CLAIMS

1. A recombinant FSH preparation, **wherein** the recombinant FSH in the preparation has 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 20 %; and/or

   (ii) a relative amount of glycans carrying fucose of at least 30%; and/or

   (iii) a relative amount of 2,6-coupled sialic acid of at least 30%; and/or

   (iv) it is a diverse glycosylation pattern.

2. The recombinant FSH preparation 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 preparation obtainable by production in the human cell line GT-5s or a cell line derived therefrom or a cell line homologous thereto.

4. The recombinant FSH preparation 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) the glycosylation pattern comprises a relative amount of at least tetraantennary glycans of at least 18%;

   (c) a Z-number of at least 200;

   (d) it is human recombinant FSH; and/or

   (e) it is produced by a human cell line or human cells.

5. The recombinant FSH preparation 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 25% to about 50%;

   (ii) a relative amount of at least tetraantennary glycans of at least 16%;

   (iii) a relative amount of glycans carrying fucose of at least 35%;

(iv) a relative amount of 2,6-coupled sialic acid of at least 53%;

(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%;

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

(ix) a relative amount of glycans carrying a sulfate group of at least 3%;

(x) it comprises at least 45 different glycan structures, wherein each one of the different glycan structures has a relative amount of at least 0.05 % of the total amount of glycan structures of the FSH in the preparation;

(xi) it comprises at least 35 different glycan structures, wherein each one of the different glycan structures has a relative amount of at least 0.1 % of the total amount of glycan structures of the FSH in the preparation;

(xiii) it comprises at least 20 different glycan structures, wherein each one of the different glycan structures has a relative amount of at least 0.5 % of the total amount of glycan structures of the FSH in the preparation; and/or

(xiv) it comprises at least 40 % more different glycan structures than FSH obtained from CHO cells in a corresponding preparation, wherein each one of the different glycan structures has a relative amount of at least 0.05 % of the total amount of glycan structures of the FSH in the respective preparation.

6. The recombinant FSH preparation 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-acetylglucosamine (bisGlcNAc) in the range of from about 25% to about 50%;

(ii) a relative amount of at least tetraantennary glycans of at least 16%;

(iii) a relative amount of glycans carrying fucose of at least 35%;

(iv) a relative amount of 2,6-coupled sialic acid in the range of from about 53% to about 99%; and

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

7. The recombinant FSH preparation according to any one of claims 1 to 6, **wherein** the recombinant FSH in the preparation has a glycosylation pattern according to any one of the embodiments listed in table 1.

8. The recombinant FSH preparation according to any one of claims 1 to 7, **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.

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

10. The recombinant FSH preparation according to any one of claims 1 to 9, **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 $5*10^4$ to about $1*10^5$ 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 $5*10^4$ to about $1*10^5$ 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 $5*10^4$ to about $1*10^5$ 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.

11. The recombinant FSH preparation according to any one of claims 1 to 9, **wherein** the FSH is capable of inducing follicle growth in a female human being after administration of a single dose, wherein the single dose preferably comprises 25 to 500 IU FSH and preferably is administered parenteral, in particular by subcutaneous injection.

12. A pharmaceutical composition comprising the recombinant FSH preparation according to any one of claims 1 to 11.

13. The recombinant FSH preparation according to any one of claims 1 to 11 or the pharmaceutical composition according to claim 12 for use in infertility treatment.

14. The recombinant FSH preparation or pharmaceutical composition according to claim 13, **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.

15. The recombinant FSH preparation according to any one of claims 1 to 11 or the pharmaceutical composition according to claim 12 for inducing and/or stimulating the secretion of sex steroids also independent of cAMP.

16. The recombinant FSH preparation according to any one of claims 1 to 11 or the pharmaceutical composition according to claim 12 for stimulating or co-stimulating germ cell maturation by a biological process which is independent of cAMP signaling.

17. The recombinant FSH preparation according to any one of claims 1 to 11 or the pharmaceutical composition according to claim 12 for inducing and/or stimulating the secretion of sex steroids at FSH concentrations at which no significant cAMP release is induced.

18. The recombinant FSH preparation or the pharmaceutical composition according to any one of claims 13 to 16 for use in infertility treatment, **wherein** the infertility treatment includes assisted reproductive technologies, ovulation induction, in-vitro fertilization, for example in-vitro fertilization with intracytoplasmic sperm injection, gamete intrafallopian transfer, intrauterine insemination, treatment of anovulatory disorder in women, treatment of severe hormone deficiency disorder for egg maturation in woman, treatment of sperm production deficiencies in men, and/or the enablement or improvement of germ cell maturation such as folliculogenesis and spermatogenesis, in particular follicle maturation in women, for example during in vitro fertilization stimulation protocols and/or for anovulatory disorder treatment.

19. The recombinant FSH preparation or the pharmaceutical composition according to any one of claims 13 to 18, wherein the recombinant FSH preparation or the pharmaceutical composition has one or more of the following characteristics:

    (i) it is capable of inducing follicular growth and/or ovular maturation after the administration of only a single dose; and/or

    (ii) it has a lower circulation half-life in one or more of humans, cynomolgus monkeys, rats and/or mice than FSH preparations obtained from human urine and/or expressed in CHO cells; and/or

    (iii) it has a lower bioavailability in one or more of humans, cynomolgus monkeys, rats and/or mice than FSH preparations obtained from human urine and/or expressed in CHO cells; and/or

    (iv) it has a therapeutic efficacy in one or more of humans, cynomolgus monkeys, rats and/or mice which is similar to or higher than that of FSH preparations obtained from human urine and/or expressed in CHO cells.

20. Use of the recombinant FSH preparation according to any one of claims 1 to 11 or the pharmaceutical composition according to claim 12 for the induction of follicle growth and/or ovular maturation, and/or a use as defined in any one of claims 13 to 19.

CLAIMS

1. A recombinant FSH preparation, wherein the recombinant FSH in the preparation has a glycosylation pattern comprising the following characteristics:

   (i) a relative amount of glycans carrying bisecting N-acetylglucosamine (bisGlcNAc) of at least 20 % of the total amount of glycans attached to FSH in the preparation; and

   (ii) a relative amount of 2,6-coupled sialic acid of at least 40% of the total amount of sialic acids.

2. The recombinant FSH preparation according to claim 1, wherein the glycosylation pattern further

   (iii) comprises a relative amount of glycans carrying fucose of at least 30% of the total amount of glycans attached to FSH in the preparation; and/or

   (iv) is a diverse glycosylation pattern, wherein the FSH in the preparation comprises at least 35 different glycan structures, wherein each one of these different glycan structures has a relative amount of at least 0.1% of the total amount of glycan structures of the FSH in the preparation.

3. A recombinant FSH preparation obtainable by production in the human cell line GT-5s or a cell line derived therefrom or a cell line homologous thereto.

4. The recombinant FSH preparation according to any one of claims 1 to 3, wherein the recombinant FSH in the preparation has a glycosylation pattern comprising the following characteristics:

   (i) a relative amount of glycans carrying bisecting N-acetylglucosamine (bisGlcNAc) of at least 25 % of the total amount of glycans attached to FSH in the preparation; and

   (ii) a relative amount of 2,6-coupled sialic acid of at least 50% of the total amount of sialic acids.

5. The recombinant FSH preparation according to claim 4, wherein the recombinant FSH in the preparation has a glycosylation pattern comprising the following characteristics:

   (i) a relative amount of glycans carrying bisecting N-acetylglucosamine (bisGlcNAc) of at least 30 % of the total amount of glycans attached to FSH in the preparation; and

   (ii) a relative amount of 2,6-coupled sialic acid of at least 53% of the total amount of sialic acids.

6. The recombinant FSH preparation according to any one of claim 1 to 5, 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) the glycosylation pattern comprises a relative amount of at least tetraantennary glycans of at least 18%;

(c) a Z-number of at least 200;

(d) it is human recombinant FSH;

(e) it is produced by a human cell line or human cells; and/or

(f) the FSH in the preparation comprises an alpha subunit having the amino acid sequence of SEQ ID NO: 1 and a beta subunit having the amino acid sequence of SEQ ID NO: 2.

7. The recombinant FSH preparation according to any one of claims 1 to 6, 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 25% to about 50%;

(ii) a relative amount of at least tetraantennary glycans of at least 16%;

(iii) a relative amount of glycans carrying fucose of at least 35%;

(iv) a relative amount of 2,6-coupled sialic acid of at least 53%;

(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%;

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

(ix) a relative amount of glycans carrying a sulfate group of at least 3%;

(x) it comprises at least 45 different glycan structures, wherein each one of the different glycan structures has a relative amount of at least 0.05 % of the total amount of glycan structures of the FSH in the preparation;

(xi) it comprises at least 35 different glycan structures, wherein each one of the different glycan structures has a relative amount of at least 0.1 % of the total amount of glycan structures of the FSH in the preparation;

(xiii) it comprises at least 20 different glycan structures, wherein each one of the different glycan structures has a relative amount of at least 0.5 % of the total amount of glycan structures of the FSH in the preparation; and/or

(xiv) it comprises at least 40 % more different glycan structures than FSH obtained from CHO cells in a corresponding preparation, wherein each one of the different glycan structures has a relative amount of at least 0.05 % of the total amount of glycan structures of the FSH in the respective preparation.

8. The recombinant FSH preparation according to any one of claims 1 to 7, wherein the glycosylation pattern comprises the following characteristics:

(i) a relative amount of glycans carrying bisecting N-acetylglucosamine (bisGlcNAc) in the range of from about 25% to about 50%;

(ii) a relative amount of at least tetraantennary glycans of at least 16%;

(iii) a relative amount of glycans carrying fucose of at least 35%;

(iv) a relative amount of 2,6-coupled sialic acid in the range of from about 53% to about 99%; and

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

9. The recombinant FSH preparation according to any one of claims 1 to 8, 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.

10. The recombinant FSH preparation according to any one of claims 1 to 9, wherein said FSH is capable of stimulating or co-stimulating germ cell maturation by a biological process which is independent of cAMP signaling.

11. The recombinant FSH preparation according to any one of claims 1 to 10, 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 $5*10^4$ to about $1*10^5$ 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 $5*10^4$ to about $1*10^5$ 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 $5*10^4$ to about $1*10^5$ 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.

12. The recombinant FSH preparation according to any one of claims 1 to 10, wherein the FSH is capable of inducing follicle growth in a female human being after administration of a single dose, wherein the single dose preferably comprises 25 to 500 IU FSH and preferably is administered parenteral, in particular by subcutaneous injection.

13. A pharmaceutical composition comprising the recombinant FSH preparation according to any one of claims 1 to 12.

14. The pharmaceutical composition according to claim 13 being in the form of a single unit dose comprising about 50 IU to about 400 IU FSH.

15. Use of the recombinant FSH preparation according to any one of claims 1 to 12 or the pharmaceutical composition according to claim 13 or 14 for the manufacture of a medicament for use in infertility treatment.

16. The use of the recombinant FSH preparation according to claim 15, 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.

17. Use of the recombinant FSH preparation according to any one of claims 1 to 12 or the pharmaceutical composition according to claim 13 or 14 for the manufacture of a medicament for inducing and/or stimulating the secretion of sex steroids also independent of cAMP.

18. Use of the recombinant FSH preparation according to any one of claims 1 to 12 or the pharmaceutical composition according to claim 13 or 14 for the manufacture of a medicament for stimulating or co-stimulating germ cell maturation by a biological process which is independent of cAMP signaling.

19. Use of the recombinant FSH preparation according to any one of claims 1 to 12 or the pharmaceutical composition according to claim 13 or 14 for the manufacture of a medicament for inducing and/or stimulating the secretion of sex steroids at FSH concentrations at which no significant cAMP release is induced.

20. The use according to any one of claims 15 to 19, wherein the medicament is for use in infertility treatment, wherein the infertility treatment includes assisted reproductive technologies, ovulation induction, in-vitro fertilization, for example in-vitro fertilization with intracytoplasmic sperm injection, gamete intrafallopian transfer, intrauterine insemination, treatment of anovulatory disorder in women, treatment of severe hormone deficiency disorder for egg maturation in woman, treatment of sperm production deficiencies in men, and/or the enablement or improvement of germ cell maturation such as folliculogenesis and spermatogenesis, in particular follicle maturation in women, for example during in vitro fertilization stimulation protocols and/or for anovulatory disorder treatment.

21. The use according to any one of claims 15 to 20, wherein the recombinant FSH preparation or the pharmaceutical composition has one or more of the following characteristics:

    (i) it is capable of inducing follicular growth and/or ovular maturation after the administration of only a single dose; and/or

    (ii) it has a lower circulation half-life in one or more of humans, cynomolgus monkeys, rats and/or mice than FSH preparations obtained from human urine and/or expressed in CHO cells; and/or

    (iii) it has a lower bioavailability in one or more of humans, cynomolgus monkeys, rats and/or mice than FSH preparations obtained from human urine and/or expressed in CHO cells; and/or

    (iv) it has a therapeutic efficacy in one or more of humans, cynomolgus monkeys, rats and/or mice which is similar to or higher than that of FSH preparations obtained from human urine and/or expressed in CHO cells.

22. Use of the recombinant FSH preparation according to any one of claims 1 to 12 or the pharmaceutical composition according to claim 13 or 14 for the manufacture of a medicament for the induction of follicle growth and/or ovular maturation.