Description

This invention relates to the use of recombinant DNA techniques to produce human follicle stimulating hormone (FSH), which is a heteropolymeric protein hormone, and its beta subunit.

Various polypeptide chains have been expressed, via recombinant DNA technology, in host cells such as bacteria, yeast, and cultured mammalian cells. Fiddes, J.C. and Goodman, H.M. (1979) Nature Vol. 281, p.351-356 and Fiddes, J.C. and Goodman, H.M. (1980) Nature Vol. 286, p.684-687 describe the cloning of, respectively, the alpha and beta subunits of human choriogonadotropin (hCG).

US-A-4383036 (Kaname) describes a process for producing hCG in which human lymphoblastoid cells are implanted into a laboratory animal, harvested from the animal, and cultured in vitro; accumulated hCG is then harvested from the culture.

According to a first aspect of the invention, there is provided a process for the preparation of biologically active, heterodimeric human follicle stimulating hormone (FSH), having an alpha subunit and a beta subunit, wherein the beta subunit has the sequence:

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Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu,
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the process comprising commonly synthesising each of the subunits in a eukaryotic cell transfected with one or more expression vectors comprising heterologous DNA encoding each of the subunits.

The invention relates to the production of biologically active heterodimeric FSH from a single culture of transformed cells. The production of both subunits of FSH in the same cell eliminates the necessity of recombining subunits from separate cultures to assemble an active heterodimeric molecule. The system also allows production of FSH, in a single culture, which undergoes, in the culture, post-translational modification, eg glycosylation and proteolytic processing, for activity or stability.

According to a second aspect of the invention, there is provided the immature beta subunit of human FSH, which has the sequence:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys Asn Ser Cys Glu
Leu Thr Asn Ile Thr Ile Ala Ile Glu Lys Glu
Glu Cys Arg Phe Cys Ile Ser Ile Asn Thr Thr
Trp Cys Ala Gly Tyr Cys Tyr Thr Arg Asp Leu
Val Tyr Lys Asp Pro Ala Arg Pro Lys Ile Gln
Lys Thr Cys Thr Phe Lys Glu Leu Val Tyr Glu
Thr Val Arg Val Pro Gly Cys Ala His His Ala
Asp Ser Leu Tyr Thr Tyr Pro Val Ala Thr Gln
Cys His Cys Gly Lys Cys Asp Ser Asp Ser Thr
Asp Cys Thr Val Arg Gly Leu Gly Pro Ser Tyr
Cys Ser Phe Gly Glu Met Lys Glu.

According to a third aspect of the invention, there is provided recombinant DNA, which may be comprised in a vector, preferably an expression vector capable of transfecting a eukaryotic cell, the recombinant DNA comprising heterologous DNA encoding the beta subunit of human FSH, wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

The expression vector may also comprise heterologous DNA encoding the alpha subunit of human FSH or may form a set of expression vectors together with a second expression vector comprising such heterologous DNA.

The term "expression vector" refers to a cloning vector which includes heterologous (to the vector) DNA under the control of sequences which permit expression in a host cell. Such vectors include replicating viruses, plasmids, and phages. Preferred vectors are those containing at least the 69% transforming region, and most preferably all, of the bovine papilloma virus genome.

Expression vectors within the scope of the invention include:

an expression vector capable of transfecting a eukaryotic cell, the vector comprising heterologous DNA encoding the subunits of biologically active, heterodimeric FSH; and

a pair of expression vectors, each vector being capable of transfecting a eukaryotic cell, and each vector comprising heterologous DNA encoding a respective subunit of FSH, wherein both the subunits of FSH are encoded by heterologous DNA in the vectors.

A preferred vector has been deposited at the NRRL under Accession No. B-15923.

In preferred embodiments, each expression vector is autonomously replicating, ie, not integrated into the chromosome of the host cell. The use of autonomously replicating expression vectors prevents undesirable influence of the desired coding regions by control sequences in the host chromosome.

According to a fourth aspect of the invention, there is provided a cell transfected with an expression vector, the cell being capable of producing biologically active FSH, the alpha and beta subunits of FSH being encoded by heterologous DNA in the expression vector(s), wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

It is preferred that the expression vector, or a second expression vector with which the cell is transfected, encode the alpha subunit of FSH and that the cell be capable of producing biologically active heterodimeric human FSH. The cell may be a mammalian cell; in some embodiments a mouse cell is used.

According to a fifth aspect of the invention, there is provided a process for preparing the beta subunit of human FSH comprising culturing host cells transfected with an expression vector comprising heterologous DNA encoding the beta subunit of FSH, wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

According to a sixth aspect of the invention, there is provided a process for preparing biologically active, heterodimeric human FSH comprising culturing mammalian cells transfected with one or more expression vector(s) comprising heterologous DNA encoding the alpha and beta subunits of FSH, wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

According to a seventh aspect of the invention, there is provided a process for preparing a pharmaceutical composition, the process comprising preparing recombinant FSH as described above and admixing the recombinant FSH so prepared with a carrier therefor.

For a better understanding of the invention, and to show how it may be put into effect, preferred embodiments will now be described with reference to the accompanying drawings, in which:

FIGURE 1 is a diagrammatic illustration of the construction of the plasmid pRF375;

FIGURE 2 is a partial restriction map of the lambda clone 15B and the beta FSH-containing 6.8 kb EcoRI-BamHI fragment that is inserted into pBR322;

FIGURE 3 is a partial restriction map of the beta FSH coding region and the BamHI fragment that is inserted into a BPV based expression vector; and

FIGURE 4 is a diagrammatic illustration of the construction of the BPV-containing plasmid CL28FSH2.8BPV, encoding the beta subunit of FSH.

Structure

The cloning vectors of the invention have the general structure referred to above. Preferred vectors have the structures shown in the Figures, and are described in more detail below.

Construction of Cloning Vectors

Isolation of cDNA Clones Encoding the Common Alpha Subunit

In order to produce the heterodimeric FSH of the invention, the alpha subunit of human chorionic gonadotropin (hCG) first is isolated; the alpha subunit is common to the fertility hormones hCG, luteinizing hormone (LH), and FSH. Fiddes and Goodman (J. Mol. Appl. Gen. 1(1), 3-18 (1981)) discloses the cloning and sequence of the common alpha subunit.

All of the techniques used herein are described in detail in Maniatis et al (1982) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory), hereby incorporated by reference.

RNA is extracted from placental tissue by the following method. Homogenization of the tissue is carried out in a 1:1 mixture of phenol:100mM Na-acetate (pH 5.5) containing 1mM EDTA, that has been warmed to 60°C for 20 min. After cooling on ice for 10 min, the phases are separated by centrifugation. The hot phenol extraction is repeated twice more followed by two extractions with chloroform.

RNA is precipitated from the final aqueous phase by the addition of 2.5 volumes of ethanol.

In order to enrich for poly A+ mRNA, placental RNA is passed over oligo (dT)-cellulose in 0.5M NaCl buffered with 10mM Tris-HCl, pH 7.5, and washed with the same solution. Poly A+ mRNA is eluted with 10mM Tris-HCl (pH 7.5), 1mM EDTA, 0.05% SDS and precipitated twice with ethanol. Typical initial yields are 1.5-2.0 mg of total RNA per g of tissue, of which about 2% is poly A+ mRNA.

Placental cDNA libraries are constructed by reverse transcription of placental mRNA, second strand synthesis using E. coli DNA polymerase I (large fragment), treatment with SI nuclease, and homopolymer

tailing (dC) with terminal deoxynucleotidyl transferase; all such procedures are by conventional techniques.

In a typical preparation, 20-30% conversion of mRNA to single strand (ss) cDNA; 70% resistance to digestion with nuclease SI after second strand synthesis; and dC "tails" of ten to twenty-five bases in length, are obtained. These cDNA molecules are then annealed to DNA fragments of the plasmid pBR 322, which has been digested with PstI, and to which dG "tails" have been added. These recombinant plasmids are then used to transform E. coli cells to generate a cDNA library (transformed cells are selected on the basis of tetracycline resistance).

In order to identify the human alpha hCG clone, a 219 bp fragment of a mouse alpha thyroid stimulating hormone (TSH) clone is used as a hybridization probe. This probe has 77% sequence homology with the human clone. It is radioactively labeled by nick translation and hybridized to the cDNA library under conditions that take into account the extent of homology. Strongly hybridizing clones are analyzed by restriction mapping and clones containing the complete coding sequence of alpha hCG are verified by DNA sequencing.

Construction of Plasmid pRF375

Referring to Fig. 1, the plasmid CL28 (identical to plasmid JYMMT(E); Hamer et al. (1983) J. Mol. Applied Gen. 1, 273-288), containing the murine metallothionein promoter, SV40 DNA, and pBR322 sequences, is cut with the restriction endonuclease BglII. At this site is inserted the cDNA clone of alpha hCG, containing untranslated regions of about 10 and 220 bp at its 5' and 3' ends, respectively. This clone has been genetically engineered by the addition of synthetic BamHI linkers at its termini.

The resulting plasmid pRF302 is digested with restriction enzymes BamHI and SalI to release the SV40 DNA sequence.

Plasmid pB2-2, which contains the entire BPV genome, and some pBR322 sequences, is digested with BamHI and SalI to yield the BPV genome with BamHI/SalI ends; this fragement is ligated into pRF302 containing the metallothionein-hCG sequences.

Following transformation of E. coli, plasmid CRF375 is identified and isolated. It encodes the common alpha subunit under the control of the mouse metallothionein promoter.

Isolation of the Human beta FSH Gene

A human genomic library in phage lambda (Lawn et al., 1978, Cell 15, p. 1157-1174) is screened using "guessed" long probes. The idea behind such probes, set forth in Jaye et al. (1983) Nucleic Acids Research 11(8), 2325, is that if the amino acid sequence of a desired protein is at least partially known, a long probe can be constructed in which educated guesses are made as to the triplet encoding any amino acid which can be encoded by more than one, and not more than four, different triplets. Any correct guesses increase the amount of homology, and improve the specificity, of the results.

To isolate desired regions of DNA, two labeled 45-mer probes are used: TB36, homologous with amino acids 56-70 of human beta FSH; and TB21, homologous with amino acids 73-87. These probes have the following nucleotide compositions (corresponding amino acids are also given):

```
TB36:                Val-Tyr-Glu-Thr-Val-Lys-Val-
(AA56-70)      3'    CAC ATG CTC TGG CAC TCT CAC

                     Pro-Gly-Cys-Ala-His-His-Ala-Asp
                     GGT CCG ACG CGG GTG GTG CGA CTG 5'

TB21:                Tyr-Thr-Tyr-Pro-Val-Ala-Thr-
(AA73-87)      3'    ATG TGC ATG GGT CAC CGA TGT

                     Glu-Cys-His-Cys-Gly-Lys-Cys-Asp
                     CTC ACA GTG ACG CCG TTT ACG CTG 5'
```

The above probes are used to screen the human genomic library as follows. TB21 is labeled with ^{32}P and used to screen approximately 5 x 10^5 lambda plaques on duplicate filters by the in situ plaque hybridization technique of Benton and Davis (1977) Science 196, 180-182. The prehybridization solution is maintained at 55°C for several hours and has the following composition: 0.75M NaCl; 0.15M Tris/HCl, pH 8.0; 10mM EDTA; 5 x Denhardt's Solution; 0.1% sodium pyrophosphate; o.1% SDS; 100 microgram/ml E. coli t-RNA. The hybridization solution has the same composition except that it is maintained overnight at 45°C, and contains labeled probe in a concentration of about 0.5 x 10^6 cpm/ml. After hybridization, the filters are washed four times in 1 X SSC (= 0.15M NaCl, 0.015M Na_3-citrate) and exposed to x-ray film.

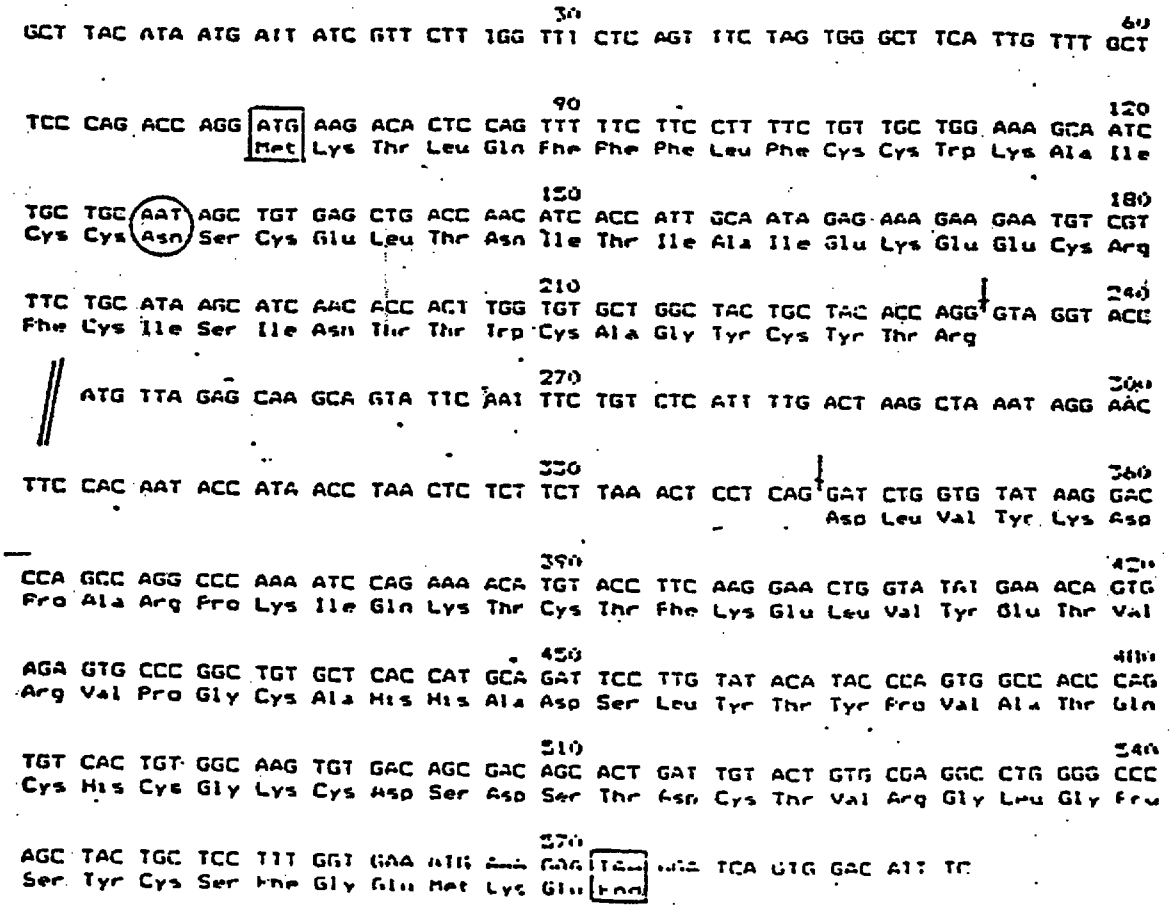
This screening procedure yields 50 plaques which hybridize to TB21 on both sets of filters. These 50 individual plaques are picked and combined into 10 culture pools containing 5 plaques each. The 10 cultures are grown and DNA is isolated from 50ml phage lysates. This DNA is then digested with EcoRI and fractionated on two identical 1% agarose gels, after which it is transferred to nitrocellulose paper according to the method of Southern (1975) J. Mol. Biol. 98, 503-517.

The DNAs on the two filters are hybridized to ^{32}P labeled TB21 and TB36, respectively. Individual plaques from the pool containing a restriction fragment which strongly hybridizes to both probes are then screened according to the above procedure, except that the DNAs are digested with EcoRI, BamHI, and EcoRI plus BamHI. In this way the 6.8kb EcoRI-BamHI fragment containing human beta FSH is isolated.

A partial restriction map of clone 15B, containing the 6.8kb EcoRI-BamHI fragment, is shown in Fig. 2. In order to locate the position of the beta FSH sequences within the clone, the 6.8 kb EcoRI-BamHI fragment of clone 15B is subcloned into pBR322 to yield plasmid p15B6.8R/B (Fig. 2). p15B6.8R/B is then digested with various restriction enzymes and the products are fractionated by agarose gel electrophoresis using conventional methods. The DNA is blotted to nitrocellulose paper and hybridized to fragments of a porcine beta FSH cDNA clone labeled with ^{32}P by nick translation.

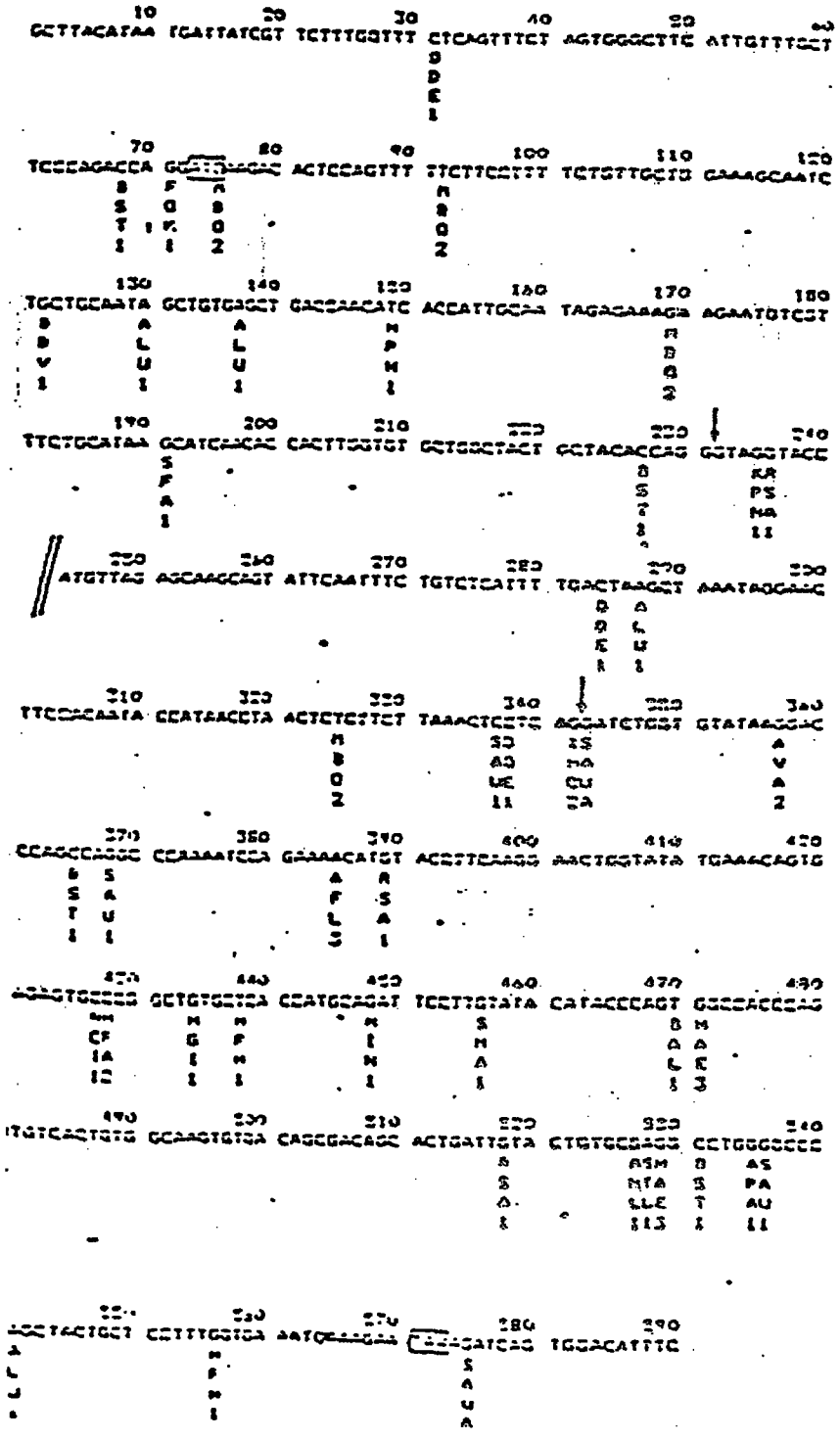
As shown in Fig. 2, the porcine beta FSH probe hybridizes to only two fragments of the human DNA, namely a 1.1kb HindIII-KpnI and a 1.4kb PstI fragment. Partial DNA sequencing of these two fragments shows that this DNA indeed codes for human beta FSH and that the entire coding region for beta FSH is contained in these two fragments.

As shown by the restriction map of Fig. 3, the beta FSH coding sequence is interrupted by an intervening sequence of approximately 1.6kb between amino acids 35 and 36 of mature beta FSH. The nucleotide sequence of the entire human beta FSH coding region and some of the flanking and intervening sequences are given below. The amino acid sequence deduced from the nucleotide sequence is given for the coding region.



Still referring to the above sequence, there is a box around the ATG initiation codon of the 18 amino acid signal peptide, which is cleaved post-translationally. The mature protein begins with the amino acid Asn encoded by the circled triplet AAT. The exon-intron boundaries are marked by arrows; they are flanked by the concensus sequence GT for the splice donor and AG for the splice acceptor site. There is a box around the stop codon TAA, the end of the coding region.

Below is a reproduction of the above sequence not broken into triplets, showing restriction sites; the ATG beginning and the TAA ending the coding region are boxed and the exon-intron junctions are marked by arrows.



Insertion of the Beta FSH DNA into a BPV-Based Expression Vector

Referring to Fig. 3, a synthetic BamHI linker is inserted at the DdeI site of p15B6.8R/B, which is located 42 nucleotides 5' of the ATG initiation codon. Referring to Fig. 4, p15B6.8R/B is digested with DdeI and treated with E. coli DNA polymerase (Klenow), after which it is ligated to synthetic BamHI linkers and digested with BamHI. The 295 bp fragment containing the first exon of FSH is isolated and cloned into the BamHI site of pBR322. The resulting plasmid pBR295Bam is digested with KpnI plus EcoRI plus AvaI and ligated to p15B6.8R/B which has been digested with KpnI plus EcoRI plus SmaI. The ligation mix is then

used to transform E. coli, and the plasmid pBR2.8Bam containing the human beta FSH DNA sequence as a BamHI fragment is identified from among the transformants by restriction mapping.

As shown in Fig. 4, expression plasmid CL28FSH2.8BPV is prepared according to the same method used to prepare pRF375 (Fig. 1), except that the 2.8 kb BamHI fragment of pBR2.8Bam is used in place of the alpha hCG cDNA clone. Plasmid CL28FSH2.8BPV can be used to transform mammalian host cells—using conventional methods, and human beta FSH can be isolated and purified.

Transfection of Mouse Cells

To produce heterodimeric FSH using a mixed transfection, five μg of each BPV plasmid, i.e., pRF375 (alpha subunit) and CL28FSH2.8BPV (beta FSH), are mixed and added to 0.5 ml of a 250 mM $CaCl_2$ solution containing 10 μg of salmon sperm DNA as carrier. This mixture is bubbled into 0.5 ml of 280 mM NaCl, 50 mM Hepes and 1.5 mM sodium phosphate. The calcium phosphate precipitate is allowed to form for 30-40 minutes at room temperature.

24 hours prior to transfection, $5x10^5$ cells of mouse C127 cells (available from Dr. Dean Hamer, National Cancer Institute, NIH, Bethesda, MD, USA) are placed in a 100 mm dish or T-75 flask. Immediately before adding the exogenous DNA, the cells are fed with fresh medium (Dulbecco's Modified Medium, 10% foetal calf serum). One ml of calcium phosphate precipitate is added to each dish (10 ml), and the cells are incubated for 6-8 hours at 37°C.

The medium is aspirated and replaced with 5 ml of 20% glycerol in phosphate buffered saline, pH 7.0 (PBS) for 2 minutes at room temperature. The cells are washed with PBS, fed with 10ml of medium, and incubated at 37°C. After 20-24 hours, the medium is changed and subsequent refeeding of the cells is carried out every 3-4 days. Individual clones are grown in T-25 flasks. After 7-21 days, cell clones can be transferred to larger flasks for analysis.

Deposits

The following, described above, has been deposited in the Agricultural Research Culture Collection (NRRL), Peoria, IL 61604:

CL28FSH2.8BPV in E. coli, NRRL B-15923.

The following, described above, has been deposited in the American Type Culture Collection, Rockville, MD:

pRF375 in C127 cells, ATCC CRL 8401;

The transformed cell lines of the invention are used to produce the beta subunit of FSH or glycosylated, biologically active heterodimeric human FSH, which is purified from the cells and/or their culture media using conventional purification techniques. FSH has a number of well-known medical uses associated with human fertility. For example, FSH can be used, alone or in conjunction with hCG or LH, to induce ovulation, or superovulation for in vitro fertilization.

In addition, heterodimeric FSH, or the beta subunit alone, can be used in diagnostic tests for fertility and pituitary functions.

FSH produced by recombinant cells has the advantage, compared to FSH obtained from natural sources, of being free from contamination by other human proteins, in particular other fertility hormones.

Other embodiments are within the following claims. For example, rather than producing heterodimeric FSH by culturing cells containing two separate expression vectors, one encoding the alpha subunit and the other encoding the beta subunit, DNA encoding both subunits can be included in the same expression vector.

Claims

Claims for the following Contracting States : BE, FR, DE, IT, LU, NL, SE, CH, LI, GB

1. A process for the preparation of biologically active, heterodimeric human follicle stimulating hormone (FSH), having an alpha subunit and a beta subunit, wherein the mature beta subunit has the sequence:

used to transform E. coli, and the plasmid pBR2.8Bam containing the human beta FSH DNA sequence as a BamHI fragment is identified from among the transformants by restriction mapping.

As shown in Fig. 4, expression plasmid CL28FSH2.8BPV is prepared according to the same method used to prepare pRF375 (Fig. 1), except that the 2.8 kb BamHI fragment of pBR2.8Bam is used in place of the alpha hCG cDNA clone. Plasmid CL28FSH2.8BPV can be used to transform mammalian host cells using conventional methods, and human beta FSH can be isolated and purified.

Transfection of Mouse Cells

To produce heterodimeric FSH using a mixed transfection, five µg of each BPV plasmid, i.e., pRF375 (alpha subunit) and CL28FSH2.8BPV (beta FSH), are mixed and added to 0.5 ml of a 250 mM $CaCl_2$ solution containing 10 µg of salmon sperm DNA as carrier. This mixture is bubbled into 0.5 ml of 280 mM NaCl, 50 mM Hepes and 1.5 mM sodium phosphate. The calcium phosphate precipitate is allowed to form for 30-40 minutes at room temperature.

24 hours prior to transfection, $5x10^5$ cells of mouse C127 cells (available from Dr. Dean Hamer, National Cancer Institute, NIH, Bethesda, MD, USA) are placed in a 100 mm dish or T-75 flask. Immediately before adding the exogenous DNA, the cells are fed with fresh medium (Dulbecco's Modified Medium, 10% foetal calf serum). One ml of calcium phosphate precipitate is added to each dish (10 ml), and the cells are incubated for 6-8 hours at 37°C.

The medium is aspirated and replaced with 5 ml of 20% glycerol in phosphate buffered saline, pH 7.0 (PBS) for 2 minutes at room temperature. The cells are washed with PBS, fed with 10ml of medium, and incubated at 37°C. After 20-24 hours, the medium is changed and subsequent refeeding of the cells is carried out every 3-4 days. Individual clones are grown in T-25 flasks. After 7-21 days, cell clones can be transferred to larger flasks for analysis.

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The transformed cell lines of the invention are used to produce the beta subunit of FSH or glycosylated, biologically active heterodimeric human FSH, which is purified from the cells and/or their culture media using conventional purification techniques. FSH has a number of well-known medical uses associated with human fertility. For example, FSH can be used, alone or in conjunction with hCG or LH, to induce ovulation, or superovulation for in vitro fertilization.

In addition, heterodimeric FSH, or the beta subunit alone, can be used in diagnostic tests for fertility and pituitary functions.

FSH produced by recombinant cells has the advantage, compared to FSH obtained from natural sources, of being free from contamination by other human proteins, in particular other fertility hormones.

Other embodiments are within the following claims. For example, rather than producing heterodimeric FSH by culturing cells containing two separate expression vectors, one encoding the alpha subunit and the other encoding the beta subunit, DNA encoding both subunits can be included in the same expression vector.

Claims

Claims for the following Contracting States : BE, FR, DE, IT, LU, NL, SE, CH, LI, GB

1. A process for the preparation of biologically active, heterodimeric human follicle stimulating hormone (FSH), having an alpha subunit and a beta subunit, wherein the mature beta subunit has the sequence:

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu,

the process comprising commonly synthesising each of the subunits in a eukaryotic cell transfected with one or more expression vectors comprising heterologous DNA encoding each of the subunits.

2. A process as claimed in claim 1, wherein the cell is transfected with more than one expression vector, each vector comprising heterologous DNA encoding a respective subunit.

3. A process as claimed in claim 1, wherein the cell is transfected with a single expression vector comprising heterologous DNA encoding each subunit.

4. The immature beta subunit of human FSH, which has the sequence:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys Asn Ser Cys Glu
Leu Thr Asn Ile Thr Ile Ala Ile Glu Lys Glu
Glu Cys Arg Phe Cys Ile Ser Ile Asn Thr Thr
Trp Cys Ala Gly Tyr Cys Tyr Thr Arg Asp Leu
Val Tyr Lys Asp Pro Ala Arg Pro Lys Ile Gln
Lys Thr Cys Thr Phe Lys Glu Leu Val Tyr Glu
Thr Val Arg Val Pro Gly Cys Ala His His Ala
Asp Ser Leu Tyr Thr Tyr Pro Val Ala Thr Gln
Cys His Cys Gly Lys Cys Asp Ser Asp Ser Thr
Asp Cys Thr Val Arg Gly Leu Gly Pro Ser Tyr
Cys Ser Phe Gly Glu Met Lys Glu.

5. Recombinant DNA encoding the beta subunit of human FSH, wherein the mature beta subunit has the sequence:

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

6. Recombinant DNA as claimed in claim 5, which is comprised in a vector.

7. Recombinant DNA as claimed in claim 6 wherein the vector is an expression vector capable of transforming a eukaryotic cell.

8. A vector having NRRL Accession No. B-15923.

9. Recombinant DNA as claimed in claim 7, which additionally encodes the following leader sequence of the beta subunit:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

10. An expression vector capable of transfecting a eukaryotic cell, the vector comprising heterologous DNA encoding the alpha and beta subunits of biologically active, heterodimeric FSH, wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

* C L M S *

11. A pair of expression vectors comprising a first expression vector which is as claimed in claim 7, 8 or 9 and a second expression vector comprising heterologous DNA encoding the alpha subunit of human FSH.

12. A pair of expression vectors, each vector being capable of transfecting a eukaryotic cell, and each vector comprising heterologous DNA encoding a respective subunit of FSH, wherein both the subunits of FSH are encoded by heterologous DNA in the vectors, wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

13. A eukaryotic cell transfected with one or more expression vectors, the cell being capable of producing biologically active FSH, the alpha and beta subunits of FSH being encoded by heterologous DNA in the expression vector(s), wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

14. A cell as claimed in claim 13, wherein the expression vector, or one of the expression vectors, additionally codes for the following leader sequence of the beta subunit:

* C L M S *

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

15. A cell as claimed in claim 13 or 14, which is a mammalian cell.

16. A cell as claimed in claim 13, 14 or 15, wherein the vector or each of the vectors comprises at least the 69% transforming region of the bovine papilloma virus genome.

17. A cell as claimed in any one of claims 13 to 16, which is a mouse cell.

18. A cell as claimed in any one of claims 13 to 17, wherein the or each vector comprises a plasmid.

19. A process for preparing the beta subunit of human FSH comprising culturing host cells transfected with an expression vector comprising heterologous DNA encoding the beta subunit of FSH, wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

20. A process for the preparation of the immature beta subunit of human FSH, the process comprising culturing host cells transfected with an expression vector comprising heterologous DNA encoding the immature beta subunit of FSH, wherein the immature beta subunit has the sequence of the mature beta subunit set forth in claim 19 and additionally has the leader sequence:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

21. A process as claimed in claim 1, 19 or 20, wherein the host cells are as claimed in any one of claims 13 to 18.

22. A process as claimed in claim 1, the process comprising recovering the FSH from a culture of the cells.

23. A process for preparing a pharmaceutical composition, the process comprising preparing recombinant FSH by a process as claimed in claim 1 or 22 and admixing the recombinant FSH so prepared with a

* C L M S *

carrier therefor.

Claims for the following Contracting State : AT

1. A process for the preparation of biologically active, heterodimeric human follicle stimulating hormone (FSH), having an alpha subunit and a beta subunit, wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu

the process comprising commonly synthesising each of the subunits in a eukaryotic cell transfected with one or more expression vectors comprising heterologous DNA encoding each of the subunits.

2. A process for preparing the beta subunit of human FSH comprising culturing host cells transfected with an expression vector comprising heterologous DNA encoding the beta subunit of FSH, wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr

Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

3. A process for preparing the immature beta subunit of human FSH, the process comprising culturing host cells transfected with an expression vector comprising heterologous DNA encoding the immature beta subunit of FSH, wherein the immature beta subunit has the sequence of the mature beta subunit

* C L M S *

set forth in claim 2 and additionally has the leader sequence:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

4. A process as claimed in claim 1, the beta subunit of the FSH being encoded by heterologous DNA of a first expression vector and the alpha subunit of the FSH being encoded by heterologous DNA of a second expression vector transfected into the host cell.

5. A process as claimed in claim 1, both subunits of the FSH being encoded by heterologous DNA of a single expression vector.

6. A process for the preparation of DNA encoding the beta subunit of human FSH, wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu,

the process comprising DNA ligation and/or synthesis.

7. A process as claimed in claim 6, wherein the recombinant DNA is in the form of a vector.

8. A process as claimed in claim 7, wherein the vector is an expression vector capable of transforming a eukaryotic cell.

9. A process as claimed in claim 7, wherein the vector has NRRL Accession No. B-15923.

10. A process as claimed in claim 7, wherein the recombinant DNA additionally encodes the leader sequence of the beta subunit:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

11. A process for preparing a pair of expression vectors comprising preparing a first expression vector comprising heterologous DNA encoding the beta subunit of human FSH by DNA ligation and/or synthesis, wherein the mature beta subunit has the sequence:

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu

and preparing a second expression vector comprising heterologous DNA encoding the alpha subunit of human FSH by DNA ligation and/or synthesis.

12. A process for preparing an expression vector, the vector comprising heterologous DNA encoding the alpha and beta subunits of biologically active, heterodimeric FSH, the process comprising DNA ligation and/or synthesis, wherein the mature beta subunit has the sequence:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp

Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

13. A process for preparing a transfected eukaryotic cell, the process comprising transfecting the cell with one or more expression vectors, the transfected cell being capable of producing the biologically active, heterodimeric FSH, the alpha and beta subunits of FSH being encoded by heterologous DNA in the expression vector(s), wherein the mature beta subunit has the sequence:

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

14. A process as claimed in claim 13, wherein the expression vector additionally encodes the following leader sequence of the beta subunit:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

15. A process as claimed in claim 13 or 14, wherein the cell which is a mammalian cell.

16. A process as claimed in claim 13, 14 or 15, wherein the vector or each of the vectors comprises at least the 69% transforming region of the bovine papilloma virus genome.

17. A process as claimed in any one of claims 13 to 16, which is a mouse cell.

18. A process as claimed in any one of claims 13 to 17, wherein the or each vector comprises a plasmid.

19. A process as claimed in claim 1, the process comprising recovering FSH from a culture of the cells.

20. A process for preparing a pharmaceutical composition, the process comprising preparing recombinant FSH by a process as claimed in claim 1 and admixing the recombinant FSH so prepared with a carrier therefor.

Patentansprüche
Patentansprüche für folgende Vertragsstaaten : BE, FR, DE, IT, LU, NL, SE, CH, LI, GB

1. Verfahren zur Herstellung von biologisch aktivem, heterodimeren, menschlichen follikelstimulierenden Hormon (FSH), welches eine Alpha-Untereinheit und eine Beta-Untereinheit besitzt, wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu,

und das Verfahren die gemeinsame Synthese jeder der Untereinheiten in einer eukaryotischen Zelle umfaßt, die mit einem oder mehr Expressionsvektoren transfektiert ist, der heterologe DNA zur Codierung jeder der Untereinheiten beinhaltet.

2. Verfahren nach Anspruch 1, wobei die Zelle mit mehr als einem Expressionsvektor transfektiert ist und jeder Vektor heterologe DNA, die eine bestimmte Untereinheit codiert, umfaßt.

3. Verfahren nach Anspruch 1, wobei die Zelle mit einem einzigen Expressionsvektor transfektiert ist, der heterologe DNA, die jede Untereinheit codiert, umfaßt.

4. Unreife Beta-Untereinheit von menschlichem FSH, die folgende Sequenz aufweist:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys Asn Ser Cys Glu
Leu Thr Asn Ile Thr Ile Ala Ile Glu Lys Glu
Glu Cys Arg Phe Cys Ile Ser Ile Asn Thr Thr
Trp Cys Ala Gly Tyr Cys Tyr Thr Arg Asp Leu
Val Tyr Lys Asp Pro Ala Arg Pro Lys Ile Gln
Lys Thr Cys Thr Phe Lys Glu Leu Val Tyr Glu
Thr Val Arg Val Pro Gly Cys Ala His His Ala
Asp Ser Leu Tyr Thr Tyr Pro Val Ala Thr Gln
Cys His Cys Gly Lys Cys Asp Ser Asp Ser Thr
Asp Cys Thr Val Arg Gly Leu Gly Pro Ser Tyr
Cys Ser Phe Gly Glu Met Lys Glu.

5. Rekombinante DNA, welche die Beta-Untereinheit von menschlichem FSH codiert, wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

6. Rekombinante DNA nach Anspruch 5, die in einem Vektor enthalten ist.

7. Rekombinante DNA nach Anspruch 6, wobei der Vektor ein Expressionsvektor ist, der eine eukaryotische Zelle transformieren kann.

8. Vektor mit der NRRL-Zugriffsnummer B-15923.

9. Rekombinante DNA nach Anspruch 7, die zusätzlich die folgende Leader-Sequenz der Beta-Untereinheit codiert:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

10. Expressionsvektor, der eine eukaryotische Zelle transfektieren kann, wobei der Vektor heterologe DNA umfaßt, welche die Alpha- und Beta-Untereinheiten des biologisch aktiven, heterodimeren FSH codiert, wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

* C L M S *

11. Expressionsvektorenpaar, das einen ersten Expressionsvektor nach Anspruch 7, 8 oder 9 und einen zweiten Expressionsvektor umfaßt, der heterologe DNA, welche die Alpha-Untereinheit von menschlichem FSH codiert, beinhaltet.

12. Expressionsvektorenpaar, wobei jeder Vektor eine eukaryotische Zelle transfektieren kann und jeder Vektor heterologe DNA, welche die jeweilige Untereinheit von menschlichem FSH codiert, beinhaltet, wobei beide Untereinheiten von FSH von heterologer DNA in den Vektoren codiert werden und wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

13. Eukaryotische Zelle, die mit einem oder mehr Expressionsvektoren transfektiert ist, wobei die Zelle imstande ist, biologisch aktives FSH zu erzeugen, wobei die Alpha- und Beta-Untereinheiten von FSH von heterologer DNA in dem oder den Expressionsvektor(en) codiert werden und wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

14. Zelle nach Anspruch 13, wobei der Expressionsvektor oder einer der Expressionsvektoren zusätzlich für die folgende Leader-Sequenz der Beta-Untereinheit codiert:

* C L M S *

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

15. Zelle nach Anspruch 13 oder 14, die eine Säugetierzelle ist.

16. Zelle nach Anspruch 13, 14 oder 15, wobei der Vektor oder jeder der Vektoren zumindest 69% des Transformationsbereichs des Rinderpapillomavirusgenoms enthält.

17. Zelle nach einem der Ansprüche 13 bis 16, die eine Mauszelle ist.

18. Zelle nach einem der Ansprüche 13 bis 17, wobei der oder jeder Vektor ein Plasmid umfaßt.

19. Verfahren zur Herstellung der Beta-Untereinheit von menschlichem FSH, welches das Kultivieren von Wirtszellen umfaßt, die mit einem Expressionsvektor transfektiert sind, der heterologe DNA umfaßt, welche die Beta-Untereinheit von FSH codiert, wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

20. Verfahren zur Herstellung der unreifen Beta-Untereinheit von menschlichem FSH, welches das Kultivieren von Wirtszellen umfaßt, die mit einem Expressionsvektor transfektiert sind, der heterologe DNA umfaßt, welche die unreife Beta-Untereinheit von FSH codiert, wobei die unreife Beta-Untereinheit die in Anspruch 19 angeführte Sequenz der reifen Beta-Untereinheit besitzt und zusätzlich die folgende Leader-Sequenz aufweist:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

21. Verfahren nach Anspruch 1, 19 oder 20, wobei die Wirtszellen solche sind, die in einem der Ansprüche 13 bis 18 beansprucht sind.

22. Verfahren nach Anspruch 1, welches die Gewinnung von FSH aus einer Kultur der Zellen umfaßt.

23. Verfahren zur Herstellung einer pharmazeutischen Zusammensetzung, welches die Herstellung von rekombinantem FSH durch ein Verfahren nach Anspruch 1 oder 22 und das Zusetzen des so

* C L M S *

hergestellten FSH zu einem Träger umfaßt.

Patentansprüche für folgenden Vertragsstaat : AT

1. Verfahren zur Herstellung von biologisch aktivem, heterodimeren, menschlichen follikelstimulierenden Hormon (FSH), welches eine Alpha-Untereinheit und eine Beta-Untereinheit besitzt, wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu

und das Verfahren die gemeinsame Synthese jeder der Untereinheiten in einer eukaryotischen Zelle umfaßt, die mit einem oder mehr Expressionsvektoren transfektiert ist, der heterologe DNA zur Codierung jeder der Untereinheiten beinhaltet.

2. Verfahren zur Herstellung der Beta-Untereinheit von menschlichem FSH, welches das Kultivieren von Wirtszellen umfaßt, die mit einem Expressionsvektor transfektiert sind, der heterologe DNA beinhaltet, welche die Beta-Untereinheit von FSH codiert, wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

3. Verfahren zur Herstellung der unreifen Beta-Untereinheit von menschlichem FSH, welches das Kultivieren von Wirtszellen umfaßt, die mit einem Expressionsvektor transfektiert sind, der heterologe DNA beinhaltet, welche die unreife Beta-Untereinheit von FSH codiert, wobei die unreife Beta-Untereinheit die in Anspruch 2 angeführte Sequenz der reifen Beta-Untereinheit besitzt und zusätzlich die folgende

* C L M S *

Leader-Sequenz aufweist:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

4. Verfahren nach Anspruch 1, wobei die Beta-Untereinheit des FSH durch heterologe DNA eines ersten Expressionsvektors codiert wird und die Alpha-Untereinheit des FSH durch heterologe DNA eines zweiten Expressionsvektors codiert wird, der in die Wirtszelle transfektiert ist.

5. Verfahren nach Anspruch 1, wobei beide Untereinheiten des FSH von heterologer DNA eines einzigen Expressionsvektors codiert werden.

6. Verfahren zur Herstellung von DNA, welche die Beta-Untereinheit von menschlichem FSH codiert, wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu,

und das Verfahren DNA-Ligation und/oder Synthese umfaßt.

7. Verfahren nach Anspruch 6, wobei die rekombinante DNA die Form eines Vektors aufweist.

8. Verfahren nach Anspruch 7, wobei der Vektor ein Expressionsvektor ist, der eine eukaryotische Zelle transformieren kann.

9. Verfahren nach Anspruch 7, wobei der Vektor die NRRL-Zugriffsnummer B-15923 besitzt.

10. Verfahren nach Anspruch 7, wobei die rekombinante zusätzlich die folgende Leader-Sequenz der Beta-Untereinheit codiert:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

11. Verfahren zur Herstellung von zwei Expressionsvektoren, das die Herstellung eines ersten Expressionsvektors, der heterologe DNA, welche die Beta-Untereinheit von menschlichem FSH codiert, beinhaltet, durch DNA-Ligation und/oder Synthese umfaßt, wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu

sowie die Herstellung eines zweiten Expressionsvektors, der heterologe DNA, welche die Alpha-Untereinheit von menschlichem FSH codiert, beinhaltet, durch DNA-Ligation und/oder Synthese.

12. Verfahren zur Herstellung eines Expressionsvektors, wobei der Vektor heterologe DNA umfaßt, welche die Alpha- und Beta-Untereinheiten des biologisch aktiven, heterodimeren FSH codiert, und das Verfahren DNA-Ligation und/oder Synthese umfaßt und wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

13. Verfahren zur Herstellung einer transfektierten eukaryotischen Zelle, welches die Transfektion der Zelle mit einem oder mehr Expressionsvektoren umfaßt, wobei die transfektierte Zelle imstande ist, das biologisch aktive heterodimere FSH zu erzeugen, wobei die Alpha- und Beta-Untereinheiten von FSH von heterologer DNA in dem oder den Expressionsvektor(en) codiert werden und wobei die reife Beta-Untereinheit die folgende Sequenz aufweist:

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

14. Verfahren nach Anspruch 13, wobei der Expressionsvektor zusätzlich für die folgende Leader-Sequenz der Beta-Untereinheit codiert:

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

15. Verfahren nach Anspruch 13 oder 14, wobei die Zelle eine Säugetierzelle ist.

16. Verfahren nach Anspruch 13, 14 oder 15, wobei der Vektor oder jeder der Vektoren zumindest 69% des Transformationsbereichs des Rinderpapillomavirusgenoms enthält.

17. Verfahren nach jedem der Ansprüche 13 bis 16, wobei die Zelle eine Mauszelle ist.

18. Verfahren nach jedem der Ansprüche 13 bis 17, wobei der oder jeder Vektor ein Plasmid umfaßt.

19. Verfahren nach Anspruch 1, welches die Gewinnung von FSH aus einer Kultur der Zellen umfaßt.

20. Verfahren zur Herstellung einer pharmazeutischen Zusammensetzung, welches die Herstellung von rekombinantem FSH durch ein Verfahren nach Anspruch 1 und das Zusetzen des so hergestellten FSH zu einem Träger umfaßt.

Revendications

Revendications pour les Etats contractants suivants: BE, FR, DE, IT, LU, NL, SE, CH, LI, GB

1. Procédé de préparation d'une hormone folliculo-stimulante (FSH) humaine hétérodimère, biologiquement active, comportant une sous-unité alpha et une sous-unité bêta, la sous-unité bêta mature ayant la séquence :

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu,

ce procédé comprenant la synthèse en commun de chacune des sous-unités dans une cellule eucaryotique transfectée avec un ou plusieurs vecteurs d'expression de l'ADN hétérologue encodant chacune des sous-unités.

2. Procédé suivant la revendication 1, dans lequel la cellule est transfectée avec plus d'un vecteur d'expression, chaque vecteur comprenant de l'ADN hétérologue encodant une sous-unité respective.

3. Procédé suivant la revendication 1, dans lequel la cellule est transfectée avec un vecteur d'expression unique comprenant de l'ADN hétérologue encodant chaque sous-unité.

4. La sous-unité bêta immature de FSH humaine, qui comporte la séquence :

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys Asn Ser Cys Glu
Leu Thr Asn Ile Thr Ile Ala Ile Glu Lys Glu
Glu Cys Arg Phe Cys Ile Ser Ile Asn Thr Thr
Trp Cys Ala Gly Tyr Cys Tyr Thr Arg Asp Leu
Val Tyr Lys Asp Pro Ala Arg Pro Lys Ile Gln
Lys Thr Cys Thr Phe Lys Glu Leu Val Tyr Glu
Thr Val Arg Val Pro Gly Cys Ala His His Ala
Asp Ser Leu Tyr Thr Tyr Pro Val Ala Thr Gln
Cys His Cys Gly Lys Cys Asp Ser Asp Ser Thr
Asp Cys Thr Val Arg Gly Leu Gly Pro Ser Tyr
Cys Ser Phe Gly Glu Met Lys Glu.

5. ADN recombinant encodant la sous-unité bêta de la FSH humaine, dans lequel la sous-unité bêta mature comporte la séquence :

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

6. ADN recombinant suivant la revendication 5, qui est compris dans un vecteur.

7. ADN recombinant suivant la revendication 6, dans lequel le vecteur est un vecteur d'expression capable de transformer une cellule eucaryotique.

8. Vecteur ayant le numéro d'accès B-15923 à la NRRL.

9. ADN recombinant suivant la revendication 7, qui encode en outre la séquence de tête suivante de la sous-unité bêta.

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

10. Vecteur d'expression capable de transfecter une cellule eucaryotique, ce vecteur comprenant de l'ADN hétérologue encodant les sous-unités alpha et bêta d'une FSH hétérodimère, biologiquement active, la sous-unité bêta mature comportant la séquence :

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

* C L M S *

11. Paire de vecteurs d'expression, comprenant un premier vecteur d'expression qui est tel que revendiqué dans la revendication 7, 8 ou 9, et un second vecteur d'expression comprenant de l'ADN hétérologue encodant la sous-unité alpha de FSH humaine.

12. Paire de vecteurs d'expression, chaque vecteur étant capable de transfecter une cellule eucaryotique, et chaque vecteur comprenant de l'ADN hétérologue encodant une sous-unité correspondante de FSH, les deux sous-unités de FSH étant encodées par de l'ADN hétérologue dans les vecteurs, la sous-unité bêta mature comportant la séquence :

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

13. Cellule eucaryotique transfectée avec un ou plusieurs vecteurs d'expression, cette cellule étant capable de produire de la FSH biologiquement active, les sous-unités alpha et bêta de FSH étant encodées par de l'ADN hétérologue dans le ou les vecteurs d'expression, la sous-unité bêta mature comportant la séquence :

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

14. Cellule suivant la revendication 13, suivant laquelle le vecteur d'expression ou l'un des vecteurs d'expression, assure en outre le codage de la séquence de tête suivante de la sous-unité bêta :

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

* C L M S *

15. Cellule suivant la revendication 13 ou la revendication 14, qui est une cellule de mammifère.

16. Cellule suivant la revendication 13, 14 ou 15, dans laquelle le vecteur ou chacun des vecteurs comprend au moins la région de transformation de 69 % du génome de virus de papillome bovin.

17. Cellule suivant l'une quelconque des revendications 13 à 16, qui est une cellule de souris.

18. Cellule suivant l'une quelconque des revendications 13 à 17, dans laquelle le ou chaque vecteur comprend un plasmide.

19. Procédé de préparation de la sous-unité bêta de FSH humaine, comprenant la culture de cellules hôtes transfectées avec un vecteur d'expression comprenant de l'ADN hétérologue encodant la sous-unité bêta de la FSH, la sous-unité bêta mature comportant la séquence :

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

20. Procédé de préparation de la sous-unité bêta immature de la FSH humaine, ce procédé comprenant la culture de cellules hôtes transfectées avec un vecteur d'expression comprenant de l'ADN hétérologue encodant la sous-unité bêta immature de la FSH, la sous-unité bêta immature comportant la séquence de la sous-unité bêta mature mise en évidence dans la revendication 19 et comportant en outre la séquence de tête :

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

21. Procédé suivant la revendication 1, 19 ou 20, dans lequel les cellules hôtes sont telles que revendiquées dans l'une quelconque des revendications 13 à 18.

22. Procédé suivant la revendication 1, comprenant la récupération de la FSH à partir d'une culture des cellules.

23. Procédé de préparation d'une composition pharmaceutique, ce procédé comprenant la préparation d'une FSH recombinante par un procédé suivant la revendication 1 ou la revendication 22, et le mélange de cette FSH recombinante ainsi préparée avec un véhicule lui convenant.

Revendications pour l'Etat contractant suivant : AT

1. Procédé de préparation d'une hormone folliculo-stimulante (FSH) humaine hétérodimère, biologiquement active, comportant une sous-unité alpha et une sous-unité bêta, la sous-unité bêta mature ayant la

* C L M S *

séquence :

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu

ce procédé comprenant la synthèse en commun de chacune des sous-unités dans une cellule eucaryotique transfectée avec un ou plusieurs vecteurs d'expression de l'ADN hétérologue encodant chacune des sous-unités.

2. Procédé de préparation de la sous-unité bêta de FSH humaine, comprenant la culture de cellules hôtes transfectées avec un vecteur d'expression comprenant de l'ADN hétérologue encodant la sous-unité bêta de la FSH, la sous-unité bêta mature comportant la séquence :

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu

Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

3. Procédé de préparation de la sous-unité bêta immature de la FSH humaine, comprenant la culture de cellules hôtes transfectées avec un vecteur d'expression comprenant de l'ADN hétérologue encodant la sous-unité bêta immature de la FSH, la sous-unité bêta immature présentant la séquence de la sous-unité bêta mature présentée dans la revendication 2 et comportant en plus la séquence de tête :

* C L M S *

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

4. Procédé suivant la revendication 1, dans lequel la sous-unité bêta de la FSH est encodée par de l'ADN hétérologue d'un premier vecteur d'expression, et la sous-unité alpha de la FSH est encodée par de l'ADN hétérologue d'un second vecteur d'expression transfecté dans la cellule hôte.

5. Procédé suivant la revendication 1, dans lequel les deux sous-unités de la FSH sont encodées par de l'ADN hétérologue d'un seul vecteur d'expression.

6. Procédé de préparation d'ADN encodant la sous-unité bêta de la FSH humaine, dans lequel la sous-unité bêta mature a la séquence :

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu,

le procédé comprenant la ligation et/ou la synthèse de l'ADN.

7. Procédé suivant la revendication 6, dans lequel l'ADN recombinant est sous la forme d'un vecteur.

8. Procédé suivant la revendication 7, dans lequel le vecteur est un vecteur d'expression capable de transformer une cellule eucaryotique.

9. Procédé suivant la revendication 7, dans lequel le vecteur a le numéro d'accès B-15923 à la NRRL.

10. Procédé suivant la revendication 7, dans lequel l'ADN recombinant encode en outre la séquence de tête de la sous-unité bêta :

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

11. Procédé de préparation d'une paire de vecteurs d'expression, comprenant la préparation d'un premier vecteur d'expression comprenant de l'ADN hétérologue encodant la sous-unité bêta de la FSH humaine par une ligation et/ou une synthèse d'ADN, la sous-unité bêta mature comportant la séquence :

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu

et la préparation d'un second vecteur d'expression comprenant de l'ADN hétérologue encodant la sous-unité alpha de la FSH humaine par une ligation et/ou une synthèse d'ADN.

12. Procédé de préparation d'un vecteur d'expression, ce vecteur comprenant de l'ADN hétérologue encodant les sous-unités alpha et bêta de la FSH hétérodimère, biologiquement active, ce procédé comprenant la ligation et/ou la synthèse de l'ADN, la sous-unité bêta mature comportant la séquence :

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp
Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

13. Procédé de préparation d'une cellule eucaryotique transfectée, ce procédé comprenant la transfection de la cellule avec un ou plusieurs vecteurs d'expression, la cellule transfectée étant capable de produire la FSH hétérodimère, biologiquement active, les sous-unités alpha et bêta de la FSH étant encodées par de l'ADN hétérologue dans le ou les vecteurs d'expression, la sous-unité bêta mature présentant la séquence :

* C L M S *

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala
Ile Glu Lys Glu Glu Cys Arg Phe Cys Ile Ser
Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys Tyr
Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg
Pro Lys Ile Gln Lys Thr Cys Thr Phe Lys Glu
Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys
Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro
Val Ala Thr Gln Cys His Cys Gly Lys Cys Asp

Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
Glu.

14. Procédé suivant la revendication 13, dans lequel le vecteur d'expression encode en outre la séquence de tête suivante de la sous-unité bêta :

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys
Cys Trp Lys Ala Ile Cys Cys.

15. Procédé suivant la revendication 13 ou 14, dans lequel la cellule est une cellule de mammifère.

16. Procédé suivant la revendication 13, 14 ou 15, dans lequel le vecteur ou chacun des vecteurs comprend au moins la région de transformation de 69 % du génome de virus de papillome bovin.

17. Procédé suivant l'une quelconque des revendication 13 à 16, dans lequel la cellule est une cellule de souris.

18. Procédé suivant l'une quelconque des revendications 13 à 17, dans lequel le ou chaque vecteur comprend un plasmide.

19. Procédé suivant la revendication 1, comprenant la récupération de la FSH au départ d'une culture des cellules.

20. Procédé de préparation d'une composition pharmaceutique, comprenant la préparation de FSH recombinante par un procédé suivant la revendication 1, et le mélange de cette FSH recombinante ainsi préparée avec un véhicule qui lui convient.

* C L M S *