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(54) **METHOD FOR PRODUCING AND PURIFYING HYBRID OR NON-HYBRID RECOMBINANT GLYCOPROTEIN HORMONES, HYBRID OR NON-HYBRID RECOMBINANT GLYCOPROTEIN HORMONES, EXPRESSION VECTORS AND USES OF THE RECOMBINANT GLYCOPROTEIN HORMONES**

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(57) **ABSTRACT**

Disclosed is a method for producing hybrid or non-hybrid recombinant glycoprotein hormones, for example the recombinant equine chorionic gonadotropin (r-eCG), the hybrid recombinant chorionic gonadotropin, the recombinant thyroid-stimulating hormone (r-TSH), the recombinant luteinising hormone (r-LH), the luteinising hormone and the recombinant follicle-stimulating hormone (r-FSH). In addition, the present invention relates to the recombinant glycoprotein hormones comprising the equine  $\alpha$  and  $\beta$  subunits, inter alia, the  $\alpha$  subunit of mammals and equine  $\beta$  subunit, where the two subunits are fused in a simple chain, and chain-modifying agents, which hormones are easier to purify, more homogeneous, easier to produce on an industrial scale without using animals, in comparison with the wild glycoprotein hormone. The hormones are useful for inducing animal reproduction, ovulation induction, super-ovulation induction, follicle growth, oestrus induction, anoestrus reversal, puberty induction in animals, both with and without commercial interest.

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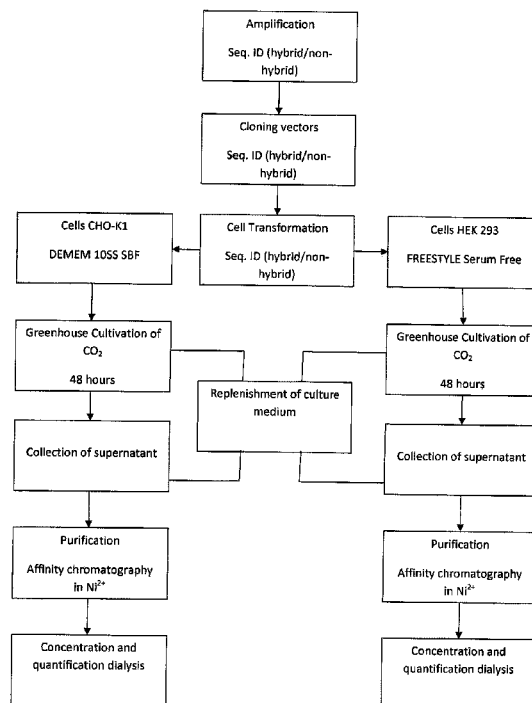
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(2) Date: **Sep. 18, 2018**

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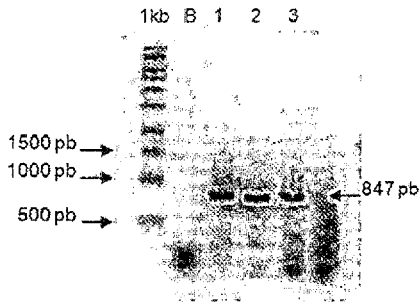


FIG. 1

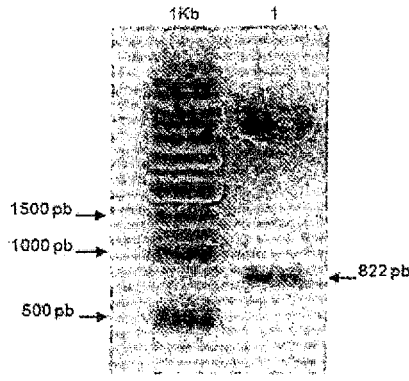


FIG. 2

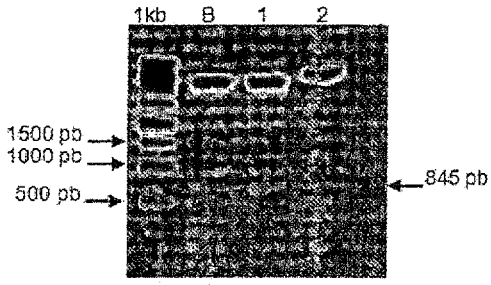


FIG. 3

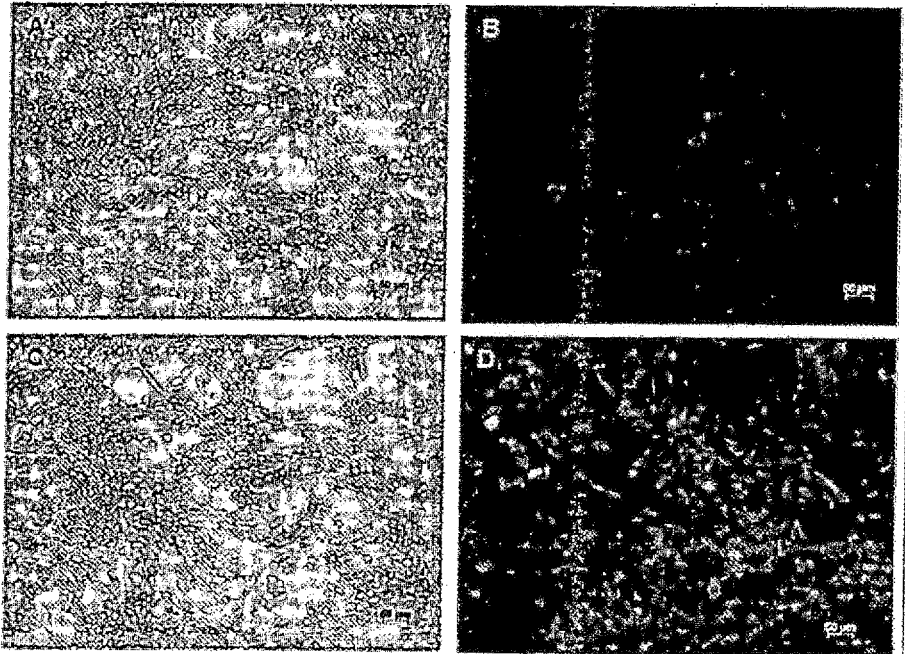


FIG. 4

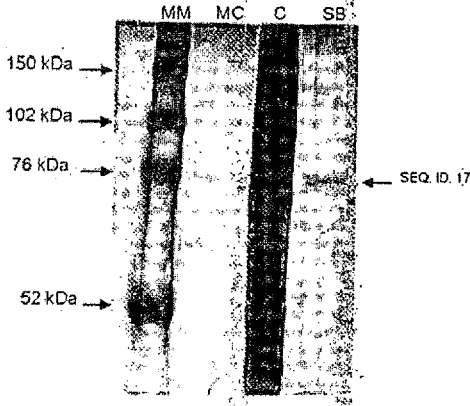


FIG. 5

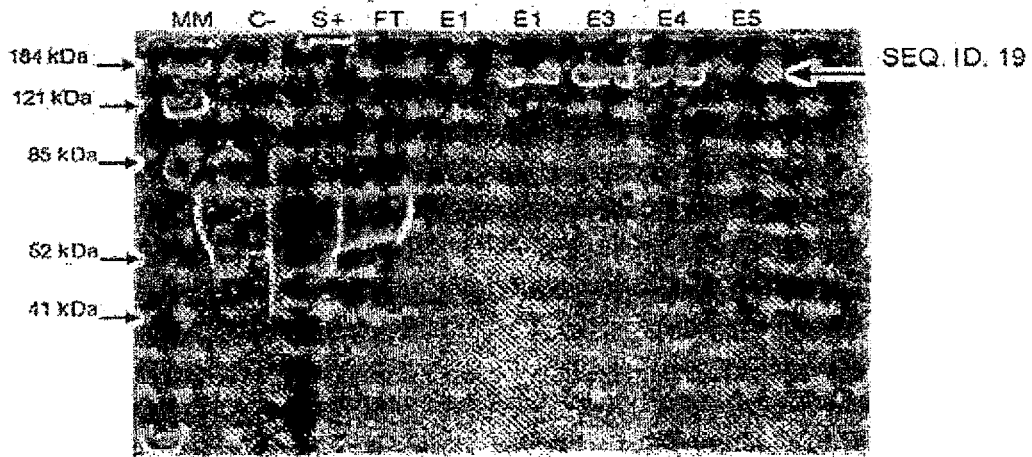


FIG. 6

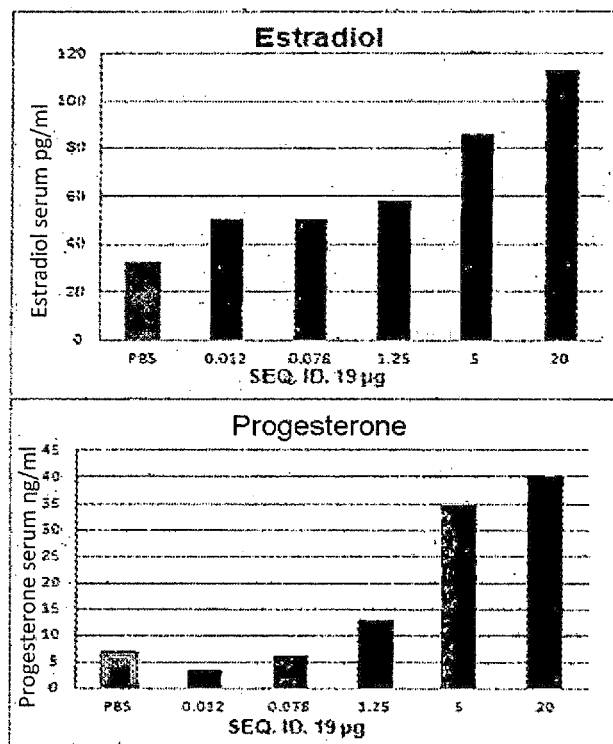


FIG. 7

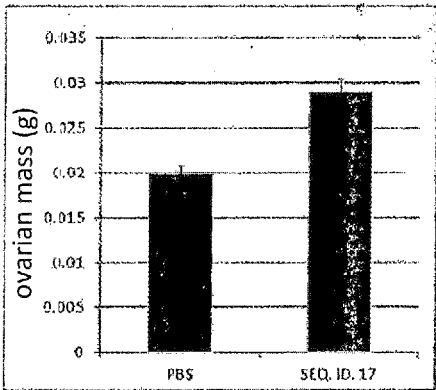


FIG. 8

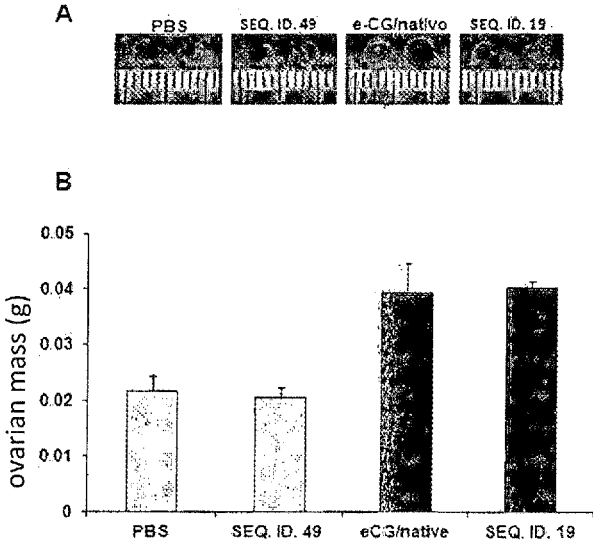


FIG. 9

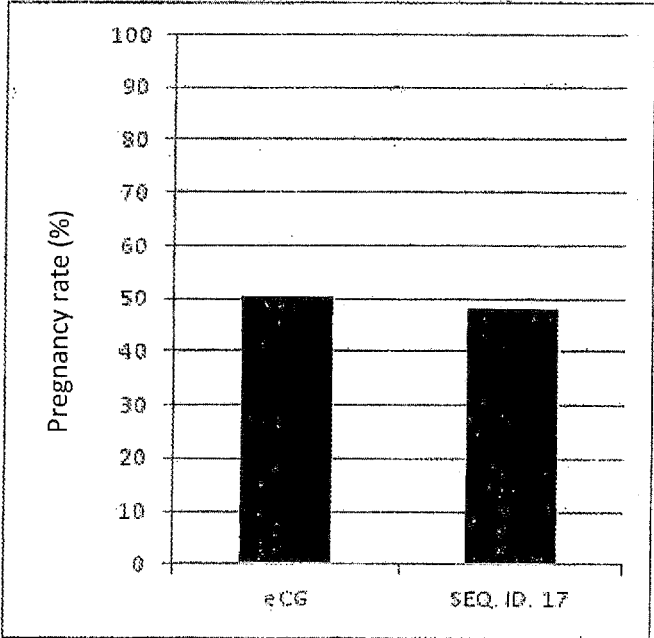


FIG. 10

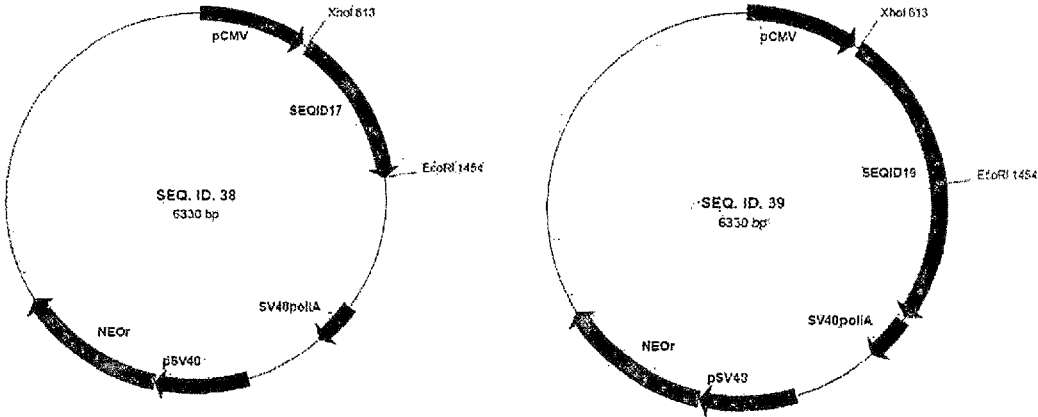


FIG. 11

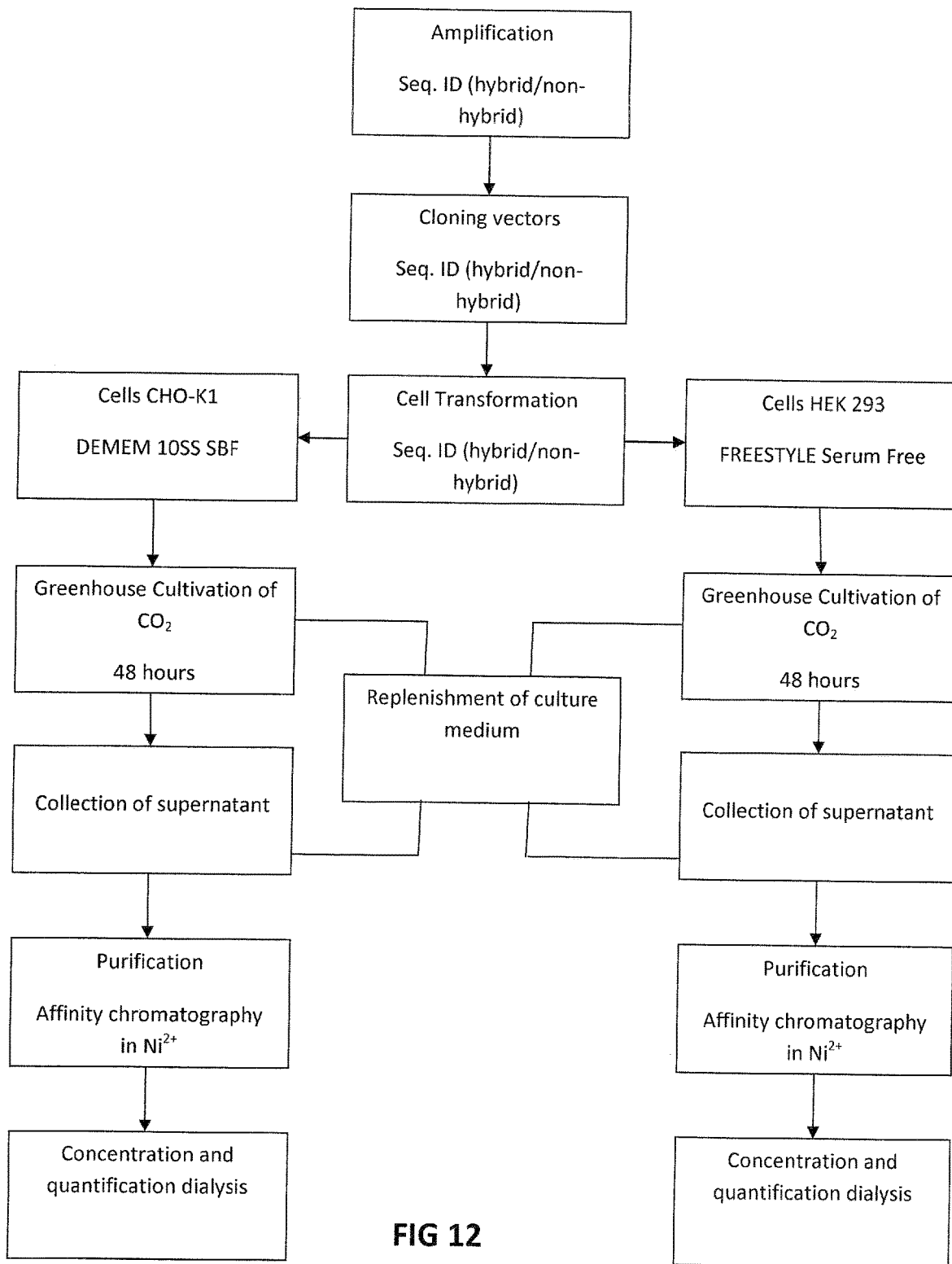


FIG 12

**METHOD FOR PRODUCING AND  
PURIFYING HYBRID OR NON-HYBRID  
RECOMBINANT GLYCOPROTEIN  
HORMONES, HYBRID OR NON-HYBRID  
RECOMBINANT GLYCOPROTEIN  
HORMONES, EXPRESSION VECTORS AND  
USES OF THE RECOMBINANT  
GLYCOPROTEIN HORMONES**

FIELD OF THE INVENTION

**[0001]** The present invention belongs to the field of processes for producing peptide hormones; specifically, it belongs to the field of processes for producing peptide hormones containing more than 20 amino acids; and describes a process for producing and purifying hybrid or non-hybrid recombinant glycoprotein hormones, hybrid or non-hybrid recombinant glycoprotein hormones, including their expression vectors, and uses thereof.

BACKGROUND OF THE INVENTION

**[0002]** In recent years, numerous biotechnological processes of production and purification of protein and glycoprotein hormones have been developed. All processes developed until then have their own strategies that vary according to the hormone to be produced and that aim at increasing the production of the hormone, or are aimed at facilitating the purification step.

**[0003]** In the production of recombinant glycoproteins, the state of the art uses mammalian cells due to their ability to promote the correct folding and post-translational processing. Several factors are involved in the optimization of protein expression in mammalian cells. One of these factors is the expression vectors for generation of recombinant cell lines using strong promoters of viral or cellular origin, such as the cytomegalovirus (CMV) promoter (Gopalkrishnan et al., 1999). Currently, most of the high protein production processes for the Biopharmaceutical industry (about 60-70%) are based on cells grown in suspension (Moritz, et al., 2015).

**[0004]** Equine Chorionic Gonadotropin (eCG) is a glycoprotein hormone produced in the trophoblast of pregnant mares, consisting of 2 subunits ( $\alpha$  and  $\beta$ ), with similar action on the follicle stimulating hormone (FSH) and luteinizing hormone (LH), both from the hypophysis and with important action in the events of induction of follicular growth and luteinization, respectively (Murphy, 2012). This bi-functional hormonal activity occurs after the administration of eCG in species of mammals other than horses, such as cattle, swine, sheep and goats (Murphy, 2012). The N-glycosylation sites of the alpha chain of the eCG is fundamental for the expression of its LH activity (Min et al. 1996; Min et al., 2004; Bousfield et al., 2004; Murphy, 2012). The loop region of the eCG protein structure and a sequence of amino acid residues (104-109) of the C-terminal region of the beta chain of this hormone are associated with the bi-functional action of eCG (LH and FSH activity) and its FSH function, respectively (Moyle et al. 1994; Galet et al. 2009).

**[0005]** eCG is used in different protocols of assisted animal reproduction. The use of eCG in other mammals may induce the production of anti-eCG antibodies and they may decrease the biological actions of this hormone in these animals (Hervé et al. 2004, Forcada et al. 2011). The alpha

chain of the eCG molecule is the major antigenic portion of this hormone (Chopineau et al, 1993).

**[0006]** The eCG gene is present on chromosome 10 in *Equus caballus*, and its expression generates the subunits, (i) gonadotropin alpha 1 subunit (chr10: 39937900-39940069; Gene ID: 100034174), its transcription undergoes splicing of 3 exons generating a messenger (mRNA) of nearly 2 Kb and an open reading frame (ORF) of 363 nucleotides translated into a mature protein of 120 amino acids and approximately 13.8 kDa, and (ii) chorionogonadotropin subunit beta (chr10: 18963366-18964444; Gene ID: 100054774), its transcription undergoes splicing of 3 exons generating a mRNA of approximately 520 kb and its open reading frame (ORF) of 510 nucleotides is translated into a mature protein of 169 amino acids and approximately 17.8 kDa (<http://genome.ucsc.edu>, <http://www.ncbi.nlm.nih.gov>).

**[0007]** The CGA gene of the *Bos taurus* species is present on chromosome 9 at position chr9: 63692501-63694585, and its expression generates the subunit, gonadotropin alpha 1 subunit (Gene ID: 280749), its transcription undergoes splicing of 4 exons generating a mRNA of approximately 742 base pairs (bp) (provisional to date) and open reading frame (ORF) of 363 nucleotides translated into a mature protein of 120 amino acids and approximately 13.6 kDa (<http://genome.ucsc.edu>, <http://www.ncbi.nlm.nih.gov>).

**[0008]** The CGA gene of the *Sus scrofa* species is present on chromosome 10 at position chr10: 62246069-62248001, and its expression generates the subunit, gonadotropin alpha 1 subunit (Gene ID: 406869), its transcription undergoes splicing of 3 exons generating a mRNA of approximately 363 base pairs (bp) (provisional to date) and open reading frame (ORF) of 363 nucleotides translated into a mature protein of 120 amino acids and approximately 13.5 kDa (<http://genome.ucsc.edu>, <http://www.ncbi.nlm.nih.gov>).

**[0009]** The CGA gene of the *Ovis aries* species is present on chromosome 8 at position chr8: 49919904-49921988, and its expression generates the subunit, gonadotropin alpha 1 subunit (Gene ID: 443538), its transcription undergoes splicing of 4 exons generating a mRNA of approximately 716 base pairs (bp) (provisional to date) and open reading frame (ORF) of 363 nucleotides translated into a mature protein of 120 amino acids and approximately 13.5 kDa (<http://genome.ucsc.edu>, <http://www.ncbi.nlm.nih.gov>).

**[0010]** The CGA gene of the *Capra hircus* species is present on chromosome 8 at position chr8: 49919901-49921988, and its expression generates the subunit, gonadotropin alpha 1 subunit (Gene ID: 100860817), its transcription undergoes splicing of 3 exons generating a mRNA of approximately 366 base pairs (bp) (provisional to date) and open reading frame (ORF) of 363 nucleotides translated into a mature protein of 120 amino acids and approximately 13.5 kDa (<http://genome.ucsc.edu>, <http://www.ncbi.nlm.nih.gov>).

STATE OF THE ART

**[0011]** Several patent documents relate to processes of producing chorionic gonadotrophin from equine and other mammals. For example:

**[0012]** Document EP0974599 discloses a recombinant equine chorionic gonadotropin hormone in which the  $\alpha$  and  $\beta$  chains of equine chorionic gonadotropin are bonded. This patent also claims the veterinary uses of this recombinant molecule.



[0013] On the other hand, document PI 0108556-5 describes a purification process of recombinant human Chorionic Gonadotropin (rhCG) produced in CHO cell cultures, which comprises the combined use of ion exchange chromatography and reverse phase HPLC. Such document also claims a pharmaceutical composition containing rhCG for subcutaneous administration.

[0014] Documents PI 9814880-0 and PI 9914670-3 relate to single-chain recombinant glycoprotein hormones, the process of producing the same without the use of a purification system by affinity chromatography, of fluorescent label and a polypeptide characteristic of cell secretion.

[0015] Document U.S. Pat. No. 5,526,0421 discloses a method of promoting site-directed mutagenesis in glycoproteins in general, for the production of hormones, such as luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone, and chorionic gonadotrophin. Document U.S. Pat. No. 6,469,139 discloses a modified human chorionic gonadotrophin at specific sites of the amino acid sequence, and its medical use as an immunological contraceptive.

[0016] Document WO9532216 discloses a method of producing biologically active glycoprotein hormones in prokaryotic cells which employs a redox thiol buffer to form structurally active subunits of the hormone.

[0017] Documents JPH1036398 and JPH1036399 relate to processes of producing recombinant equine chorionic gonadotropin, in which the subunits are not fused in a single-chain. The difference between them is due to the fact that the former claims the use of r-eCG in AI procedures or superovulation in cattle, while the latter claims the activity of stimulating the production of FSH.

[0018] Document WO2014183175 discloses methods for the production and purification of follicle stimulating hormone (FSH) using a parent or mutant HEK 293 cell platform.

[0019] Thus, no reports were found in the state of the art concerning the methods of obtaining and using artificial insemination and superovulation protocols related to the hybrid forms of chorionic gonadotrophin composed by the association of non-equine alpha chains and equine beta chains.

#### SUMMARY OF THE INVENTION

[0020] The present invention aims to propose a process for producing and purifying hybrid or non-hybrid recombinant glycoprotein hormones.

[0021] In addition, the present invention proposes recombinant glycoprotein hormones of equine origin (r-eCG) and other hybrids containing portions of equine ( $\beta$  chain) and target mammalian origin ( $\alpha$  chain), resulting in chimeric glycoprotein hormones specific to the target species, aiming at obtaining a hormonal composition that possesses the LH and FSH activities and without immunotoxicity to the target species.

[0022] In addition, the present invention further proposes the use of hybrid and non-hybrid recombinant glycoprotein hormones obtained with the use of their expression vectors and their pharmaceutical compositions in assisted animal reproduction of target species of commercial or non-commercial interest, mammals in general, like cattle, sheep, goats, pigs, horses, mules, bubainos, bison, antelopes, domestic and wild species of canines and felines, cetaceans, ursids and primates.

#### BRIEF DESCRIPTION OF DRAWINGS

[0023] FIG. 1 shows the electrophoretic analysis of the amplification procedure of the gene fragment referring to SEQ. ID. 16;

[0024] FIG. 2 shows the electrophoretic analysis of cleavage products of recombinant clones (SEQ. ID. 16) of SEQ. ID. 38;

[0025] FIG. 3 shows the electrophoretic analysis of cleavage products of recombinant clones (SEQ. ID. 16) of SEQ. ID. 39;

[0026] FIG. 4 shows the expression analysis of expression of the GFP molecule in CHO-K1 cells transfected with SEQ. ID. 39, by fluorescence microscopy, where (A) and (C) illustrate DIC (Differential Interference Contrast) images and show a similar cell growth pattern between the two cell populations, while (B) and (D) illustrate the fluorescence related to the presence of the GFP protein;

[0027] FIG. 5 shows the electrophoretic analysis of cell culture supernatant containing the SEQ. ID. 17 (non-fused recombinant r-eCG to GFP molecule); in which it is possible to observe in MM—Molecular Marker; MC—Culture medium (Freestyle Serum Free, C—HEK 293 cells after 48 hour culture; SB—Supernatant of HEK 293 cell culture after 48 hour culture, where it is possible to observe the SEQ. ID. 17 band;

[0028] FIG. 6 shows the electrophoretic analysis of purified preparation of SEQ. ID. 19. 12% SDS-PAGE, where it is possible to observe in MM—Molecular Marker; C—Culture Medium (DMEM containing 10% fetal bovine serum); S+—Cell culture supernatant; FT—Flow Through, proteins that did not bind to the His-Trap column; E1 to 5—Fractions eluted from the column, where it is possible to observe the band referring to the SEQ. ID. 19;

[0029] FIG. 7 shows purified preparations of SEQ. ID. 19 inducing the release of estradiol and progesterone in the serum of rats;

[0030] FIG. 8 shows the analysis of in vivo activity of SEQ. ID. 17 corresponding to non-fused recombinant eCG to GFP and purified from culture of the supernatant of HEK 293 cells cultured in the absence of fetal bovine serum using the Freestyle Serum Free culture medium;

[0031] FIG. 9 shows the functional comparative analysis between the recombinant forms of the control molecule (SEQ. ID: 49), of native eCG and SEQ. ID. 19, where (A) represents images of the ovaries of intramuscularly treated prepubertal rats with PBS (negative control), SEQ. ID. 49, native eCG and SEQ. ID. 19;

[0032] FIG. 10 shows the comparative analysis of the pregnancy rate in females, for the evaluation of the activity of SEQ. ID. 17 in large animals (cattle). Graphic representation of the percentage of pregnancy rate of females induced to estrus through hormonal protocols performed by the administration of eCG 300 IU and of SEQ. ID. 17 30  $\mu$ g. The analysis was performed by ultrasonography after 30 days of insemination of *Bos taurus indicus* females, with homogeneous groups for the animal category (race, age, calving at least 1 time), with n=127 for the eCG group and n=50 for the group SEQ. ID. 17 group.

[0033] FIG. 11 shows the graphic representation of vectors relating to SEQ. ID. 38 and SEQ. ID. 39, which represent all the vectors described in this invention.

[0034] FIG. 12 shows the organization chart of the steps of the production and purification process of recombinant glycoprotein hormones of this invention.

DETAILED DESCRIPTION OF THE  
INVENTION

**[0035]** The present invention relates to a process for the production and purification of recombinant glycoprotein hormones comprising the steps of:

**[0036]** (a) amplification, modification and cloning of the hybrid or non-hybrid molecules;

**[0037]** (b) construction of the expression vectors of recombinant glycoprotein hormones;

**[0038]** (c) transfection, expression and analysis of cells expressing the recombinant glycoprotein hormones;

**[0039]** (d) purification of recombinant glycoprotein hormones by affinity chromatography;

**[0040]** (e) dialysis and sterilization of recombinant glycoprotein hormones.

**[0041]** Recombinant glycoprotein hormone (r-eCG), whether or not fused to the GFP molecule, and its hybrid forms of the present invention, are selected from the group consisting of recombinant equine chorionic gonadotrophin (r-eCG), recombinant bovine chorionic gonadotrophin (r-bCG), recombinant suine chorionic gonadotrophin (r-sCG), recombinant ovary chorionic gonadotrophin (r-oCG), recombinant goat chorionic gonadotrophin (r-cCG), recombinant thyroid stimulating hormone (r-TSH), recombinant luteinizing hormone (r- and recombinant follicle stimulating hormone (r-FSH). Preferably, the glycoprotein hormone eCG, fused or not to the GFP molecule, and its obtained hybrid forms represent, respectively, the nucleotide and glycoprotein corresponding to recombinant equine chorionic gonadotrophin (r-eCG) and their hybrid forms.

**[0042]** (a) Amplification, Modification and Cloning of the Hybrid or Non-Hybrid Molecules;

**[0043]** The step of PCR amplification of the r-eCG gene fragments (SEQ.ID. 16) or r-eCG-GFP (SEQ.ID. 18) by PCR (Mullis et al., 1986) comprises the use of primer oligonucleotides of SEQ. ID. 1, SEQ. ID. 2 and SEQ. ID. 3 complementary to the different forms of native chorionic gonadotrophin to obtain a gene fragment relating to the fusion between the beta subunit DNA sequence of the native eCG (SEQ. ID. 6) and the native eCG alpha subunit DNA sequence (SEQ. ID. 4), wherein the SEQ. ID. 6 and 4 correspond to the  $\alpha$  and  $\beta$  subunits of eCG and additional sequences corresponding to their total in SEQ. ID. 16 or SEQ. ID. 18 validated by agarose gel electrophoresis.

**[0044]** SEQ. ID. 1, SEQ. ID. 2 and SEQ. ID. 3, in addition to promoting the amplification of the genes related to eCG subunits  $\alpha$  and  $\beta$  subunits, present additional nucleotide sequences associated with restriction enzyme cleavage sites and coding sequences for a histidine tail for poly-histidine sequence translation (6x His-Tag), and a proteolytic site, such as the Tobacco Etch Virus (TEV-Tag) protease site, associated with cloning of the gene sequences, purification of recombinant hormones and protein editing of these hormones respectively, thereby generating a fragment of DNA with 847 bp.

**[0045]** Amplification occurs by PCR, the polynucleotide of SEQ. ID. 16 being obtained, which is then purified on chelating resin (Sambrook et al, 1989).

**[0046]** (b) Construction of Vectors for the Expression of Recombinant Glycoprotein Hormones in Eukaryotic Cells (CHO-K1 and HEK 293)

**[0047]** The construction of vectors for the expression of recombinant glycoprotein hormones in eukaryotic cells (CHO-K1 and HEK 293) is initiated by the cloning of SEQ.

ID. 16 or SEQ. ID. 18 in prokaryotic cells (*E. coli* DH5a). This cloning step begins with the insertion of the sequences SEQ. ID. 16 or SEQ. ID. 18 in a commercial cloning vector that is used to transform DH5 $\alpha$  competent cells by thermal shock. Finally, the selection of bacterial recombinant clones containing the recombined cloning vector is performed with the sequences SEQ. ID. 16 or SEQ. ID. 18, whose presences are validated by agarose gel electrophoresis and DNA chemical sequencing. It should be clear for a person skilled in the art that various techniques and reagents may be used without the difference between the techniques and reagents being able to generate significant differences in the final process. In the present invention, the transformation of competent *E. coli* DH5a prokaryotes is accomplished by the introduction of cloning vectors by thermal shock, and the selection of recombinant clones is performed by cleavage for the detection of SEQ. ID. 16 and confirmation of the sequence by a chemical method of nucleotide sequencing, described in the literature (Sanger et al, 1997).

**[0048]** (c) Transfection, Expression and Analysis of Cells that Produce Recombinant Glycoprotein Hormones.

**[0049]** Expression vectors obtained after the cloning step of the hybrid and non-hybrid hormones are then used to transiently transfect eukaryotic cells with the aid of liposomes. Alternatively, the recombinant hormone gene sequences used in the composition of the expression vectors for unstable eukaryotic cell transformation may be used in the construction of expression vectors for stable transformation of these cells with the use of sequences. SEQ. ID. 16 or SEQ. ID. 18, via lentiviral vectors or biologically safe systems, of non-random gene integration and without the need for selective agents (antibiotics and other chemical substances) such as Transcription Activator-Like Effector Nucleases (TALENs), Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and other systems with these properties, for the generation of expression cell lines and their analysis by fluorescence microscopy for visualization of the protein expression of SEQ. ID. 19 and/or by other methodologies (immunodetection or gene sequencing) for detecting expression of the protein of SEQ. ID. 17 and SEQ. ID. 19. On the other hand, stable integration systems such as the use of lentiviral vectors can be used for these same purposes.

**[0050]** It is possible to detect, in the fluorescence microscope analysis, the change in fluorescence of the cells of the culture medium, since SEQ. ID. 19 is exported to culture medium due to its signaling peptide of SEQ. ID. 21, present between amino acids 1-20 of SEQ. ID. 19. In control cells transfected with SEQ. ID. 48, the presence of fluorescence is only detectable in the cell cytoplasm.

**[0051]** (d) Purification of Recombinant Glycoprotein Hormones

**[0052]** Purification occurs by collecting the supernatant from the culture medium of mammalian cells transfected with the sequences SEQ. ID. 16 or SEQ. ID. 18 and that secrete the SEQ. ID. 17 or SEQ. ID. 19, transiently or stably, followed by centrifugation between 1200 and 1800 g between 7 and 15 minutes at 4° C. for the removal of cells in suspension and subsequent purification of SEQ. ID 17 or SEQ. ID. 19 by affinity chromatography on nickel resins and after elution, an approximate yield of 15 to 17 mg of purified hormone is obtained for each liter of culture.

**[0053]** (e) Dialysis and Sterilization of Recombinant Glycoprotein Hormones.

**[0054]** The final step to obtain the recombinant glycoprotein hormones is carried out by dialysis in concentrators and/or by tangential centrifugation (cut off of 10 to 60 kDa), where the buffer is used in the production and purification steps of glycoprotein hormones in PBS pH 7.4 is changed, followed by sterilization of the solution containing the hormones produced with filters (0.22  $\mu\text{m}$ ) suitable for the final volume.

**[0055]** The invention also relates to a recombinant glycoprotein hormone comprising the subunits  $\alpha$  and  $\beta$  fused in a single chain and chain modifier agents at the amino and carboxy terminal moieties, such as a fusion site to a fluorescein, such as GFP; a purification marker, such as a poly-His tail; peptide signaling the secretion of the molecule; dimerization interface peptide and a specific proteolytic site, such as the proteolytic cleavage site with the TEV (Tobacco etch virus) proteolytic enzyme, and optionally, a fluorescent label. These modifications provide the recombinant molecule with features not presented by wild-type hormones, such as nickel affinity, alpha- and beta-strand fusion and/or fluorescence emission, which favor its production and purification processes.

**[0056]** All cloning, expression and purification steps described in steps (a) to (d) related the preparation of r-eCG were efficient and effective and should also be used in the cloning, expression and purification steps of the hybrid forms of these recombinant glycoprotein hormones.

**[0057]** Preferably, this production process is used for the production of SEQ. ID. 17 and SEQ. ID. 19, referring to recombinant equine chorionic gonadotrophin which, *in vivo*, had a bioactivity of approximately 10,000 IU/mg and close to the bioactivity of native eCG preparations.

**[0058]** Aiming at a reduction of the immunogenicity of SEQ. ID. 17 and SEQ. ID. 19 for other species, this invention aimed at obtaining hybrid forms of these glycoprotein hormones composed of the alpha chain of the target species and the equine beta chain with and/or without fusion with the GFP molecule.

**[0059]** Examples for obtaining functional analysis of r-eCG

#### EXAMPLE 1

##### Construction of the Cloning Vector of SEQ. ID. 16

**[0060]** The elaborated gene fragment related to SEQ. ID. 16 was commercially synthesized and amplified by PCR (FIG. 1), using the oligonucleotides (SEQ. ID. 1, SEQ. ID. 2 and SEQ. ID. 3). The electrophoretic analysis of the amplification procedure was performed on agarose gel (1%) and with the use of a 1 Kb molecular size marker. The samples tested were: negative control of the PCR reaction (lane B); and gene fragment (SEQ. ID. 16) amplified of approximately 847 bp (lanes 1, 2 and 3).

**[0061]** SEQ. ID. 16, was cloned into a plasmid vector (CloneJet-Thermo) and used to transform W5a competent *E. coli* cells by heat shock, and, after selection of recombinant clones by cleavage, the sequence confirmation of SEQ. ID., 16 was performed by a chemical nucleotide sequencing method.

#### EXAMPLE 2

##### Construction of the Expression Vectors of SEQ. ID. 38 and SEQ. ID. 39

**[0062]** Once the SEQ. ID. 16 was confirmed, the recombinant clones were cleaved with the XhoI and EcoRI enzymes, for removing the fragment of the SEQ. ID. 16, and cloned for generating SEQ. ID. 38 and SEQ. ID. 39. Selection of recombinant clones was done by cleavage of SEQ. ID. 38 and SEQ. ID. 39. The electrophoresis of clone cleavage products was done on agarose gel (1%) using a 1 Kb molecular marker. As seen in FIG. 2, the clone after cleavage with XhoI and EcoRI is found in lane 1, where the band relative to SEQ. ID. 16 is seen. In FIG. 3, the samples tested were: negative control of the PCR reaction (lane B); where the clones after cleavage of SEQ. ID. 39 are in lanes 1 and 2), where the band referring to SEQ. ID. 16 is seen. Confirmation of the perfect sequence of SEQ. ID. 16 was carried out by chemical DNA sequencing.

#### EXAMPLE 3

##### Transfection of Mammalian Cells

**[0063]** For the generation of expression cell lines (HEK 293 and CHO-K1), 6-well 500  $\mu\text{L}$  plates (24-well plates) were used for transfecting 800  $\mu\text{g}$  of SEQ. ID. 38 and SEQ. ID. 39, using 2  $\mu\text{L}$  of lipofectamine 2000 (Thermo). As a control, cells were transfected under the same conditions with SEQ. ID. 48. Cells were cultured on Freestyle Serum Free (Thermo) or DMEM medium (Sigma) containing 10% fetal bovine serum and 1 $\times$  antibiotic/antimycotic solution for 24 hours for further addition of 400  $\mu\text{g}/\text{mL}$  geneticin (G418, Sigma-Aldrich).

#### EXAMPLE 4

##### Selection of Transfected Mammalian Cell Clones

**[0064]** Transfected cells were selected over a period of 3 weeks, with geneticin concentration (G418) changes for elimination of non-transfected clones. Cells were then analyzed using fluorescence microscopy for the expression of SEQ. ID. 19 and of SEQ. ID. 49. The analysis was made by observing the change in the fluorescence of the cells and the culture medium, since SEQ. ID. 19 is exported to the medium due to its signal peptide present between amino acids 1-20 (SEQ. ID. 21). The control cells showed the presence of fluorescence only within the cells, due to the expression of SEQ. ID. 49 (FIG. 4).

**[0065]** Cells transfected with SEQ. ID. 39 express and export SEQ. ID. 19 to the culture medium (FIG. 4 (B)). Cells transfected with SEQ. ID. 48 express the GFP protein (SEQ. ID. 49) in the cells (FIG. 4 (D)).

#### EXAMPLE 5

##### Purification and Electrophoretic Analysis of SEQ. ID. 17 and SEQ. ID. 19

**[0066]** The selected HEK 293 cells were transferred to Spinner with 100 mL of Freestyle (Thermo) culture medium containing 400  $\mu\text{g}/\text{mL}$  geneticin for propagation, increasing the number of cells in the highest culture volume and consequently the concentration of recombinant protein expressed for 96 hours.

**[0067]** The selected CHO-K1 cells were transferred to 75 cm<sup>2</sup> culture bottles containing DEMEN (Sigma) containing 10% fetal bovine serum and geneticin 400 mg/mL for propagation, increasing the number of cells in the highest culture volume and consequently the concentration of recombinant protein expressed for 8 days of propagation with collections of supernatant (culture medium) every 48 hours.

**[0068]** After culture of the HEK 293 and CHO-K1 cells, centrifugation (1500×g/10 min./4° C.) of the culture media was carried out for the removal of cells in suspension, concentration and dialysis in appropriate concentrators, and for further purification of SEQ. ID. 17 and SEQ. ID. 19 by His-Trap column affinity chromatography on a suitable chromatograph. After elution with imidazole gradient (Sigma) (5 to 500 mM), the fractions were analyzed on 12% SDS-PAGE, as seen in FIG. 6. After concentration of the eluted fractions, quantification by absorbance measurement at 280 nm and correction by the correction factor (calculated by the molar extinction coefficient) of 1.29 for SEQ. ID. 17 e 1.34 for SEQ. ID. 19, an approximate yield of 15 mg of purified SEQ. ID. 17 and 17 mg of purified SEQ. ID. 19 for each liter of culture was estimated.

#### EXAMPLE 6

##### Verification of the Ability of SEQ. ID. 19 to Induce the Production of Hormones In Vivo

**[0069]** The hormonal effects of SEQ. ID. 19 were evaluated by rat assays and quantified by the chemiluminescence technique. FIG. 7 shows the induction of the production of estradiol (17β-estradiol) and progesterone measured in the serum of immature (Wistar) rats at 4-6 weeks of age after 6 and 18 hours, respectively, of the intramuscular injection of decreasing doses of SEQ. ID. 19 (0.012 to 20 μg), using as a control 4-6 week old immature rats injected with phosphate buffered saline, pH=7.4 (PBS); the procedures used in the hormone induction experiments in rats were approved by the Ethics Committee on the Use of Animals of the Ribeirao Preto Campus of the University of Sao Paulo (CEUA)—(Protocol No. 14.1.479.53.0).

#### EXAMPLE 7

##### Analysis of the Ability of SEQ. ID. 17 and of SEQ. ID. 19 to Induce Increased Ovarian Mass in Rats

**[0070]** The assessment of the ability of SEQ. ID. 17 and SEQ. ID. 19 to promote activity in vivo related to the hormonal function of chorionic gonadotrophin was analyzed by the measurement of the ovarian mass of rats treated with SEQ. ID. 17 [r-eCG without GFP (10 up)] and SEQ. ID. 19 [r-eCG with GFP (20 ug)]. The effects of SEQ. ID. 17 on ovarian growth induction were then evaluated by measuring the ovarian mass of rats (Wistar) at 21 days after intramuscular injection of 10 μg of SEQ. ID. 17 (FIG. 8). Each experimental group contained 4 animals (total of 8 ovaries per group). The procedures used in the induction experiments of ovarian mass increase in rats were approved by CEUA (Protocol No. 14.1.479.53.0).

**[0071]** Likewise, FIG. 9 shows the functional analysis (induction of ovarian mass increase) comparative between the recombinant forms of Green Fluorescent Protein (GFP, SEQ. ID. 49), the native form of eCG (SEQ. ID. 5 and SEQ.

ID. 7) and of SEQ. ID. 19. Each experimental group contained 4 animals (total of 8 ovaries per group) and only ovaries from each experimental group, randomized, are represented. The scale associated with the images is dimensioned in centimeters.

**[0072]** The results are expressed as ovarian mass in grams (g) and indicate that the recombinant forms of eCG (SEQ. ID. 17 and SEQ. ID. 19) exhibit in vivo bioactivity similar to native eCG (SEQ. ID. 5 and SEQ. ID. 7). These examples aid in the rationale for including hybrid forms of the hormone (composed of the alpha chain of equine and target animals) in this patent, since SEQ. ID. 17 and 19 show genetic similarity above 97% and structural similarities with the hybrid forms, which may be indicated for use in several species of mammals.

#### EXAMPLE 8

##### Field Tests for the Evaluation of the Activity of SEQ. ID. 17 in Large Animals (Cattle)

**[0073]** The hormonal activity of SEQ. ID. 17 was evaluated in estrus synchronization protocols (IATF) in large mammals (Bovine) by Ultrasound (GE-Logiq and Transrectal transducer mod 1-739, 8-12 Mhz) in females induced to estrus through hormonal protocols carried out by administration of eCG 300 IU and SEQ. ID. 17 30 μg.

**[0074]** The experimental model was based on Bos taurus indicus females, in homogeneous groups for the animal category (race, age, calving at least 1 time), with n=127 for the eCG group and n=50 for the SEQ. ID. 17 group. The signs of estrus were verified by clinical evaluation and by comparison of follicular waves and ovulation (ultrasound).

**[0075]** The IATF protocol consisted of the introduction of the vaginal device for progesterone release (day 1), administration of eCG 300 IU and SEQ. ID. 17 30 μg and uterine evaluation by ultrasonography (day 8). Insemination was performed after the analysis by ultrasonography and follicular wave observation, where at least one follicle presented growth (1.4 mm/day) for each animal of both groups (day 10).

**[0076]** FIG. 10 shows comparison of pregnancy rate which was performed 30 days post-insemination by transrectal ultrasonography in animals belonging to the inseminated groups from the IATF protocol using the eCG and SEQ. ID. 17. The results obtained showed a pregnancy rate of 50.23% for eCG, and of 48% for SEQ. ID. 17, where the national average pregnancy rate per IATF is 42%.

**[0077]** The analyzes showed the formation of cysts in 5.5% and twin formation in 1.59% in the eCG group, where the group SEQ. ID. 17 did not present the formation of cysts and twins.

**[0078]** Applications

**[0079]** From an effective amount of the recombinant glycoprotein hormones of SEQ. ID. 17 and SEQ. ID. 19, for example, from 0.001 to 10,000 μg, together with pharmaceutically acceptable adjuvants, such as hormone scavengers or permeants and Nanotechnology-based Release Systems, it is possible to propose a pharmaceutical composition. These adjuvants aim to ensure pharmacokinetic and pharmacodynamic quality by ensuring the adequate bioactivity of these recombinant hormones in different animal reproduction protocols. Such composition is used for assisted animal reproduction comprising an effective amount of recombinant glycoprotein hormones. Such compositions

will be used in induction of ovulation; induction of super-ovulation; follicular growth; induction of estrus; reversal of anestrus; induction of puberty in animals of commercial interest or not, mammals in general, such as cattle, sheep, goats, pigs, horses, buffaloes, bison, antelopes, domestic and wild species of canines and felines, cetaceans, ursids and primates.

**[0080]** In addition, there is also the possibility of elaborating kits for induction of ovulation; induction of super-ovulation; follicular growth; induction of estrus; reversal of anestrus; induction of puberty; for use in IATF protocols (Fixed Time Artificial Insemination) FIC (In vitro Fertilization), TETF (Fixed Time Embryo Transfer) in animals of commercial interest or not.

**[0081]** Considering that chorionic gonadotrophins can be immunogenic (induce antibody production) and antigenic (recognized by antibodies) (Hervé et al. 2004, Forcada et al. 2011; Chopineau et al., 1993) it is possible to propose that the recombinant glycoprotein hormones in question can be used to obtain native (monoclonal and/or polyclonal) or recombinant (phage display) antibodies and that both these antibodies and recombinant glycoprotein hormones, and their derivatives (conjugates to enzymes, radiolabels and/or fluorochromes), may comprise kits for the detection of these two categories of molecules (hormones and anti-hormones) in biological samples or not.

**[0082]** Although the invention has been widely described, one person skilled in the art would find obvious that many changes and modifications may be made without covering said modifications by the scope of the invention.

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#### SEQUENCE LISTING

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 Gln Asp Cys Pro Glu Cys Lys Leu Arg Glu Asn Lys Tyr Phe Phe Lys  
 35 40 45  
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 50 55 60  
 Tyr Pro Thr Pro Ala Arg Ser Arg Lys Thr Met Leu Val Pro Lys Asn  
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	20							25				30			
Asn	Ala	Thr	Leu	Ala	Ala	Glu	Lys	Glu	Ala	Cys	Pro	Ile	Cys	Ile	Thr
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Phe	Thr	Thr	Ser	Ile	Cys	Ala	Gly	Tyr	Cys	Pro	Ser	Met	Val	Arg	Val
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Met	Pro	Ala	Ala	Leu	Pro	Ala	Ile	Pro	Gln	Pro	Val	Cys	Thr	Tyr	Arg
	65			70				75						80	
Glu	Leu	Arg	Phe	Ala	Ser	Ile	Arg	Leu	Pro	Gly	Cys	Pro	Pro	Gly	Val
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Asp	Pro	Met	Val	Ser	Phe	Pro	Val	Ala	Leu	Ser	Cys	His	Cys	Gly	Pro
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Cys	Gln	Ile	Lys	Thr	Thr	Asp	Cys	Gly	Val	Phe	Arg	Asp	Gln	Pro	Leu
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Ala	Cys	Ala	Pro	Gln	Ala	Ser	Ser	Ser	Ser	Lys	Asp	Pro	Pro	Ser	Gln
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Pro	Leu	Thr	Ser	Thr	Ser	Thr	Pro	Thr	Pro	Gly	Ala	Ser	Arg	Arg	Ser
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 ttctccaggg cataccccac tccagcgagg tctaagaaga caatggtggt ccccaagaac 240  
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Gln Gly Cys Pro Glu Cys Lys Leu Lys Glu Asn Lys Tyr Phe Ser Lys
35          40          45
Leu Gly Ala Pro Ile Tyr Gln Cys Met Gly Cys Cys Phe Ser Arg Ala
50          55          60
Tyr Pro Thr Pro Ala Arg Ser Lys Lys Thr Met Leu Val Pro Lys Asn
65          70          75          80
Ile Thr Ser Glu Ala Thr Cys Cys Val Ala Lys Ala Phe Thr Lys Ala
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atcacctcgg aagccacatg ttgtgtggcc aaagcattta ccaaggccac agtgcagggga 300
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 Tyr Pro Thr Pro Ala Arg Ser Lys Lys Thr Met Leu Val Pro Lys Asn  
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ggcgtcccga tttaccagtg taagggctgc tgcttttcca gaccgtaccc cactccagca 660  
aggtccagga agacaatggt ggtcccaaag aacatcacct cagaatccac atgctgtgtg 720  
gccccagcat ttatcagggt cacagtgatg ggaaacatca agttggagaa ccacaccag 780  
tgctattgca gcaacttgcta tcaccacaag atttaa 816

<210> SEQ ID NO 17  
<211> LENGTH: 271  
<212> TYPE: PRT  
<213> ORGANISM: Unknown  
<220> FEATURE:  
<223> OTHER INFORMATION: r-eCG

<400> SEQUENCE: 17

Met His His His His His His Glu Thr Leu Gln Gly Leu Leu Leu Trp  
1 5 10 15

Met Leu Leu Ser Val Gly Gly Val Trp Ala Ser Arg Gly Pro Leu Arg  
20 25 30

Pro Leu Cys Arg Pro Ile Asn Ala Thr Leu Ala Ala Glu Lys Glu Ala  
35 40 45

Cys Pro Ile Cys Ile Thr Phe Thr Thr Ser Ile Cys Ala Gly Tyr Cys  
50 55 60

Pro Ser Met Val Arg Val Met Pro Ala Ala Leu Pro Ala Ile Pro Gln  
65 70 75 80

Pro Val Cys Thr Tyr Arg Glu Leu Arg Phe Ala Ser Ile Arg Leu Pro  
85 90 95

Gly Cys Pro Pro Gly Val Asp Pro Met Val Ser Phe Pro Val Ala Leu  
100 105 110

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Ser Cys His Cys Gly Pro Cys Gln Ile Lys Thr Thr Asp Cys Gly Val  
 115 120 125

Phe Arg Asp Gln Pro Leu Ala Cys Ala Pro Gln Ala Ser Ser Ser Ser  
 130 135 140

Lys Asp Pro Pro Ser Gln Pro Leu Thr Ser Thr Ser Thr Pro Thr Pro  
 145 150 155 160

Gly Ala Ser Arg Arg Ser Ser His Pro Leu Pro Ile Lys Thr Ser Phe  
 165 170 175

Pro Asp Gly Glu Phe Thr Thr Gln Asp Cys Pro Glu Cys Lys Leu Arg  
 180 185 190

Glu Asn Lys Tyr Phe Phe Lys Leu Gly Val Pro Ile Tyr Gln Cys Lys  
 195 200 205

Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr Pro Ala Arg Ser Arg Lys  
 210 215 220

Thr Met Leu Val Pro Lys Asn Ile Thr Ser Glu Ser Thr Cys Cys Val  
 225 230 235 240

Ala Lys Ala Phe Ile Arg Val Thr Val Met Gly Asn Ile Lys Leu Glu  
 245 250 255

Asn His Thr Gln Cys Tyr Cys Ser Thr Cys Tyr His His Lys Ile  
 260 265 270

<210> SEQ ID NO 18  
 <211> LENGTH: 1605  
 <212> TYPE: DNA  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: r-eCG-GFP

<400> SEQUENCE: 18

```

atgcatcatc atcatcatca tgagacgctc caggggctgc tgctgtggat gctgctgagt    60
gttggcgggg tctgggcatc cagggggcca ctgcggccac tgtgccggcc catcaacgcc    120
actctggctg ctgagaagga ggccctgcccc atctgcatca ccttcaccac cagcatctgt    180
gccggctact gccccagcat ggtgcggtg atgccagctg ccctgccggc cattccccag    240
ccagtgtgca cctaccgtga gctgcgctt gcttccatcc ggctccccgg ctgcccgcct    300
ggtgtggacc ccattggtct cttccccctg gccctcagtt gtcactgcgg gccctgccag    360
atcaagacca ctgactgcgg ggttttcaga gaccagccct tggcctgtgc cccccaggcc    420
tcctcttcct ctaaggatcc cccatcccaa cctctcacat ccacatccac cccaactcct    480
ggggccagca gacgttctc tcattcccctc ccaataaaga cttcttttcc tgatggagag    540
tttacaacgc aggattgcc agaatgcaag ctaagggaaa acaagtactt cttcaactg    600
ggcgtcccga tttaccagtg taagggtgc tgcttctcca gacgtaacc cactccagca    660
aggccagga agacaatggt ggtcccaaag aacatcacct cagaatccac atgctgtgtg    720
gccaaagcat ttatcaggt cacagtgat ggaacatca agttggagaa ccacaccag    780
tgctattgca gcacttgcta tcaccacaag attgagaacc tgtacttcca atcccgaatt    840
ctgcagtcca cggtagccgc ggccccggat ccaccggtcg ccaccatggt gagcaagggc    900
gaggagctgt tcaccggggt ggtgcccatc ctggtcgagc tggacggcga cgtaaacggc    960
cacaagttca gcgtgtccgg cgagggcgag ggcgatgcca cctacggcaa gctgaccctg   1020
aagttcatct gcaccaccgg caagctgccc gtgcctggc ccaccctcgt gaccaccctg   1080
    
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acctacggcg tgcagtgett cagccgctac cccgaccaca tgaagcagca cgacttcttc 1140
aagtccgccca tgccccgaagg ctacgtccag gagcgcacca tcttcttcaa ggacgacggc 1200
aactacaaga ccccgccoga ggtgaagttc gagggcgaca ccctggtgaa ccgcatcgag 1260
ctgaagggca tcgacttcaa ggaggacggc aacatcctgg ggcacaagct ggagtacaac 1320
tacaacagcc acaacgtcta tatcatggcc gacaagcaga agaacggcat caaggtgaac 1380
ttcaagatcc gccacaacat cgaggacggc agcgtgcagc tcgcccacca ctaccagcag 1440
aacacccccca tcggcgacgg ccccggtgtg ctgcccgaca accactacct gageaccagg 1500
tccgcccctga gcaaaagccc caacgagaag cgcgatcaca tggctcctgct ggagttcgtg 1560
accgcccggc ggatcactct cggcatggac gagctgtaca agtaa 1605

```

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<210> SEQ ID NO 19
<211> LENGTH: 534
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: r-eCG-GFP

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<400> SEQUENCE: 19

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Met His His His His His His Glu Thr Leu Gln Gly Leu Leu Leu Trp
 1          5          10          15
Met Leu Leu Ser Val Gly Gly Val Trp Ala Ser Arg Gly Pro Leu Arg
 20        25        30
Pro Leu Cys Arg Pro Ile Asn Ala Thr Leu Ala Ala Glu Lys Glu Ala
 35        40        45
Cys Pro Ile Cys Ile Thr Phe Thr Thr Ser Ile Cys Ala Gly Tyr Cys
 50        55        60
Pro Ser Met Val Arg Val Met Pro Ala Ala Leu Pro Ala Ile Pro Gln
 65        70        75        80
Pro Val Cys Thr Tyr Arg Glu Leu Arg Phe Ala Ser Ile Arg Leu Pro
 85        90        95
Gly Cys Pro Pro Gly Val Asp Pro Met Val Ser Phe Pro Val Ala Leu
100       105       110
Ser Cys His Cys Gly Pro Cys Gln Ile Lys Thr Thr Asp Cys Gly Val
115       120       125
Phe Arg Asp Gln Pro Leu Ala Cys Ala Pro Gln Ala Ser Ser Ser Ser
130       135       140
Lys Asp Pro Pro Ser Gln Pro Leu Thr Ser Thr Ser Thr Pro Thr Pro
145       150       155       160
Gly Ala Ser Arg Arg Ser Ser His Pro Leu Pro Ile Lys Thr Ser Phe
165       170       175
Pro Asp Gly Glu Phe Thr Thr Gln Asp Cys Pro Glu Cys Lys Leu Arg
180       185       190
Glu Asn Lys Tyr Phe Phe Lys Leu Gly Val Pro Ile Tyr Gln Cys Lys
195       200       205
Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr Pro Ala Arg Ser Arg Lys
210       215       220
Thr Met Leu Val Pro Lys Asn Ile Thr Ser Glu Ser Thr Cys Cys Val
225       230       235       240
Ala Lys Ala Phe Ile Arg Val Thr Val Met Gly Asn Ile Lys Leu Glu
245       250       255

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Asn His Thr Gln Cys Tyr Cys Ser Thr Cys Tyr His His Lys Ile Glu  
 260 265 270

Asn Leu Tyr Phe Gln Ser Arg Ile Leu Gln Ser Thr Val Pro Arg Ala  
 275 280 285

Arg Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly Glu Glu Leu Phe  
 290 295 300

Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly  
 305 310 315 320

His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly  
 325 330 335

Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro  
 340 345 350

Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser  
 355 360 365

Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met  
 370 375 380

Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly  
 385 390 395 400

Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val  
 405 410 415

Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile  
 420 425 430

Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile  
 435 440 445

Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg  
 450 455 460

His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln  
 465 470 475 480

Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr  
 485 490 495

Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp  
 500 505 510

His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly  
 515 520 525

Met Asp Glu Leu Tyr Lys  
 530

<210> SEQ ID NO 20  
 <211> LENGTH: 59  
 <212> TYPE: DNA  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Export sequence

<400> SEQUENCE: 20

gagacgctcc aggggctgct gctgtggatg ctgctgagtg ttggcggggt ctgggcate 59

<210> SEQ ID NO 21  
 <211> LENGTH: 20  
 <212> TYPE: PRT  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Export sequence

<400> SEQUENCE: 21

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Glu Thr Leu Gln Gly Leu Leu Leu Trp Met Leu Leu Ser Val Gly Gly  
 1 5 10 15

Val Trp Ala Ser  
 20

<210> SEQ ID NO 22  
 <211> LENGTH: 798  
 <212> TYPE: DNA  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: r-beCG

<400> SEQUENCE: 22

atggagacgc tccaggggct gctgctgtgg atgctgctga gtgttgccgg ggtctgggca 60  
 tccagggggc cactgcggcc actgtgccgg cccatcaacg ccaactctggc tgctgagaag 120  
 gaggcctgcc ccactctgcat caccttcacc accagcatct gtgccggcta ctgccccagc 180  
 atggtgctgg tgatgccagc tgcctgccg gccattcccc agccagtgtg cacctaccgt 240  
 gagctgcgct ttgcttccat ccggctcccc ggctgccgc ctggtgtgga ccccatggtc 300  
 tccttccccg tggccctcag ttgtcactgc gggccctgcc agatcaagac cactgactgc 360  
 ggggttttca gagaccagcc cttggcctgt gccccccagg cctcctcttc ctetaaggat 420  
 cccccatccc aacctctcac atccacatcc accccaactc ctggggccag cagacgttcc 480  
 tctcatcccc tcccaataaa gacttctttt cctgatggag agtttacaat gcagggtgtg 540  
 cctgaatgca agctaaaaga aaacaatac ttctccaagc cagatgctcc aatctatcag 600  
 tgcatggggt gctgcttctc cagggcatac cccactccag cgaggtctaa gaagacaatg 660  
 ttggtcccca agaacatcac ctgcgaagct acatgctgtg tggccaaagc atttaccag 720  
 gccacagtga tgggaaatgt cagagtggag aaccacaccg agtgccactg cagcacttgt 780  
 tattatcaca aatcctaa 798

<210> SEQ ID NO 23  
 <211> LENGTH: 265  
 <212> TYPE: PRT  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: r-beCG

<400> SEQUENCE: 23

Met Glu Thr Leu Gln Gly Leu Leu Leu Trp Met Leu Leu Ser Val Gly  
 1 5 10 15

Gly Val Trp Ala Ser Arg Gly Pro Leu Arg Pro Leu Cys Arg Pro Ile  
 20 25 30

Asn Ala Thr Leu Ala Ala Glu Lys Glu Ala Cys Pro Ile Cys Ile Thr  
 35 40 45

Phe Thr Thr Ser Ile Cys Ala Gly Tyr Cys Pro Ser Met Val Arg Val  
 50 55 60

Met Pro Ala Ala Leu Pro Ala Ile Pro Gln Pro Val Cys Thr Tyr Arg  
 65 70 75 80

Glu Leu Arg Phe Ala Ser Ile Arg Leu Pro Gly Cys Pro Pro Gly Val  
 85 90 95

Asp Pro Met Val Ser Phe Pro Val Ala Leu Ser Cys His Cys Gly Pro  
 100 105 110

Cys Gln Ile Lys Thr Thr Asp Cys Gly Val Phe Arg Asp Gln Pro Leu

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115		120				125									
Ala	Cys	Ala	Pro	Gln	Ala	Ser	Ser	Ser	Ser	Lys	Asp	Pro	Pro	Ser	Gln
130						135					140				
Pro	Leu	Thr	Ser	Thr	Ser	Thr	Pro	Thr	Pro	Gly	Ala	Ser	Arg	Arg	Ser
145					150					155					160
Ser	His	Pro	Leu	Pro	Ile	Lys	Thr	Ser	Phe	Pro	Asp	Gly	Glu	Phe	Thr
			165						170					175	
Met	Gln	Gly	Cys	Pro	Glu	Cys	Lys	Leu	Lys	Glu	Asn	Lys	Tyr	Phe	Ser
			180					185						190	
Lys	Pro	Asp	Ala	Pro	Ile	Tyr	Gln	Cys	Met	Gly	Cys	Cys	Phe	Ser	Arg
		195					200						205		
Ala	Tyr	Pro	Thr	Pro	Ala	Arg	Ser	Lys	Lys	Thr	Met	Leu	Val	Pro	Lys
	210					215					220				
Asn	Ile	Thr	Ser	Glu	Ala	Thr	Cys	Cys	Val	Ala	Lys	Ala	Phe	Thr	Lys
	225				230						235				240
Ala	Thr	Val	Met	Gly	Asn	Val	Arg	Val	Glu	Asn	His	Thr	Glu	Cys	His
				245					250					255	
Cys	Ser	Thr	Cys	Tyr	Tyr	His	Lys	Ser							
			260					265							

<210> SEQ ID NO 24  
 <211> LENGTH: 1605  
 <212> TYPE: DNA  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: r-beCG-GFP

<400> SEQUENCE: 24

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atgcatcatc atcatcatca tgagacgctc caggggctgc tgctgtggat gctgctgagt    60
gttggcgggg tctgggcctc cagggggcca ctgcccgcac tgtgccggcc catcaacgcc    120
actctggctg ctgagaagga ggccctgccc atctgcatca ccttcaccac cagcatctgt    180
gccggctact gccccagcat ggtgcccgtg atgccagctg ccctgccggc cattccccag    240
ccagtgtgca cctaccgtga gctgcgcttt gcttccatcc ggctccccgg ctgcccgcct    300
ggtgtggacc ccatggctct cttccccgtg gccctcagtt gtcactgcgg gccctgccag    360
atcaagacca ctgactgogg ggttttcaaga gaccagccct tggcctgtgc cccccaggcc    420
tcctcttctc ctaaggatcc cccatcccaa cctctcacat ccacatccac cccaactcct    480
ggggccagca gacgttccct tcateccctc ccaataaaga cttcttttcc tgatggagag    540
tttacaatgc agggctgtcc tgaatgcaag ctaaagaaa acaataactt ctccaagcca    600
gatgtcccaa tctatcagtg catgggggtc tgcttctcca gggcataccc cactccagcg    660
aggtctaaga agacaatggt ggtcccccaag aacatcacct cggaagctac atgctgtgtg    720
gccaagcat ttaccaaggc cacagtgatg ggaaatgtca gagtggagaa ccacaccgag    780
tgccactgca gcacttgcta ttatcacaaa tccgagaacc tgtacttcca atcccgaatt    840
ctgcagtcga cgggtaccgag ggcccgggat ccaccggctg ccaccatggt gagcaagggc    900
gaggagctgt tcaccggggt ggtgcccctc ctggtcagag tggacggcga cgtaaacggc    960
cacaagttca gcgtgtccgg cgagggcgag ggcgatgcca cctacggcaa gctgaccctg   1020
aagttcatct gcaccaccgg caagctgcc gtgccctggc ccaccctcgt gaccaccctg   1080
acctacggog tgcagtgtt cagccgctac cccgaccaca tgaagcagca cgacttcttc   1140
    
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aagtccgcca tgcccgaagg ctacgtccag gagegcacca tcttcttcaa ggacgacggc 1200
aactacaaga cccgcgcoga ggtgaagttc gagggcgaca ccctggtgaa ccgcacgcag 1260
ctgaagggca tcgacttcaa ggaggacggc aacatcctgg ggcacaagct ggagtacaac 1320
tacaacagcc acaacgtota tatcatggcc gacaagcaga agaacggcat caaggtgaac 1380
ttcaagatcc gccacaacat cgaggacggc agcgtgcagc tcgcccacca ctaccagcag 1440
aacacccccca tcggcgacgg ccccggtgctg ctgcccgcaca accactacct gagcaccag 1500
tccgcctga gcaagaccc caacgagaag cgcgatcaca tggctctgct ggagttcgtg 1560
accgcccgcg ggatcactct cggcatggac gagctgtaca agtaa 1605

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&lt;210&gt; SEQ ID NO 25

&lt;211&gt; LENGTH: 534

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Unknown

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: r-beCG-GFP

&lt;400&gt; SEQUENCE: 25

```

Met His His His His His His Glu Thr Leu Gln Gly Leu Leu Leu Trp
1          5          10          15
Met Leu Leu Ser Val Gly Gly Val Trp Ala Ser Arg Gly Pro Leu Arg
20          25          30
Pro Leu Cys Arg Pro Ile Asn Ala Thr Leu Ala Ala Glu Lys Glu Ala
35          40          45
Cys Pro Ile Cys Ile Thr Phe Thr Thr Ser Ile Cys Ala Gly Tyr Cys
50          55          60
Pro Ser Met Val Arg Val Met Pro Ala Ala Leu Pro Ala Ile Pro Gln
65          70          75          80
Pro Val Cys Thr Tyr Arg Glu Leu Arg Phe Ala Ser Ile Arg Leu Pro
85          90          95
Gly Cys Pro Pro Gly Val Asp Pro Met Val Ser Phe Pro Val Ala Leu
100         105         110
Ser Cys His Cys Gly Pro Cys Gln Ile Lys Thr Thr Asp Cys Gly Val
115         120         125
Phe Arg Asp Gln Pro Leu Ala Cys Ala Pro Gln Ala Ser Ser Ser Ser
130         135         140
Lys Asp Pro Pro Ser Gln Pro Leu Thr Ser Thr Ser Thr Pro Thr Pro
145         150         155         160
Gly Ala Ser Arg Arg Ser Ser His Pro Leu Pro Ile Lys Thr Ser Phe
165         170         175
Pro Asp Gly Glu Phe Thr Met Gln Gly Cys Pro Glu Cys Lys Leu Lys
180         185         190
Glu Asn Lys Tyr Phe Ser Lys Pro Asp Ala Pro Ile Tyr Gln Cys Met
195         200         205
Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr Pro Ala Arg Ser Lys Lys
210         215         220
Thr Met Leu Val Pro Lys Asn Ile Thr Ser Glu Ala Thr Cys Cys Val
225         230         235         240
Ala Lys Ala Phe Thr Lys Ala Thr Val Met Gly Asn Val Arg Val Glu
245         250         255
Asn His Thr Glu Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser Glu

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260			265			270									
Asn	Leu	Tyr	Phe	Gln	Ser	Arg	Ile	Leu	Gln	Ser	Thr	Val	Pro	Arg	Ala
	275						280					285			
Arg	Asp	Pro	Pro	Val	Ala	Thr	Met	Val	Ser	Lys	Gly	Glu	Glu	Leu	Phe
	290						295				300				
Thr	Gly	Val	Val	Pro	Ile	Leu	Val	Glu	Leu	Asp	Gly	Asp	Val	Asn	Gly
	305				310					315					320
His	Lys	Phe	Ser	Val	Ser	Gly	Glu	Gly	Glu	Gly	Asp	Ala	Thr	Tyr	Gly
			325					330						335	
Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys	Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro
		340						345					350		
Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	Thr	Tyr	Gly	Val	Gln	Cys	Phe	Ser
		355					360						365		
Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	His	Asp	Phe	Phe	Lys	Ser	Ala	Met
	370					375					380				
Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly
	385				390					395					400
Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val
			405						410						415
Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile
		420						425					430		
Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile
		435					440					445			
Met	Ala	Asp	Lys	Gln	Lys	Asn	Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg
	450					455						460			
His	Asn	Ile	Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln
	465			470						475					480
Asn	Thr	Pro	Ile	Gly	Asp	Gly	Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr
			485					490							495
Leu	Ser	Thr	Gln	Ser	Ala	Leu	Ser	Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp
		500						505					510		
His	Met	Val	Leu	Leu	Glu	Phe	Val	Thr	Ala	Ala	Gly	Ile	Thr	Leu	Gly
		515					520					525			
Met	Asp	Glu	Leu	Tyr	Lys										
	530														

<210> SEQ ID NO 26  
 <211> LENGTH: 816  
 <212> TYPE: DNA  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: r-seCG

<400> SEQUENCE: 26

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atgcatcatc atcatcatca tgagacgctc caggggctgc tgctgtggat gctgctgagt    60
gttgcggggg tctgggcac cagggggcca ctgcgccac tgtgccggcc catcaacgcc    120
actctggctg ctgagaagga ggccctgccc atctgcatca ccttcaccac cagcatctgt    180
gccggctact gccccagcat ggtgcggggtg atgccagctg ccctgcccgc cattccccag    240
ccagtgtgca cctaccgtga gctgcgcttt gettccatcc ggetccccgg ctgcccgcct    300
ggtgtggacc ccatggtctc cttccccgtg gccctcagtt gtcactgctg gccctgccag    360
atcaagacca ctgactgctg ggttttcaga gaccagccct tggcctgtgc cccccaggcc    420
    
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tctcttctct	ctaaggatcc	cccatcccaa	cctctcacat	ccacatccac	cccaactcct	480
ggggccagca	gacgttcctc	tcatccctc	ccaataaaga	cttcctttcc	tgatggagag	540
tttacaatgc	agggtgccc	agaatgcaag	ctaaaggaaa	acaagtactt	ctccaagctg	600
ggtgccccaa	tctatcagtg	catgggctgc	tgctttccca	gagcgtaccc	aactccagcg	660
aggtccaaga	agacaatggt	ggttccaaag	aacatcacct	cggaagccac	atgctgtgtg	720
gccaaagcat	ttaccaaggc	cacagtaatg	ggaaatgcca	gagtgagaaa	ccacaccgaa	780
tgccactgca	gtacttgtaa	ttatcacaaa	tcttaa			816

<210> SEQ ID NO 27						
<211> LENGTH: 271						
<212> TYPE: PRT						
<213> ORGANISM: Unknown						
<220> FEATURE:						
<223> OTHER INFORMATION: r-seCG						

<400> SEQUENCE: 27															
Met	His	His	His	His	His	Glu	Thr	Leu	Gln	Gly	Leu	Leu	Leu	Trp	
1			5					10					15		
Met	Leu	Leu	Ser	Val	Gly	Gly	Val	Trp	Ala	Ser	Arg	Gly	Pro	Leu	Arg
			20					25					30		
Pro	Leu	Cys	Arg	Pro	Ile	Asn	Ala	Thr	Leu	Ala	Ala	Glu	Lys	Glu	Ala
		35				40						45			
Cys	Pro	Ile	Cys	Ile	Thr	Phe	Thr	Thr	Ser	Ile	Cys	Ala	Gly	Tyr	Cys
	50					55					60				
Pro	Ser	Met	Val	Arg	Val	Met	Pro	Ala	Ala	Leu	Pro	Ala	Ile	Pro	Gln
65					70					75					80
Pro	Val	Cys	Thr	Tyr	Arg	Glu	Leu	Arg	Phe	Ala	Ser	Ile	Arg	Leu	Pro
			85					90						95	
Gly	Cys	Pro	Pro	Gly	Val	Asp	Pro	Met	Val	Ser	Phe	Pro	Val	Ala	Leu
			100					105					110		
Ser	Cys	His	Cys	Gly	Pro	Cys	Gln	Ile	Lys	Thr	Thr	Asp	Cys	Gly	Val
		115					120					125			
Phe	Arg	Asp	Gln	Pro	Leu	Ala	Cys	Ala	Pro	Gln	Ala	Ser	Ser	Ser	Ser
	130					135					140				
Lys	Asp	Pro	Pro	Ser	Gln	Pro	Leu	Thr	Ser	Thr	Ser	Thr	Pro	Thr	Pro
145					150					155					160
Gly	Ala	Ser	Arg	Arg	Ser	Ser	His	Pro	Leu	Pro	Ile	Lys	Thr	Ser	Phe
			165						170					175	
Pro	Asp	Gly	Glu	Phe	Thr	Met	Gln	Gly	Cys	Pro	Glu	Cys	Lys	Leu	Lys
		180						185					190		
Glu	Asn	Lys	Tyr	Phe	Ser	Lys	Leu	Gly	Ala	Pro	Ile	Tyr	Gln	Cys	Met
		195					200					205			
Gly	Cys	Cys	Phe	Ser	Arg	Ala	Tyr	Pro	Thr	Pro	Ala	Arg	Ser	Lys	Lys
	210					215					220				
Thr	Met	Leu	Val	Pro	Lys	Asn	Ile	Thr	Ser	Glu	Ala	Thr	Cys	Cys	Val
225					230					235					240
Ala	Lys	Ala	Phe	Thr	Lys	Ala	Thr	Val	Met	Gly	Asn	Ala	Arg	Val	Glu
			245						250					255	
Asn	His	Thr	Glu	Cys	His	Cys	Ser	Thr	Cys	Tyr	Tyr	His	Lys	Ser	
		260						265					270		

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<210> SEQ ID NO 28  
 <211> LENGTH: 1605  
 <212> TYPE: DNA  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: r-seCG-GFP

<400> SEQUENCE: 28

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atgcatcatc atcatcatca tgagacgctc caggggctgc tgctgtggat gctgctgagt      60
gttgcggggg tctgggcate cagggggcca ctgcgggcac tgtgccggcc catcaacgcc      120
actctggctg ctgagaagga ggccctgccc atctgcatca ccttcaccac cagcatctgt      180
gccggctact gccccagcat ggtgcgggtg atgccagctg ccctgccggc cattccccag      240
ccagtgtgca cctaccgtga gctgcgcttt gcttccatcc ggctccccgg ctgcccgcct      300
ggtgtggacc ccattggtctc ctccccctg gccctcagtt gtcactgctg gccctgccag      360
atcaagacca ctgactgctg ggttttcaga gaccagccct tggcctgtgc cccccaggcc      420
tcctcttcct ctaaggatcc cccatcccaa cctctcacat ccacatccac cccaactcct      480
ggggccagca gacgttctc tcattccctc ccaataaaga ctctcttcc tgatggagag      540
tttacaatgc agggctgccc agaatgcaag ctaaaggaaa acaagtactt ctccaagctg      600
ggtgccccaa tctatcagtg catgggctgc tgcttctcca gagcgtacc aactccagcg      660
aggccaaga agacaatggt ggttccaaag aacatcacct cggaagccac atgctgtgtg      720
gccccaaagc ttaccaaggc cacagtaatg gaaaatgcca gagtggagaa ccacaccgaa      780
tgccactgca gtacttgta ttatcacaaa tctgagaacc tgtacttcca atcccgaatt      840
ctgcagtctg cggtaaccgc ggccccggat ccaccggctg ccaccatggt gagcaagggc      900
gaggagctgt tcaccggggt ggtgccctc ctggctgagc tggacggcga cgtaaaaggc      960
cacaagtcca gcgtgtccgg cgagggcgag ggcatgcca cctacggcaa gctgaccctg     1020
aagttcatct gcaccaccgc caagctgccc gtgccctggc ccaccctcgt gaccaccctg     1080
acctacggcg tgcagtgtct cagccgctac cccgaaccaca tgaagcagca cgactttctc     1140
aagtcgcca tgcccgaagg ctacgtccag gagcgcacca tcttcttcaa ggaagcggc     1200
aactacaaga cccgcgcga ggtgaagttc gagggcgaca ccctggtgaa ccgcctcgag     1260
ctgaagggca tcgacttcaa ggaggacggc aacatcctgg ggcacaagct ggagtacaac     1320
tacaacagcc acaacgtcta tatcatggcc gacaagcaga agaacggcat caaggtgaac     1380
ttcaagatcc gccacaacat cgaggacggc agcgtgcagc tcgccacca ctaccagcag     1440
aacaccccc cggcgacgg ccccgctctg ctgcccgaca accactacct gagcaccag     1500
tccgccctga gcaaagacc caacgagaag cgcgatcaca tggctcctgct ggagttcgtg     1560
accgcccgcg ggatcactct cggcatggac gagctgtaca agtaa     1605
    
```

<210> SEQ ID NO 29  
 <211> LENGTH: 534  
 <212> TYPE: PRT  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: r-seCG-GFP

<400> SEQUENCE: 29

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Met His His His His His His Glu Thr Leu Gln Gly Leu Leu Leu Trp
1           5           10          15
    
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Met Leu Leu Ser Val Gly Gly Val Trp Ala Ser Arg Gly Pro Leu Arg  
                   20                                  25                                  30  
 Pro Leu Cys Arg Pro Ile Asn Ala Thr Leu Ala Ala Glu Lys Glu Ala  
                   35                                  40                                  45  
 Cys Pro Ile Cys Ile Thr Phe Thr Thr Ser Ile Cys Ala Gly Tyr Cys  
                   50                                  55                                  60  
 Pro Ser Met Val Arg Val Met Pro Ala Ala Leu Pro Ala Ile Pro Gln  
                   65                                  70                                  75                                  80  
 Pro Val Cys Thr Tyr Arg Glu Leu Arg Phe Ala Ser Ile Arg Leu Pro  
                   85                                  90                                  95  
 Gly Cys Pro Pro Gly Val Asp Pro Met Val Ser Phe Pro Val Ala Leu  
                   100                                  105  
 Ser Cys His Cys Gly Pro Cys Gln Ile Lys Thr Thr Asp Cys Gly Val  
                   115                                  120                                  125  
 Phe Arg Asp Gln Pro Leu Ala Cys Ala Pro Gln Ala Ser Ser Ser Ser  
                   130                                  135                                  140  
 Lys Asp Pro Pro Ser Gln Pro Leu Thr Ser Thr Ser Thr Pro Thr Pro  
                   145                                  150                                  155                                  160  
 Gly Ala Ser Arg Arg Ser Ser His Pro Leu Pro Ile Lys Thr Ser Phe  
                   165                                  170                                  175  
 Pro Asp Gly Glu Phe Thr Met Gln Gly Cys Pro Glu Cys Lys Leu Lys  
                   180                                  185                                  190  
 Glu Asn Lys Tyr Phe Ser Lys Leu Gly Ala Pro Ile Tyr Gln Cys Met  
                   195                                  200                                  205  
 Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr Pro Ala Arg Ser Lys Lys  
                   210                                  215                                  220  
 Thr Met Leu Val Pro Lys Asn Ile Thr Ser Glu Ala Thr Cys Cys Val  
                   225                                  230                                  235                                  240  
 Ala Lys Ala Phe Thr Lys Ala Thr Val Met Gly Asn Ala Arg Val Glu  
                   245                                  250                                  255  
 Asn His Thr Glu Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser Glu  
                   260                                  265                                  270  
 Asn Leu Tyr Phe Gln Ser Arg Ile Leu Gln Ser Thr Val Pro Arg Ala  
                   275                                  280                                  285  
 Arg Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly Glu Glu Leu Phe  
                   290                                  295                                  300  
 Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly  
                   305                                  310                                  315                                  320  
 His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly  
                   325                                  330                                  335  
 Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro  
                   340                                  345                                  350  
 Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser  
                   355                                  360                                  365  
 Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met  
                   370                                  375                                  380  
 Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly  
                   385                                  390                                  395                                  400  
 Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val  
                   405                                  410                                  415

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Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile  
420 425 430

Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile  
435 440 445

Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg  
450 455 460

His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln  
465 470 475 480

Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr  
485 490 495

Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp  
500 505 510

His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly  
515 520 525

Met Asp Glu Leu Tyr Lys  
530

<210> SEQ ID NO 30  
<211> LENGTH: 816  
<212> TYPE: DNA  
<213> ORGANISM: Unknown  
<220> FEATURE:  
<223> OTHER INFORMATION: r-oeCG

<400> SEQUENCE: 30

atgcatcatc atcatcatca tgagacgctc caggggctgc tgctgtggat gctgctgagt 60  
gttgccgggg tctgggcatac cagggggcca ctgcccgcac tgtgccggcc catcaacgcc 120  
actctggctg ctgagaagga ggccctgcccc atctgcatca ccttcaccac cagcatctgt 180  
gccggctact gccccagcat ggtgcccgtg atgccagctg cctgcccggc cattccccag 240  
ccagtgtgca cctaccgtga gctgcgcttt gcttccatcc ggctccccgg ctgcccgcct 300  
ggtgtggacc ccattggtctc ctccccctg gccctcagtt gtcactgccc gccctgccag 360  
atcaagacca ctgactgccc ggttttccaga gaccagccct tggcctgtgc cccccaggcc 420  
tcctcttcct ctaaggatcc cccatcccaa cctctcacat ccacatccac cccaactcct 480  
ggggccagca gacgttctc tcattccctc ccaataaaga cttcctttcc tgatggagag 540  
tttacaatgc aggggtgtcc tgaatgcaag ctaaaagaaa acaataactt ctccaagcca 600  
gatgctccaa tttatcagtg catgggggtg tgctttctcca gggcataccc cactccagcg 660  
aggtctaaga agacaatggt ggttcccagg aacatcacct cggaagccac atgttgtgtg 720  
gccccaaagc ttaccaagcc cacagtgatg ggaaatgtca gagtggagaa ccacaccgag 780  
tgccactgca gtacttgtaa ttatcacaaa tcttaa 816

<210> SEQ ID NO 31  
<211> LENGTH: 271  
<212> TYPE: PRT  
<213> ORGANISM: Unknown  
<220> FEATURE:  
<223> OTHER INFORMATION: r-oeCG

<400> SEQUENCE: 31

Met His His His His His His Glu Thr Leu Gln Gly Leu Leu Trp  
1 5 10 15

Met Leu Leu Ser Val Gly Gly Val Trp Ala Ser Arg Gly Pro Leu Arg

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20			25			30									
Pro	Leu	Cys	Arg	Pro	Ile	Asn	Ala	Thr	Leu	Ala	Ala	Glu	Lys	Glu	Ala
	35						40					45			
Cys	Pro	Ile	Cys	Ile	Thr	Phe	Thr	Thr	Ser	Ile	Cys	Ala	Gly	Tyr	Cys
	50						55				60				
Pro	Ser	Met	Val	Arg	Val	Met	Pro	Ala	Ala	Leu	Pro	Ala	Ile	Pro	Gln
	65				70					75					80
Pro	Val	Cys	Thr	Tyr	Arg	Glu	Leu	Arg	Phe	Ala	Ser	Ile	Arg	Leu	Pro
				85						90				95	
Gly	Cys	Pro	Pro	Gly	Val	Asp	Pro	Met	Val	Ser	Phe	Pro	Val	Ala	Leu
		100						105					110		
Ser	Cys	His	Cys	Gly	Pro	Cys	Gln	Ile	Lys	Thr	Thr	Asp	Cys	Gly	Val
		115					120						125		
Phe	Arg	Asp	Gln	Pro	Leu	Ala	Cys	Ala	Pro	Gln	Ala	Ser	Ser	Ser	Ser
	130						135				140				
Lys	Asp	Pro	Pro	Ser	Gln	Pro	Leu	Thr	Ser	Thr	Ser	Thr	Pro	Thr	Pro
	145				150					155					160
Gly	Ala	Ser	Arg	Arg	Ser	Ser	His	Pro	Leu	Pro	Ile	Lys	Thr	Ser	Phe
				165						170					175
Pro	Asp	Gly	Glu	Phe	Thr	Met	Gln	Gly	Cys	Pro	Glu	Cys	Lys	Leu	Lys
		180						185					190		
Glu	Asn	Lys	Tyr	Phe	Ser	Lys	Pro	Asp	Ala	Pro	Ile	Tyr	Gln	Cys	Met
		195					200						205		
Gly	Cys	Cys	Phe	Ser	Arg	Ala	Tyr	Pro	Thr	Pro	Ala	Arg	Ser	Lys	Lys
	210						215				220				
Thr	Met	Leu	Val	Pro	Lys	Asn	Ile	Thr	Ser	Glu	Ala	Thr	Cys	Cys	Val
	225				230					235					240
Ala	Lys	Ala	Phe	Thr	Lys	Ala	Thr	Val	Met	Gly	Asn	Val	Arg	Val	Glu
				245						250					255
Asn	His	Thr	Glu	Cys	His	Cys	Ser	Thr	Cys	Tyr	Tyr	His	Lys	Ser	
			260					265					270		

<210> SEQ ID NO 32  
 <211> LENGTH: 1605  
 <212> TYPE: DNA  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: r-oeCG-GFP

<400> SEQUENCE: 32

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atgcatcatc atcatcatca tgagacgctc caggggctgc tgctgtggat gctgctgagt      60
gttgcggggg tctgggcatac cagggggcca ctgcccacc tgtgccggcc catcaacgcc      120
actctggctg ctgagaagga ggccctgccc atctgcatca ccttcaccac cagcatctgt      180
gccggctact gccccagcat ggtgcgggtg atgccagctg ccctgccggc cattccccag      240
ccagtgtgca cctaccgtga gctgcgcttt gcttccatcc ggctccccgg ctgcccgct      300
ggtgtggacc ccattggtctc cttccccgtg gccctcagtt gtcactgctg gccctgccag      360
atcaagacca ctgactgctg ggttttcaga gaccagcctt tggcctgtgc cccccaggcc      420
tcctcttctc ctaaggatcc cccatcccaa cctctcacat ccacatccac cccaactcct      480
ggggccagca gacgttctc tcacccctc ccaataaaga cttcctttcc tgatggagag      540
tttacaatgc aggtgtgtcc tgaatgcaag ctaaaagaaa acaataactt ctccaagcca      600
    
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gatgctccaa tttatcagtg catgggggtgc tgcttctcca gggcataccc cactccagcg	660
aggtctaaga agacaatggt ggttcccaag aacatcacct cggaagccac atgttgtgtg	720
gccaagcat ttaccaaggc cacagtgatg ggaaatgtca gagtggagaa ccacaccgag	780
tgccactgca gtacttgta ttatcacaaa tctgagaacc tgtaactcca atcccgaatt	840
ctgcagtcga cgggtaccgag ggcccgggat ccaccggtcg ccaccatggt gagcaagggc	900
gaggagctgt tcaccggggt ggtgcccatc ctggtcgagc tggacggcga cgtaaacggc	960
cacaagttca gcgtgtccgg cgagggcgag ggcgatgcca cctacggcaa gctgaccctg	1020
aagttcatct gcaccacgg caagctgccc gtgccctggc ccacctcgt gaccacctg	1080
acctacggcg tgcagtgtt cagccgctac cccgaccaca tgaagcagca cgacttctt	1140
aagtcggcca tgcccgaagg ctacgtccag gagcgcacca tcttcttcaa ggacgacggc	1200
aactacaaga cccgcgcca ggtgaagttc gagggcgaca ccctggtgaa ccgcatcgag	1260
ctgaagggca tcgacttcaa ggaggacggc aacatcctgg ggcacaagct ggagtacaac	1320
tacaacagcc acaacgtcta tatcatggcc gacaagcaga agaacggcat caaggtgaac	1380
ttcaagatcc gccacaacat cgaggacggc agcgtgcagc tcgccgacca ctaccagcag	1440
aacaccccca tcggcgacgg ccccgctgtg ctgcccgaca accactacct gagcaccag	1500
tccgacctga gcaaaagacc caacgagaag cgcgatcaca tggctctgct ggagttcgtg	1560
accgcccgcg ggatcactct cggcatggac gagctgtaca agtaa	1605

<210> SEQ ID NO 33  
 <211> LENGTH: 534  
 <212> TYPE: PRT  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: r-oeCG-GFP

<400> SEQUENCE: 33

Met His His His His His His Glu Thr Leu Gln Gly Leu Leu Leu Trp
1 5 10 15
Met Leu Leu Ser Val Gly Gly Val Trp Ala Ser Arg Gly Pro Leu Arg
20 25 30
Pro Leu Cys Arg Pro Ile Asn Ala Thr Leu Ala Ala Glu Lys Glu Ala
35 40 45
Cys Pro Ile Cys Ile Thr Phe Thr Thr Ser Ile Cys Ala Gly Tyr Cys
50 55 60
Pro Ser Met Val Arg Val Met Pro Ala Ala Leu Pro Ala Ile Pro Gln
65 70 75 80
Pro Val Cys Thr Tyr Arg Glu Leu Arg Phe Ala Ser Ile Arg Leu Pro
85 90 95
Gly Cys Pro Pro Gly Val Asp Pro Met Val Ser Phe Pro Val Ala Leu
100 105 110
Ser Cys His Cys Gly Pro Cys Gln Ile Lys Thr Thr Asp Cys Gly Val
115 120 125
Phe Arg Asp Gln Pro Leu Ala Cys Ala Pro Gln Ala Ser Ser Ser Ser
130 135 140
Lys Asp Pro Pro Ser Gln Pro Leu Thr Ser Thr Ser Thr Pro Thr Pro
145 150 155 160
Gly Ala Ser Arg Arg Ser Ser His Pro Leu Pro Ile Lys Thr Ser Phe



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165					170					175					
Pro	Asp	Gly	Glu	Phe	Thr	Met	Gln	Gly	Cys	Pro	Glu	Cys	Lys	Leu	Lys
		180						185					190		
Glu	Asn	Lys	Tyr	Phe	Ser	Lys	Pro	Asp	Ala	Pro	Ile	Tyr	Gln	Cys	Met
		195					200					205			
Gly	Cys	Cys	Phe	Ser	Arg	Ala	Tyr	Pro	Thr	Pro	Ala	Arg	Ser	Lys	Lys
	210					215					220				
Thr	Met	Leu	Val	Pro	Lys	Asn	Ile	Thr	Ser	Glu	Ala	Thr	Cys	Cys	Val
225					230					235					240
Ala	Lys	Ala	Phe	Thr	Lys	Ala	Thr	Val	Met	Gly	Asn	Val	Arg	Val	Glu
			245						250						255
Asn	His	Thr	Glu	Cys	His	Cys	Ser	Thr	Cys	Tyr	Tyr	His	Lys	Ser	Glu
			260					265					270		
Asn	Leu	Tyr	Phe	Gln	Ser	Arg	Ile	Leu	Gln	Ser	Thr	Val	Pro	Arg	Ala
		275					280						285		
Arg	Asp	Pro	Pro	Val	Ala	Thr	Met	Val	Ser	Lys	Gly	Glu	Glu	Leu	Phe
	290					295					300				
Thr	Gly	Val	Val	Pro	Ile	Leu	Val	Glu	Leu	Asp	Gly	Asp	Val	Asn	Gly
305					310					315					320
His	Lys	Phe	Ser	Val	Ser	Gly	Glu	Gly	Glu	Gly	Asp	Ala	Thr	Tyr	Gly
			325						330						335
Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys	Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro
			340					345						350	
Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	Thr	Tyr	Gly	Val	Gln	Cys	Phe	Ser
		355					360						365		
Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	His	Asp	Phe	Phe	Lys	Ser	Ala	Met
	370					375					380				
Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly
385					390					395					400
Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val
				405					410						415
Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile
			420					425						430	
Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile
		435					440						445		
Met	Ala	Asp	Lys	Gln	Lys	Asn	Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg
	450					455						460			
His	Asn	Ile	Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln
465				470						475					480
Asn	Thr	Pro	Ile	Gly	Asp	Gly	Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr
				485					490						495
Leu	Ser	Thr	Gln	Ser	Ala	Leu	Ser	Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp
			500						505						510
His	Met	Val	Leu	Leu	Glu	Phe	Val	Thr	Ala	Ala	Gly	Ile	Thr	Leu	Gly
		515					520						525		
Met	Asp	Glu	Leu	Tyr	Lys										
			530												

&lt;210&gt; SEQ ID NO 34

&lt;211&gt; LENGTH: 816

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Unknown

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&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: r-ceCG

&lt;400&gt; SEQUENCE: 34

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atgcatcatc atcatcatca tgagacgctc caggggctgc tgctgtggat gctgctgagt      60
gttgcggggg tctgggcate cagggggcca ctgcgggcac tgtgccggcc catcaacgcc      120
actctggctg ctgagaagga ggcctgcccc atctgcatca ccttcaccac cagcatctgt      180
gccggctact gccccagcat ggtgcgggtg atgccagctg ccctgccggc cattccccag      240
ccagtgtgca cctaccgtga getgcgcttt gettccatcc ggctccccgg ctgccccgct      300
ggtgtggaac ccattggttc cttccccgtg gccctcagtt gtaactgctg gccctgccag      360
atcaagacca ctgactgctg ggtttttcaga gaccagccct tggcctgtgc cccccaggcc      420
tcctcttctc ctaaggatcc cccatcccaa cctctcacat ccacatccac cccaactcct      480
ggggccagca gacgttcctc tcacccctc ccaataaaga cttcctttcc tgatggagag      540
tttatgatgc aggggtgtcc tgaatgcaag ctaaaggaaa acaaatactt ctccaagcca      600
gacgtcccaa tctatcagtg catgggctgc tgcttctcca gggcataccc cactccagcg      660
aggtctaaga agacaatggt ggtccccaag aacatcacct cggaagccac atgctgtgtg      720
gccaaagcgt ttaccaaggc cacagtgacg ggaaacgtca gactggagaa ccacaccgac      780
tgccactgca gtacttgtaa ttatcacaaa tcttaa      816

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&lt;210&gt; SEQ ID NO 35

&lt;211&gt; LENGTH: 271

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Unknown

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: r-ceCG

&lt;400&gt; SEQUENCE: 35

```

Met His His His His His His Glu Thr Leu Gln Gly Leu Leu Leu Trp
 1          5          10          15
Met Leu Leu Ser Val Gly Gly Val Trp Ala Ser Arg Gly Pro Leu Arg
 20          25          30
Pro Leu Cys Arg Pro Ile Asn Ala Thr Leu Ala Ala Glu Lys Glu Ala
 35          40          45
Cys Pro Ile Cys Ile Thr Phe Thr Thr Ser Ile Cys Ala Gly Tyr Cys
 50          55          60
Pro Ser Met Val Arg Val Met Pro Ala Ala Leu Pro Ala Ile Pro Gln
 65          70          75          80
Pro Val Cys Thr Tyr Arg Glu Leu Arg Phe Ala Ser Ile Arg Leu Pro
 85          90          95
Gly Cys Pro Pro Gly Val Asp Pro Met Val Ser Phe Pro Val Ala Leu
100          105          110
Ser Cys His Cys Gly Pro Cys Gln Ile Lys Thr Thr Asp Cys Gly Val
115          120          125
Phe Arg Asp Gln Pro Leu Ala Cys Ala Pro Gln Ala Ser Ser Ser Ser
130          135          140
Lys Asp Pro Pro Ser Gln Pro Leu Thr Ser Thr Ser Thr Pro Thr Pro
145          150          155          160
Gly Ala Ser Arg Arg Ser Ser His Pro Leu Pro Ile Lys Thr Ser Phe
165          170          175

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Pro Asp Gly Glu Phe Met Met Gln Gly Cys Pro Glu Cys Lys Leu Lys  
 180 185 190

Glu Asn Lys Tyr Phe Ser Lys Pro Asp Ala Pro Ile Tyr Gln Cys Met  
 195 200 205

Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr Pro Ala Arg Ser Lys Lys  
 210 215 220

Thr Met Leu Val Pro Lys Asn Ile Thr Ser Glu Ala Thr Cys Cys Val  
 225 230 235 240

Ala Lys Ala Phe Thr Lys Ala Thr Val Thr Gly Asn Val Arg Val Glu  
 245 250 255

Asn His Thr Asp Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser  
 260 265 270

<210> SEQ ID NO 36  
 <211> LENGTH: 1605  
 <212> TYPE: DNA  
 <213> ORGANISM: Unknown  
 <220> FEATURE:  
 <223> OTHER INFORMATION: r-ceCG-GFP

<400> SEQUENCE: 36

atgcatcadc atcatcatca tgagacgctc caggggctgc tgctgtggat getgctgagt 60

gttgcggggg tctgggcadc cagggggcca ctgcgccac tgtgccggcc catcaacgcc 120

actctggctg ctgagaagga ggctgcccc atctgcatca ccttcaccac cagcatctgt 180

gccggctact gccccagcat ggtgcggtg atgccagctg ccctgccggc cattccccag 240

ccagtgtgca cctaccgtga gctgcgctt gcttccatcc ggctccccgg ctgccccct 300

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<210> SEQ ID NO 37
<211> LENGTH: 534
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: r-ceCG-GFP

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<400> SEQUENCE: 37

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Pro Leu Cys Arg Pro Ile Asn Ala Thr Leu Ala Ala Glu Lys Glu Ala
35        40        45
Cys Pro Ile Cys Ile Thr Phe Thr Thr Ser Ile Cys Ala Gly Tyr Cys
50        55        60
Pro Ser Met Val Arg Val Met Pro Ala Ala Leu Pro Ala Ile Pro Gln
65        70        75        80
Pro Val Cys Thr Tyr Arg Glu Leu Arg Phe Ala Ser Ile Arg Leu Pro
85        90        95
Gly Cys Pro Pro Gly Val Asp Pro Met Val Ser Phe Pro Val Ala Leu
100       105       110
Ser Cys His Cys Gly Pro Cys Gln Ile Lys Thr Thr Asp Cys Gly Val
115       120       125
Phe Arg Asp Gln Pro Leu Ala Cys Ala Pro Gln Ala Ser Ser Ser Ser
130       135       140
Lys Asp Pro Pro Ser Gln Pro Leu Thr Ser Thr Ser Thr Pro Thr Pro
145       150       155       160
Gly Ala Ser Arg Arg Ser Ser His Pro Leu Pro Ile Lys Thr Ser Phe
165       170       175
Pro Asp Gly Glu Phe Met Met Gln Gly Cys Pro Glu Cys Lys Leu Lys
180       185       190
Glu Asn Lys Tyr Phe Ser Lys Pro Asp Ala Pro Ile Tyr Gln Cys Met
195       200       205
Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr Pro Ala Arg Ser Lys Lys
210       215       220
Thr Met Leu Val Pro Lys Asn Ile Thr Ser Glu Ala Thr Cys Cys Val
225       230       235       240
Ala Lys Ala Phe Thr Lys Ala Thr Val Thr Gly Asn Val Arg Val Glu
245       250       255
Asn His Thr Asp Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser Glu
260       265       270
Asn Leu Tyr Phe Gln Ser Arg Ile Leu Gln Ser Thr Val Pro Arg Ala
275       280       285
Arg Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly Glu Glu Leu Phe
290       295       300
Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly
305       310       315       320

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His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly  
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Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro  
340 345 350

Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser  
355 360 365

Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met  
370 375 380

Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly  
385 390 395 400

Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val  
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Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile  
420 425 430

Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile  
435 440 445

Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg  
450 455 460

His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln  
465 470 475 480

Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr  
485 490 495

Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp  
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His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly  
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Met Asp Glu Leu Tyr Lys  
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<210> SEQ ID NO 38  
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<212> TYPE: DNA  
<213> ORGANISM: Unknown  
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<400> SEQUENCE: 38

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 <220> FEATURE:  
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&lt;210&gt; SEQ ID NO 44

&lt;211&gt; LENGTH: 5540

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Unknown

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: poeCG

&lt;400&gt; SEQUENCE: 44

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&lt;210&gt; SEQ ID NO 45

&lt;211&gt; LENGTH: 6329

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Unknown

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: poeCG-GFP

&lt;400&gt; SEQUENCE: 45

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<210> SEQ ID NO 46
<211> LENGTH: 5540
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: pceCG

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<400> SEQUENCE: 46

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<210> SEQ ID NO 48
<211> LENGTH: 4733
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: preGFP

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<400> SEQUENCE: 48

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1. A process for the production and purification of hybrid or non-hybrid recombinant glycoprotein hormones, comprising the steps of:

- (a) amplification, modification and cloning of the hybrid or non-hybrid molecules;
- (b) construction of the expression vectors of recombinant glycoprotein hormones;
- (c) transfection, expression and analysis of cells expressing the recombinant glycoprotein hormones;
- (d) purification of recombinant glycoprotein hormones by affinity chromatography;
- (e) dialysis and sterilization of recombinant glycoprotein hormones;

wherein the recombinant glycoprotein hormone (r-eCG) and its hybrid forms are selected from the group consisting of recombinant equine chorionic gonadotrophin (r-eCG), recombinant bovine chorionic gonadotrophin (r-bCG), recombinant suine chorionic gonadotrophin (r-sCG), recombinant ovary chorionic gonadotrophin (r-oCG), recombinant goat chorionic gonadotrophin (r-cCG), recombinant thyroid stimulat-

ing hormone (r-TSH), recombinant luteinizing hormone (r-LH) and recombinant follicle stimulating hormone (r-FSH).

2. The process, according to claim 1, wherein the recombinant glycoprotein hormone (r-eCG) and its hybrid forms are preferably recombinant equine chorionic gonadotrophin (r-eCG) and their hybrid forms.

3. The process, according to claim 1, wherein the amplification of the r-eCG gene fragments (SEQ. ID. 16) or r-eCG-GFP (SEQ.ID. 18), by PCR, using primer oligonucleotides (SEQ. ID. 1, SEQ. ID. 2 and SEQ. ID. 3) complementary to the different forms of native chorionic gonadotrophin to obtain a gene fragment relating to the fusion between the beta subunit DNA sequence of the native eCG (SEQ. ID. 6) and the DNA sequence of the alpha subunit of the native eCG (SEQ. ID. 4), wherein such SEQ. ID. 6 and 4 correspond to the a and 13 subunits of eCG and additional sequences corresponding to their total in SEQ. ID. 16 or SEQ. ID. ID. 18 validated by agarose gel electrophoresis.

4. The process according to claim 3, wherein SEQ. ID. 1, SEQ. ID. 2 and SEQ. ID. 3 still exhibit additional nucleotide

sequences associated with cleavage sites for restriction enzymes and coding sequences for a histidine tail and a proteolytic site for the TEV-Tag protease, associated with cloning of the gene sequences, purification of the recombinant hormones and protein editing thereof, respectively.

5. The process, according to claim 1, wherein the construction of vectors for the expression of recombinant glycoprotein hormones in eukaryotic cells (CHO-K1 and HEK 293) is initiated by the cloning of SEQ. ID. 16 or SEQ. ID. 18 in prokaryotic cells (*E. coli* DH5 $\alpha$ ).

6. The process of claim 5, wherein the cloning step is initiated with the insertion of the SEQ. ID. 16 or SEQ. ID. 18 sequences in a commercial vector which is used to transform the DH5 $\alpha$  competent cells by thermal shock, followed by selection of the bacterial clones containing the recombinant cloning vector with the SEQ. ID. 16 or SEQ. ID. 18 sequences, whose presences are validated by agarose gel electrophoresis and chemical DNA sequencing.

7. The process according to claim 5, wherein the expression vectors are used to transiently transduce eukaryotic cells with the aid of liposomes and in a stable manner, with the use of the SEQ. ID. 16 or SEQ. ID. 18 sequences via lentiviral vectors or biological safe systems, of non-random gene integration and without the need for selective agents.

8. The process according to claim 1, wherein the purification of glycoprotein hormones occurs by collecting the culture supernatant from mammalian cells transfected with the SEQ. ID. 16 or SEQ. ID. 18 sequences and that secrete the SEQ. ID. 17 or SEQ. ID. 19 sequences, transiently or stably, followed by affinity chromatography on nickel resins.

9. The process according to claim 1, wherein it is for the production of the SEQ. ID. 17, SEQ. ID. 19, SEQ. ID. 23, SEQ. ID. 25, SEQ. ID. 27, SEQ. ID. 29, SEQ. ID. 31, SEQ. ID. 33, SEQ. ID. 35 and SEQ. ID. 37 polypeptides, relating to recombinant equine chorionic gonadotrophin and its hybrid forms, from their respective DNA sequences and the use of nucleotide sequences of primers of the different forms of chorionic gonadotrophin and of cleavage sites for restriction enzymes and of DNA sequences coding for a histidine tail and a proteolytic site for TEV-Tag protease.

10. Hybrid or non-hybrid recombinant glycoprotein hormones produced by the process as defined in claim 1, comprising  $\alpha$  and  $\beta$  equine subunits or a from mammal and  $\beta$  from equine subunits, a purification marker, secretion signaling peptide of the molecule, a dimerization interface peptide, a specific proteolytic site and, optionally, a fluorescent label.

11. The hormones, according to claim 10, wherein the two subunits are fused in a single chain, and chain modifying agents in the amino and carboxy-terminal moieties.

12. The hormones, according to claim 10, wherein the chain modifying agents contain or do not contain a fusion to a fluorescence-emitting molecule, such as GFP.

13. The hormones, according to claim 10, wherein the purification label is such as the affinity sequences, such as the histidine tail.

14. The hormones, according to claim 10, wherein they are administered in an amount of 0.001 to 10,000  $\mu$ g, observing the body weight of the target animals.

15. The hormones, according to claim 10, wherein they conform with SEQ. ID. 1 to SEQ. ID. 49.

16. Expression vectors of recombinant glycoprotein hormones (hybrids and non-hybrids), wherein they are for transfection of eukaryotic cells via transient or stable transfection systems and are used as a source of homogenous and bioactive preparations of these hormones, wherein such vectors are SEQ. ID. 1 to SEQ. ID. 49, as defined in claim 10, associated with the production and purification of these recombinant glycoprotein hormones.

17. A pharmaceutical composition comprising a recombinant glycoprotein hormones, as defined in claim 10, and a carrier therefor.

18. The composition of claim 10, for use in assisted animal reproduction in mammal species generally of commercial interest or not, such as cattle, sheep, goats, swine, horses, mules, bubalins, bison, antelopes, domestic and wild species of canines and felines, cetaceans, ursids and primates.

19. The composition according to claim 17 for use in the induction of ovulation; induction of superovulation; follicular growth; induction of estrus; reversal of anestrous; puberty induction; use in IATF protocols (Fixed-Time Artificial Insemination), FIV (in vitro fertilization) protocols, TETF protocols (Fixed Time Embryo Transfer) in animals of commercial interest or not.

20. The composition according to claim 16, wherein the recombinant glycoprotein hormones are still used to obtain native (monoclonal or polyclonal) or recombinant (Phage Display) antibodies against these hormones (native and/or recombinant).

21. The composition according to claim 20, wherein as many antibodies as the recombinant glycoprotein hormones and derivatives thereof comprise hormone and anti-hormone detection kits in biological samples or not.

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