against a specific antibiotic. Further suitable selection markers are, for example, genes for enzymes such as DHFR or GS. Vectors enabling the expression of recombinant proteins including FSH as well as suitable expression cassettes and expression elements which enable the expression of a recombinant protein with high yield in a host cell are well known in the prior art and are also commercially available, and thus, need no detailed description here.

The terms "cell" and "cells" and "cell line" used interchangeably, preferably refer to one or more mammalian cells, in particular human cells. The term includes progeny of a cell or cell population. Those skilled in the art will recognize that "cells" include progeny of a single cell, and the progeny can not necessarily be completely identical (in morphology or of total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. "Cell" preferably refers to isolated cells and/or cultivated cells which are not incorporated in a living human or animal body.

The term "patient" means according to the invention a human being, a nonhuman primate or another animal, in particular a mammal such as a cow, horse, pig, sheep, goat, dog, cat or a rodent such as a mouse and rat. In a particularly preferred embodiment, the patient is a human being. In case of a human patient, the FSH preferably is human FSH. The patient may be male or female, and preferably is female.

The term "pharmaceutical composition" particularly refers to a composition suitable for administering to a human or animal, i.e., a composition containing components which are pharmaceutically acceptable. Preferably, a pharmaceutical composition comprises an active compound or a salt or prodrug thereof together with a carrier, diluent or pharmaceutical excipient such as buffer, preservative and tonicity modifier.


The term "infertility treatment" according to the invention means the treatment of a dysfunction or disease related to the reproduction or fertility of a human or animal
subject. In particular, infertility treatment includes assisted reproductive technologies, ovulation induction, in-vitro fertilization, intrauterine insemination, as well as the enablement or improvement of germ cell maturation such as folliculogenesis and spermatogenesis.

According to the invention, the term "wherein no significant amounts of cAMP are released" or similar expressions, respectively, in particular refer to the release of cAMP by cells or tissue in an amount which is less than 25%, preferably less than 20%, more preferably less than 15%, less than 10%, less than 7.5%, less than 5% or less than 2.5% of the amount of cAMP release obtained by cells or tissue after stimulation with FSH in a concentration which results in the maximum release of cAMP. These cells or tissue are susceptible or responsive to stimulation by FSH, such as granulosa cells or Sertoli cells. A cAMP release which is independent of FSH, i.e. a cAMP release which also occurs in the absence of FSH, should not be considered in this respect. Preferably, a "release of a significant amount of cAMP" or a "significant release of cAMP" is any release of cAMP above the cAMP release in the absence of FSH, in particular any detectable release of cAMP above the inaccuracy of measurement. A standard procedure for measuring cAMP release is described in the examples and may be used for determining a significant or non-significant release of cAMP. The "release of cAMP" refers to an intracellular release of cAMP and/or an extracellular release or secretion of cAMP, preferably only to a secretion of cAMP. cAMP refers to cyclic adenosine monophosphate which acts as a second messenger molecule in cellular signal transduction. cAMP is synthesized in cells from ATP by the adenylyl cyclase. A biological process or signal transduction pathway which is "independent of cAMP signaling" preferably does not involve activation of adenylyl cyclase.

"Sex steroids", also known as gonadal steroids or sex hormones, in particular refer to steroid hormones that interact with vertebrate androgen or estrogen receptors. The term "sex steroid" includes androgens such as anabolic steroids, androstenedione, dehydroepiandrosterone, dihydrotestosterone and testosterone; estrogens such as estradiol, estril and estrone; and progesterone. Preferably, sex steroids refer to naturally occurring sex steroids, more preferably to natural human sex steroids. Preferred sex steroids according to the invention are estradiol and progesterone, in particular progesterone.

The present invention is based on the finding that an improved recombinant FSH preparation having an optimal glycosylation pattern is capable of inducing secretion of sex steroids such as progesterone at low FSH concentrations at which no significant cAMP release is induced. In particular, the improved FSH according to the present invention induces sex steroid secretion at much lower concentrations than the commonly used urinary FSH or recombinant FSH obtained from CHO cells. Thereby,
the improved FSH can be administered at much lower doses which reduces adverse effects and production costs. Furthermore, if given at comparable doses, the improved FSH provides a longer activity in the patient's body compared to the commonly used FSH, since also a long time after administration when only a very low concentration of the FSH remains in the circulation, the FSH according to the present invention still exerts its biological activity and preferably, stimulates or co-stimulates germ cell maturation and/or the release of sex steroids. Moreover, at high concentrations, the improved FSH according to the present invention and the commonly used urinary or CHO-derived FSH show comparable effects. Therefore, there is no additional risk of overdosing compared to the commonly used FSH.

In view of these findings, the present invention provides, in a first aspect, a FSH having a glycosylation pattern comprising one or more of the following characteristics:

(i) a relative amount of glycans carrying bisecting N-acetylglucosamine (bisGlcNAc) of at least 35%;

(ii) a relative amount of glycans carrying fucose of at least 60%; and

(iii) a relative amount of 2,6-coupled sialic acid of at least 30%.

Preferably, said FSH is a recombinant FSH and thus, is obtained by recombinant production in a host cell, which preferably is a human host cell. Suitable human host cells which provide a respective glycosylation pattern are described subsequently.

Preferably, the glycosylation pattern comprises at least two of the features (i), (ii) and (iii) (in particular features (i) and (ii), (i) and (iii), or (ii) and (iii)), and more preferably all of the features (i), (ii) and (iii). Furthermore, the glycosylation pattern may further comprise a relative amount of at least tetraantennary glycans of at least 18%, a relative amount of glycans carrying one or more sialic acid residues of at least 85%, and/or a relative amount of glycans carrying galactose of at least 95%, and/or a relative amount of glycan branches carrying a terminal galactose unit of at least 60%. The terminal galactose unit may optionally further carry a sialic acid residue. The recombinant FSH preferably has a Z-number of at least 200.

The relative amount of glycans carrying bisGlcNAc is preferably at least 38% or at least 40%. More preferably, it is in the range of from about 35% to about 60%, in particular in the range of from about 38% to about 50% or in the range of from about 40% to about 45%. Most preferably, it is about 42%. The relative amount of glycans carrying one or more sialic acid residues is preferably at least 88%, and more preferably in the range of from about 85% to about 98% or in the range of from about 88% to about 95%, most preferably about 90%. The Z-number is preferably at least 2.10, more preferably at least
215, at least 220, at least 230 or at least 240. Preferably, the relative amount of at least
tetraantennary glycans is at least 19%, more preferably at least 20% or at least 21%.
The relative amount of at least triantennary glycans, in particular tri- and tetraantennary
glycans, preferably is at least 40%, more preferably at least 45%, at least 50% or at
least 55%. Preferably, the relative amount of glycans carrying fucose is at least 70%,
more preferably at least 75% or at least 78%. It may be in the range of from about 70% to
about 90%, in particular in the range of from about 75% to about 85%. Preferably, the
relative amount of 2,6-coupled sialic acid is at least 40%, at least 45%, at least 50% or
at least 55%, in particular in the range of about 40% to about 80%, preferably about
50% to about 60%. Preferably, the ratio of 2,3-coupled sialic acid to 2,6-coupled sialic
acid is in the range of from about 1:10 to about 7:3, more preferably from about 1:5 to
about 3:2 or from about 1:2 to about 1:1, most preferably from about 2:3 to about 1:1.
In preferred embodiments, the relative amount of 2,6-coupled sialic acids exceeds that
of 2,3-coupled sialic acids. The relative amount of glycans carrying galactose
preferably is at least 97% and most preferably is about 98%. Preferably, the relative
amount of glycan branches carrying a terminal galactose unit optionally modified by a
sialic acid residue is at least 65%, more preferably at least 70% or at least 73%. It is
preferably in the range of from about 60% to about 95%, and more preferably in the
range of from about 70% to about 80%.

In certain embodiments the glycosylation pattern of the recombinant FSH according to
the present invention comprises one or more, preferably all of the following characteristics:

(i) a relative amount of glycans carrying bisecting N-acetylglucosamine
(bisGlcNAc) in the range of from about 38% to about 50%;

(ii) a relative amount of glycans carrying fucose of at least 70%;

(iii) a relative amount of 2,6-coupled sialic acid of at least 50%;

(iv) a relative amount of glycans carrying one or more sialic acid residues of at
least 88%; and

(v) a relative amount of at least tetraantennary glycans of at least 19%.

Furthermore, the present invention provides a recombinant FSH that is obtainable by
production in a human host cell or a human cell line. Preferably, the recombinant FSH
is obtainable in the cell line GT-5s. It was found that an FSH produced in said cell line
exhibits the glycosylation pattern described above and in particular exhibits the
advantageous therapeutic and pharmacological effects described herein. Thus, the
present invention also pertains to a method for producing a recombinant FSH by
recombinantly expressing the FSH in the cell line GT-5s. The recombinant FSH respectively produced can be isolated and optionally purified.

Thus, the recombinant FSH preferably is obtainable by a process comprising the steps of:

(i) cultivating a human host cell, preferably derived from the cell line GT-5s, comprising nucleic acids coding for the FSH alpha and beta subunits under conditions suitable for expression of the FSH; and

(ii) isolating FSH.

The isolation of FSH preferably comprises the further steps of:

(a) obtaining the culture supernatant where the FSH is secreted by the human cells, or lysing the human cells where the FSH is not secreted;

(b) isolating the FSH from the culture supernatant or cell lysate using chromatographic steps such as reversed phase chromatography, size exclusion chromatography and/or hydrophobic interaction chromatography; and

(c) optionally obtaining an acidic fraction of the FSH by removing basic FSH isoforms, preferably by using anion exchange chromatography including a washing step which removes basic FSH isoforms, such as a washing step at about pH 5.0 or about pH 4.5.

The human host cell used for expression preferably is or is derived from the cell line GT-5s.

Preferably, the nucleic acid coding for the FSH alpha subunit and the nucleic acid coding for the FSH beta subunit are comprised in expression cassettes comprised in a suitable expression vector that allows the expression in the human host cell. The nucleic acid coding for the FSH alpha subunit and the nucleic acid coding for the FSH beta subunit may be comprised in the same vector, but preferably are comprised in separate vectors. Furthermore, they may also be expressed from one expression cassette using appropriate elements such as an IRES element. Preferably, the FSH is secreted by the human cells. In preferred embodiments, cultivation of the human cells is performed in a fermenter and/or under serum-free conditions.

A suitable purification process for the recombinant FSH is described, for example, in the U.S. patent application no. US 61/263,931 and the European patent application no. EP 09 014 585.5.
The recombinant FSH obtainable by production in human host cells or a human cell line preferably exhibits the features described herein with respect to the recombinant FSH according to the present invention. In particular, its glycosylation pattern comprises one or more of the characteristics described above and in claims 1 to 6.

In preferred embodiments of the aspects of the present invention, the recombinant FSH according to the present invention is recombinant human FSH (rhFSH), preferably obtainable by production in a human cell line, such as the cell line GT-5s, which comprises one or more nucleic acids encoding the human FSH subunits and elements for expressing said one or more nucleic acids in the host cell. Preferably, the alpha subunit of the rhFSH has the amino acid sequence according to SEQ ID NO: 1 and is glycosylated at amino acids Asn52 and Asn78. The beta subunit of the rhFSH preferably has the amino acid sequence according to SEQ ID NO: 2 and is glycosylated at amino acids Asn7 and Asn24.

According to one embodiment, the recombinant FSH according to the present invention is capable of stimulating the release of progesterone in granulosa cells

(a) at concentrations where no significant amounts of cAMP are released; and/or

(b) by inducing a signal transduction pathway which is independent of cAMP signaling.

According to one embodiment, the recombinant FSH according to the present invention is capable of stimulating or co-stimulating germ cell maturation by a biological process which is independent of cAMP signaling. It was surprisingly found that the glycosylation pattern described above results in a respective novel pharmacological profile of the recombinant FSH, which exhibits the pharmacological and therapeutic advantages described herein.

The recombinant FSH according to the present invention may have one or more of the subsequently described characteristics as can be determined in a granulose cell assay (as is e.g. described in Example 2). As is demonstrated by the examples, the recombinant FSH having the above described glycosylation pattern and in particular the recombinant FSH obtainable by production in the cell line GT-5s exhibit the subsequently described characteristics which result in the pharmacological and therapeutic advantages described herein.

The recombinant FSH is according to one embodiment capable of stimulating the release of progesterone in granulose cells at concentrations which are below the minimum concentration needed for the induction of cAMP release by the granulose cells. The release of progesterone, estradiol and/or cAMP mentioned below refers to
an in vitro release in about $1 \times 10^4$ to about $1 \times 10^6$ granulosa cells/ml, preferably in about $5 \times 10^4$ to about $1 \times 10^5$ granulosa cells/ml, in particular under conditions as described in example 2, below.

Preferably, the recombinant FSH according to the present invention is capable of releasing at least 100 ng/ml, at least 150 ng/ml, at least 200 ng/ml, preferably at least 250 ng/ml, at least 300 ng/ml or at least 400 ng/ml progesterone at a concentration which does not induce a cAMP release or which induces a cAMP release of less than 20 pmol/ml, less than 15 pmol/ml, less than 10 pmol/ml, less than 5 pmol/ml.

Furthermore, the recombinant FSH according to the present invention is preferably capable of releasing at least 100 ng/ml, at least 200 ng/ml, preferably at least 300 ng/ml or at least 400 ng/ml progesterone at a FSH concentration that is lower than the concentration necessary with human urinary FSH or recombinant FSH produced in CHO cells (Gonal F). Thus, it is preferably capable of releasing at least 100 ng/ml, at least 200 ng/ml, preferably at least 300 ng/ml or at least 400 ng/ml progesterone at a concentration wherein human urinary FSH or recombinant FSH produced in CHO cells (Gonal F) do not result in a corresponding, respectively equally high release of progesterone. As is demonstrated by the examples, the recombinant FSH according to the present invention induces respectively stimulates the production of progesterone more strongly than human urinary FSH or recombinant FSH produced in CHO cells (Gonal F).

Furthermore, the recombinant FSH according to the present invention is preferably capable of releasing at least 50 nmol/l, at least 75 nmol/l, at least 100 nmol/l, at least 125 nmol/l or at least 150 nmol/l estradiol at a FSH concentration which does not induce a cAMP release or which induces a cAMP release of less than 20 pmol/ml, less than 15 pmol/ml, less than 10 pmol/ml, less than 5 pmol/ml.

Furthermore, the recombinant FSH according to the present invention is preferably capable of releasing at least 50 nmol/l, at least 75 nmol/l, at least 100 nmol/l, at least 125 nmol/l, at least 150 nmol/l, at least 200 nmol/l, at least 250 nmol/l, at least 300 nmol/l or at least 350 nmol/l estradiol at a FSH concentration that is lower than the concentration necessary with human urinary FSH or recombinant FSH produced in CHO cells (Gonal F). Thus, it is preferably capable of releasing at least 50 nmol/l, at least 75 nmol/l, at least 100 nmol/l, at least 125 nmol/l, at least 150 nmol/l, at least 200 nmol/l, at least 250 nmol/l, 300 nmol/l or at least 350 nmol/l estradiol at a concentration wherein human urinary FSH or recombinant FSH produced in CHO cells (Gonal F) do not result in a corresponding, respectively equally high release of estradiol. As is demonstrated by the examples, the recombinant FSH according to the present invention induces respectively stimulates the production of estradiol more
strongly than human urinary FSH or recombinant FSH produced in CHO cells (Gonal F).

The respective characteristics described herein on the cAMP release and the expression of the sex steroids can be analysed and determined by using a granulose cell assay, as is e.g. described in example 2.

The recombinant FSH according to the present invention preferably is present in a pharmaceutical composition. Thus, another aspect of the present invention is a pharmaceutical composition comprising the recombinant FSH according to the present invention for use in infertility treatment as defined herein. The pharmaceutical composition may include further pharmaceutically active agents, in particular further agents useful in infertility treatment such as other gonadotropins, in particular LH and/or CG, preferably recombinant and/or human LH or CG. Alternatively, the pharmaceutical composition comprising the recombinant FSH may be designed for use in combination with such further pharmaceutically active agents.

Furthermore, the present invention provides the recombinant FSH according to the present invention for use in infertility treatment.

As discussed above, in certain embodiments the recombinant FSH according to the present invention is capable of stimulating or co-stimulating the release of sex steroids such as progesterone, in particular the release of progesterone in granulosa cells, already at concentrations where no significant amount of cAMP is released. In particular, the recombinant FSH according to the present invention may be capable of stimulating release of sex steroids such as progesterone in granulosa cells at concentrations which are below the minimum concentration needed for the induction of cAMP release by the granulosa cells.

Furthermore, the recombinant FSH according to the present invention is capable of stimulating release of progesterone, in particular release of progesterone in granulosa cells, by inducing a signal transduction pathway which is independent of cAMP signaling. Preferably, the infertility treatment includes the induction of a signal transduction pathway which is independent of cAMP signaling by the recombinant FSH according to the present invention, resulting in the stimulation of progesterone release. However, other signal transduction pathways including cAMP signaling may additionally be activated by the recombinant FSH. In other embodiments, the infertility treatment does not involve the induction of a significant release of cAMP by the recombinant FSH according to the present invention.
In further embodiments, as described above, the recombinant FSH according to the present invention is capable of stimulating or co-stimulating germ cell maturation by a biological process which is independent of cAMP signaling.

Thus, the present invention also pertains to the recombinant FSH or the pharmaceutical composition described above for inducing and/or stimulating the secretion of sex steroids also independent of cAMP. Furthermore, the present invention also pertains to the recombinant FSH or the pharmaceutical composition described above for stimulating or co-stimulating germ cell maturation by a biological process which is independent of cAMP signaling. Additionally, the present invention also pertains to the recombinant FSH or the pharmaceutical composition described above for inducing and/or stimulating the secretion of sex steroids at FSH concentrations at which no significant cAMP release is induced. Furthermore, the present invention also pertains to the recombinant FSH or the pharmaceutical composition described above for inducing sex steroid secretion at much lower concentrations than the commonly used urinary FSH or recombinant FSH obtained from CHO cells. The pharmacological and therapeutic advantages of the respective uses in particular for infertility treatment were discussed in detail above.

In particular, the infertility treatment may include the stimulation or co-stimulation of germ cell maturation by a biological process which is independent of cAMP signaling. However, the infertility treatment may additionally comprise the stimulation of germ cell maturation by one or more other biological processes which involve cAMP signaling. In other embodiments, the infertility treatment does not involve the stimulation of germ cell maturation by such other biological processes.

The germ cell maturation preferably includes follicular growth and/or spermatogenesis. Furthermore, the biological process by which the FSH stimulates germ cell maturation may include secretion of sex steroids, in particular progesterone, preferably by granulosa cells. Preferably, the biological process which is independent of cAMP signaling refers to the secretion of sex steroids, in particular progesterone, preferably by granulosa cells, induced by a signal transduction pathway which does not involve cAMP as messenger molecule.

In certain embodiments, the recombinant FSH according to the present invention is administered to the patient in a dose which results in an FSH concentration in the circulation of the patient of less than 5 IU/L. In preferred embodiments, the dose to be administered to the patient results in an FSH concentration in the circulation of the patient which is less than about 4 IU/L, more preferably less than about 3 IU/L, less than about 2 IU/L, less than about 1 IU/L or less than about 0.5 IU/L. Preferably, the concentration of the FSH in the patient’s circulation is in the range of about 0.01 to about 5 IU/L, more preferably about 0.05 to about 2 IU/L, even more preferably about
0.1 to about 1.5 IU/L or about 0.2 to about 1 IU/L. In particular, the FSH is administered to the patient in a dose which does not induce a significant release of cAMP.

In preferred embodiments, the infertility treatment includes assisted reproductive technologies, ovulation induction, in-vitro fertilization, intrauterine insemination, and/or the enablement or improvement of germ cell maturation such as folliculogenesis and spermatogenesis.

Preferably, the recombinant FSH according to the present invention is for parenteral administration to the patient. In particular, the recombinant FSH is to be administered by injection or infusion, for example intravenously, intramuscularly or subcutaneously. In certain embodiments of the present invention, the recombinant FSH is present in a pharmaceutical composition. Suitable dosage regimens can be determined by the skilled artisan and can be derived from the general knowledge in the field.

FIGURES

Figure 1 shows the cAMP release of isolated granulosa cells stimulated with different concentrations of the improved recombinant human FSH (FSH-GEX; preparation 1: open squares, preparation 2: closed triangles) or FSH obtained from CHO cells (Gonal F; closed diamonds).

Figure 2 shows the estradiol synthesis of isolated granulosa cells stimulated with different concentrations of the improved recombinant human FSH (FSH-GEX; preparation 1: open squares, preparation 2: closed triangles) or FSH obtained from CHO cells (Gonal F; closed diamonds).

Figure 3 shows the progesterone synthesis of isolated granulosa cells stimulated with different concentrations of the improved recombinant human FSH (FSH-GEX; preparation 1: open squares, preparation 2: closed triangles) or FSH obtained from CHO cells (Gonal F; closed diamonds).

Figure 4 shows the cAMP release of isolated granulosa cells stimulated with different concentrations of the improved recombinant human FSH (FSH-GEX; open squares) or urinary FSH (Fostimon; closed diamonds).

Figure 5 shows the estradiol synthesis of isolated granulosa cells stimulated with different concentrations of the improved recombinant human FSH (FSH-GEX; open squares) or urinary FSH (Fostimon; closed diamonds).
Figure 6 shows the progesterone synthesis of isolated granulosa cells stimulated with different concentrations of the improved recombinant human FSH (FSH-GEX; open squares) or urinary FSH (Fostimon; closed diamonds).

Figure 7 shows the results of the Steelman-Pohley assay using the improved recombinant human FSH in comparison to standard urinary FSH and standard recombinant FSH obtained from CHO cells. The ovarian weight gain in immature female rats after daily administration for three days is plotted against the used FSH concentration.

EXAMPLES

Example 1: Preparation of FSH-GEX™

FSH is produced by cultivation of GT-5s cells stably transfected with two expression constructs harbouring the alpha and beta chain of human FSH (alpha chain accession number NT_007299.13; beta chain accession number NT_009237.18). The plasmid for the expression of the FSH alpha chain is carrying the gene of a mutated version of the murine dihydrofolate reductase (dfr) with higher resistance to the enzyme inhibitor methotrexate than the native form and the second plasmid for the expression of the FSH alpha chain is carrying the puromycin resistance gene.

Transfection of the cell line for FSH-GEX™ expression was performed by nucleofection using the two expression plasmids described above. For selection and amplification of stable antibody producing cell clones puromycin and methotrexate were added at increasing concentrations. Amplified cell pools were seeded in a semi-solid matrix for single cell cloning by the Clone PixFL technology or single cell cloning by limited dilution. The clones were screened for high secretion of intact FSH molecules.

FSH is produced by fermentation of the final FSH producing GT-5s clone in batch, fed-batch or perfusion process under serum free conditions. The fermentation is usually run for 2-3 weeks.

After fermentation the supernatant is filtered through 2um filters to eliminate cells and cell debris prior to a sterile filtration step using 0.2um filters. The purification process utilizes a reverse phase chromatography (RPC) as capture step followed by a concentration step and a subsequent size exclusion chromatography (SEC). Optionally, the eluate is then applied to an anion exchange chromatography (AEC) to eliminate the less acidic FSH contents. This is done by washing the bound FSH with washing buffer at pH 5.0 ("enrichment at pH 5.0") or pH 4.5 ("enrichment at pH 4.5") to elute less acidic FSH isoforms prior to elution of the desired FSH fraction. As a
polishing step a hydrophobic interaction chromatography (HIC) is used to gain FSH at high purity.

**Example 2: Granulosa cell assay**

In order to perform a granulosa cell assay primary cells are isolated from the follicular fluid of IVF patients during the collection of the oocytes. After a Ficoll gradient centrifugation which eliminates other cell types as e.g. red blood cells the granulosa cells are seeded in 24 to 96 well plate format for 5-7 days in culture medium containing androstendione or testosterone. After that period, the cells (2 to $4 \times 10^4$ cells per well) are stimulated with FSH ranging between 1 pg/ml to 2 pg/ml in the steps shown in the diagram (400 µl medium per well). After three to four hours incubation half of the supernatant is collected for performing the cAMP assay. Another 24 h later the cells are lysed by freeze thaw in the remaining supernatant. The lysate is applied in the progesterone and estradiol assays.

**Comparison of FSH-GEX™ and Gonal F**

In the first set of experiments FSH-GEX™ is compared to Gonal F (Merck Serono SA). Gonal F is FSH recombinantly produced in CHO cells. The results are shown in Figs. 1 to 3. While the second messenger cAMP is produced at comparable FSH concentrations of Gonal F and FSH-GEX™ products in comparable amounts, the steroids progesterone and estradiol are released at much lower FSH concentrations in the case of FSH-GEX™ products compared to FSH recombinantly produced in CHO cells (Gonal F).

**Comparison of FSH-GEX™ and Fostimon**

In another set of experiments the FSH-GEX™ was compared against Fostimon (IBSA Institut Biochimique SA), the FSH product isolated out of human urine. The results are shown in Figs. 4 to 6. While the cAMP level rises similarly at comparable dose ranges of FSH for both products, the sex steroids are produced at a significantly lower concentration of FSH-GEX™ compared to Fostimon.

Note: Since the assays are performed using different donors, differences in the stimulation profile may account to the donors used in each assay.

**Example 3: Steelman-Pohley assay**

The activity of FSH was also determined by the Steelman-Pohley assay. The assay was performed according to the pharmacopeia. In particular, the ovarian weight gain in immature female rats was measured after administration of three different FSH
concentrations each given daily for three days. The potency is calculated using the parallel line evaluation. The results are shown in Fig. 7.

Table 1: Calculated activity of FSH-GEX™ after comparison with urinary FSH

<table>
<thead>
<tr>
<th>Sample</th>
<th>calculated activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH-GEX without enrichment</td>
<td>6,271 IU/mg</td>
</tr>
<tr>
<td>FSH-GEX with enrichment at pH 4.5</td>
<td>7,663 IU/mg</td>
</tr>
</tbody>
</table>

Activity of the urinary standard: 7,135 IU/mg

As demonstrated by the Steelman-Pohley assay, the in vivo activities of the FSH-GEX™ and of the urinary and recombinant standard FSH are similar in rat.

Example 4: Glycoprofiling

The glycoprofiles of the different FSH preparations were determined by structural analysis of the glycosylation. This analysis gave the following results:

Table 2: Relative amounts of the different glycosylation properties

<table>
<thead>
<tr>
<th>Sample</th>
<th>F</th>
<th>S</th>
<th>G</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH-GEX</td>
<td>80%</td>
<td>90%</td>
<td>98%</td>
<td>42%</td>
</tr>
<tr>
<td>Fostimon</td>
<td>48%</td>
<td>83%</td>
<td>91%</td>
<td>28%</td>
</tr>
<tr>
<td>Puregon</td>
<td>29%</td>
<td>91%</td>
<td>91%</td>
<td>0%</td>
</tr>
</tbody>
</table>

F: fucose; S: sialic acid; G: galactose; B: bisecting N-acetylgalactosamine


Shown are the relative amounts of N-glycans on the FSH which carry the indicated units. Puregon is another recombinant human FSH produced in CHO cells.

Table 3: Relative amounts of the sialic acid linkage

<table>
<thead>
<tr>
<th>Sample</th>
<th>2,3-linked sialic acid</th>
<th>2,6-linked sialic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH-GEX</td>
<td>43%</td>
<td>57%</td>
</tr>
<tr>
<td>Bravelle</td>
<td>75%</td>
<td>25%</td>
</tr>
<tr>
<td>Gonal F / Puregon</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

In FSH-GEX™, the sialic acid residues are coupled to the glycans by 2,3- as well as 2,6-bonds in a ratio of about 1 : 1, while in the urinary FSH Bravelle (Ferring Pharmaceuticals Inc.) the ratio is about 3 : 1 in favor of 1,3-linked sialic acid. Due to their recombinant production in CHO cells, Puregon (Organon / EssexPharma) and
Gonal F (Merck Serono) do not have any bisecting N-acetylgalactosamines and only comprises 2,3-coupled sialic acids.

**Table 4: Antennarity of the glycosylation of the different FSH**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bi</th>
<th>Tri</th>
<th>Tetra</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH-GEX</td>
<td>42 %</td>
<td>35 %</td>
<td>22 %</td>
</tr>
<tr>
<td>Fostimon</td>
<td>39 %</td>
<td>45 %</td>
<td>16 %</td>
</tr>
<tr>
<td>GonalF&lt;sup&gt;1&lt;/sup&gt;</td>
<td>~65 %</td>
<td>~25 %</td>
<td>~10 %</td>
</tr>
<tr>
<td>Puregon&lt;sup&gt;2&lt;/sup&gt;</td>
<td>53 %</td>
<td>26 %</td>
<td>12 %</td>
</tr>
</tbody>
</table>

<sup>1</sup> literature values (Gervais, A. et al. (2003) Glycobiology 13(3), 179-189)

<sup>2</sup> literature values (Hard, K. et al. (1990) European Journal of Biochemistry 193, 263-271)

Shown are the relative amounts of bi-, tri- and tetraantennary N-glycans on the FSH.

**Table 5: Relative amount of terminal galactose units**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Terminal galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH-GEX</td>
<td>75 %</td>
</tr>
<tr>
<td>Fostimon</td>
<td>43 %</td>
</tr>
</tbody>
</table>

Shown are the relative amounts of N-glycan branches on the FSH which have a galactose unit at their end.

**Table 6: Z-number of different FSH**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Z-number</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH-GEX without enrichment</td>
<td>220</td>
</tr>
<tr>
<td>FSH-GEX with enrichment of acidic isoforms</td>
<td>245</td>
</tr>
<tr>
<td>Gonal F (rFSH)</td>
<td>218</td>
</tr>
<tr>
<td>Puregon (rFSH)</td>
<td>204</td>
</tr>
<tr>
<td>Fostimon (uFSH)</td>
<td>212</td>
</tr>
<tr>
<td>Bravelle (uFSH)</td>
<td>244</td>
</tr>
</tbody>
</table>

Shown is the Z-number, i.e. the relative acidity, of the FSH preparations. A higher Z-number indicates a more acidic FSH preparation.

In conclusion, the FSH according to the present invention (FSH-GEX™) has a high degree of bisecting N-acetylgalactosamine, a high antennarity and a high degree of sialylation, in particular after enrichment of the acidic isoforms. Because of these three
glycosylation parameters, the FSH-GEX™ has a superior activity compared to the common recombinant or urinary FSH preparations.

Furthermore, the FSH-GEX™ is also highly fucosylated and has a ratio of 2,3- to 2,6-sialylation of about 1:1.
INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 5, line 33.

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet

Name of depository institution
Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)

Address of depository institution (including postal code and country)
Inhoffenstr. 7B
38124 Braunschweig
Germany

Date of deposit 2010-07-28
Accession Number not available yet

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

Accession Number of Deposit (designated GT-5s in the application)

For receiving Office use only

This sheet was received with the international application
yes

Authorized officer
Marinus-Van den Nouweland, Y.

For International Bureau use only

This sheet was received by the International Bureau on:

Authorized officer
Additional indications according to form PCT/RO/134 for deposited cell line GT-5s
Accession Number of Deposit not available yet

Applicant herewith requests for those countries which have a respective provision that the furnishing of a sample of the deposited material referred to in the application may only be made to an independent, nominated expert (request of the "expert solution" where applicable, in particular in Australia, Canada, Croatia, Denmark, Finland, Germany, Iceland, Norway, Singapore, Spain, Sweden, United Kingdom, Europe).

For Europe, applicant accordingly requests that a sample of the deposited biological material will be made available as provided in Rule 33(1)(2) EPC until the publication of the mention of the grant of the patent or for 20 years from the date of filing if the application is refused or withdrawn or deemed to be withdrawn, only by the issue of a sample to an expert nominated by the person requesting the sample (Rule 32 EPC).
CLAIMS

1. A recombinant FSH having a glycosylation pattern comprising one or more of the following characteristics:
   (i) a relative amount of glycans carrying bisecting N-acetylglucosamine (bisGlcNAc) of at least 35%;
   (ii) a relative amount of glycans carrying fucose of at least 60%; and/or
   (iii) a relative amount of 2,6-coupled sialic acid of at least 30%.

2. The recombinant FSH according to claim 1, wherein the glycosylation pattern comprises at least two of the features (i), (ii) and (iii), and preferably all of the features (i), (ii) and (iii).

3. A recombinant FSH obtainable by production in the human cell line GT-5s.

4. The recombinant FSH according to any one of claim 1 to 3, which comprises one or more of the following characteristics
   (a) the glycosylation pattern comprises a relative amount of glycans carrying one or more sialic acid residues of at least 85%;
   (b) a Z-number of at least 200;
   (c) it is human recombinant FSH; and/or
   (d) it is produced by a human cell line or human cells.

5. The recombinant FSH according to any one of claims 1 to 4, wherein the glycosylation pattern comprises one or more of the following characteristics:
   (i) a relative amount of glycans carrying bisecting N-acetylglucosamine (bisGlcNAc) in the range of from about 38% to about 50%;
   (ii) a relative amount of at least tetraantennary glycans of at least 19%;
   (iii) a relative amount of glycans carrying fucose of at least 70%;
   (iv) a relative amount of 2,6-coupled sialic acid of at least 45%;
(v) a relative amount of glycans carrying one or more sialic acid residues of at least 88%;

(vi) a Z-number of at least 220;

(vii) a relative amount of glycans carrying galactose of at least 95%; and/or

(viii) a relative amount of glycan branches carrying a terminal galactose unit optionally modified by a sialic acid residue of at least 60%.

6. The recombinant FSH according to any one of claims 1 to 5, wherein the glycosylation pattern comprises the following characteristics:

(i) a relative amount of glycans carrying bisecting N-acetylg glucosamine (bisGlcNAc) in the range of from about 38% to about 50%;

(ii) a relative amount of at least tetraantennary glycans of at least 19%;

(iii) a relative amount of glycans carrying fucose of at least 70%;

(iv) a relative amount of 2,6-coupled sialic acid in the range of from about 50% to about 60%; and

(v) a relative amount of glycans carrying one or more sialic acid residues of at least 88%.

7. The recombinant FSH according to any one of claims 1 to 6, wherein said FSH is capable of stimulating the release of progesterone in granulosa cells

(a) at concentrations where no significant amounts of cAMP are released; and/or

(b) by inducing a signal transduction pathway which is independent of cAMP signaling.

8. The recombinant FSH according to any one of claims 1 to 7, wherein said FSH is capable of stimulating or co-stimulating germ cell maturation by a biological process which is independent of cAMP signaling.

9. The recombinant FSH according to any one of claims 1 to 8, wherein the FSH has one or more of the following characteristics as can be determined in a granulose cell assay
(a) it is capable of stimulating the release of progesterone in granulose cells at concentrations which are below the minimum concentration needed for the induction of cAMP release by the granulose cells;

(b) it is capable of stimulating the release of at least 200ng/ml progesterone in about $5 \times 10^4$ to about $1 \times 10^5$ granulosa cells/ml at FSH concentrations which do not induce a cAMP release or which induce a cAMP release of less than 10 pmol/ml;

(c) it is capable of stimulating the release of at least 100ng/ml progesterone in about $5 \times 10^4$ to about $1 \times 10^5$ granulosa cells/ml at a concentration that is lower than the concentration needed by human urinary FSH or recombinant FSH produced in CHO cells (Gonal F); and/or

(d) it is capable of stimulating the release of at least 100ng/ml progesterone in about $5 \times 10^4$ to about $1 \times 10^5$ granulosa cells/ml at a concentration wherein human urinary FSH or recombinant FSH produced in CHO cells (Gonal F) do not result in a corresponding release of progesterone.

10. A pharmaceutical composition comprising the recombinant FSH according to any one of claims 1 to 9.

11. The recombinant FSH according to any one of claims 1 to 9 or the pharmaceutical composition according to claim 10 for use in infertility treatment.

12. The recombinant FSH according to claim 11, wherein the dose to be administered to the patient results in an FSH concentration in the circulation of the patient in the range of about 0.05 to about 2 IU/L, preferably about 0.1 to about 1 IU/L.

13. The recombinant FSH according to any one of claims 1 to 9 or the pharmaceutical composition according to claim 10 for inducing and/or stimulating the secretion of sex steroids also independent of cAMP.

14. The recombinant FSH according to any one of claims 1 to 9 or the pharmaceutical composition according to claim 10 for stimulating or co-stimulating germ cell maturation by a biological process which is independent of cAMP signaling.

15. The recombinant FSH according to any one of claims 1 to 9 or the pharmaceutical composition according to claim 10 for inducing and/or stimulating the secretion of sex steroids at FSH concentrations at which no significant cAMP release is induced.
16. The recombinant FSH or the pharmaceutical composition according to any one of claims 11 to 14 for use in infertility treatment, wherein the infertility treatment includes assisted reproductive technologies, ovulation induction, in-vitro fertilization, intrauterine insemination, and/or the enablement or improvement of germ cell maturation such as folliculogenesis and spermatogenesis.
Figure 2

Estradiol

- - - Gonal F
- - ○ FSH-GEX preparation 1
- - ▲ FSH-GEX preparation 2

Concentration FSH [ng/ml]

Estradiol [nmol/l]
A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/435 A61K38/24
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)
EPO-Internal , EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT
Category Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No.

X wo 2009/127826 AI (FERRIN6 INTERNAT CT SA [CH] ; COTTINGHAM IAN [GB]; PLASKIN DANIEL [IL] ;) 22 October 2009 (2009-10-22) 1,3-16
Y page 5, line 9 - line 11
page 10, line 19 - line 22
page 11, line 13 - line 20
page 18, line 22 - page 19, line 26
page 6, line 11 - line 24

X wo 03/035686 A2 (APPLIED RESEARCH SYSTEMS [NL]; LOUMAYE ERNEST [FR]; GIARTOSIO CARLO EM) 1 May 2003 (2003-05-01 ) 3-5 ,7-16
Y page 7, line 16 - page 8, line 25
page 15, line 1 - line 9
page 19, line 20 - line 27
page 21, line 15 - line 28
page 22, line 10 - line 25

* Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :
* A document defining the general state of the art which is not considered to be of particular relevance
* E earlier document but published on or after the International filing date
* L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
* O document referring to an oral disclosure, use, exhibition or other means
* P document published prior to the international filing date but later than the priority date claimed

* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve a inventive step when the document is taken alone
* Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* a document member of the same patent family

Date of the actual completion of the International search 13 December 2010
Date of mailing of the International search report 28/12/2010

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer
Bonenlo, Steve

Form PCT/ISA/2/10 (second sheet) (April 2005)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>
### International Search Report

**Information on patent family members**

<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AU 2009237479 A1</td>
<td>22-10-2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT 329930 T</td>
<td>15-07-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 2002340562 B2</td>
<td>23-10-2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2464368 A1</td>
<td>01-05-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 1608078 A</td>
<td>20-04-2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 60212425 T2</td>
<td>09-11-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DK 1438336 T3</td>
<td>02-10-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1438336 A2</td>
<td>21-07-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1621549 A2</td>
<td>01-02-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ES 2261740 T3</td>
<td>16-11-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR 20040278 A2</td>
<td>30-04-2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL 161235 A</td>
<td>20-07-2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2005515974 T</td>
<td>02-06-2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MX PA04003352 A</td>
<td>08-07-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO 20042061 A</td>
<td>13-07-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT 1438336 E</td>
<td>31-08-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 200402279 A</td>
<td>01-08-2005</td>
</tr>
</tbody>
</table>

**International application No**
PCT/EP2010/004769

Form PCT/ISA/210 (patent family annex) (April 2005)