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SOUS UNITE DE GONADOTROPHINE B CHRONIQUE HUMAINE ET UTILISATIONS DE CELLE-CI
(54) Title: RECOMBINANT BIFUNCTIONAL PROTEIN OF HUMAN LUTROPIN RECEPTOR AND HUMAN CHORIONIC
GONADOTROPIN B-SUBUNIT AND USES THEREOF

(57) **Abrégé/Abstract:**

The present invention relates to a chimeric nucleic acid molecule encoding a fusion protein having a human lutropin hormone-receptor domain and a human chorionic gonadotropin- β subunit domain, the isolated human lutropin hormone-receptor/human chorionic gonadotropin- β fusion protein, and antibodies which recognize part or all the domains of the fusion protein. Also provided are compositions having the human lutropin hormone-receptor/human chorionic gonadotropin- β fusion protein and a pharmaceutical carrier and compositions including antibodies to the fusion protein and a pharmaceutical carrier. The present invention also relates to methods of treating androgen-excess-mediated disease conditions by administering to a subject an antibody to the human lutropin hormone-receptor/human chorionic gonadotropin- β fusion protein under conditions suitable to treat the conditions, and methods of immunocontraception that involve administering to a subject the chimeric nucleic acid molecule encoding the human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein, the fusion protein in whole or in part, or antibodies thereto.



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**RECOMBINANT BIFUNCTIONAL PROTEIN OF HUMAN LUTROPIN
RECEPTOR AND HUMAN CHORIONIC GONADOTROPIN B-SUBUNIT
AND USES THEREOF**

5 **[0001]** This application claims the benefit of U.S. Provisional Patent
Application Serial No. 60/613,246, filed September 27, 2004.

[0002] The subject matter of this application was made with support from
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1 R01 HD37109-01A1. The U.S. Government may have certain rights.

10

FIELD OF THE INVENTION

[0003] The present invention relates to a chimeric nucleic acid molecule
encoding a human lutropin hormone receptor/human chorionic gonadotropin- β
fusion protein, an expressed recombinant human lutropin hormone
15 receptor/human chorionic gonadotropin- β fusion protein, antibodies to the protein,
and uses thereof.

BACKGROUND OF THE INVENTION

[0004] Pituitary lutropin hormone (LH) is suppressed during the luteal
20 phase of the menstrual cycle by negative feedback from ovarian steroids (Leung et
al., "Interactions of Steroids and Gonadotropins in the Control of Steroidogenesis
in the Ovarian Follicle," *Annu Rev Physiol* 42:71-82 (1980); Christenson et al.,
"Maturation of Ovarian Follicles in the Prepubertal Gilt," *J Reprod Fertil Suppl*
33:21-36 (1985) and Abraham et al., "Simultaneous Radioimmunoassay of
25 Plasma FSH, LH, Progesterone, 17-Hydroxyprogesterone, and Estradiol-17 β
During the Menstrual Cycle," *J Clin Endocrinol* 34:312-318 (1972)). Human
chorionic gonadotropin (hCG), a placental hormone produced in the female at the
time of implantation, provides a backup for pituitary LH to rescue the corpus
luteum of pregnancy. hCG is a heterodimeric glycoprotein hormone consisting of
30 an α and a β subunit that stimulates intracellular levels of cAMP via a G-protein-
coupled receptor. A distinct feature of the hCG- β subunit is the presence of a C-

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terminal extension (CTP) of 24 amino acids, which produce specific antibodies to hCG- β with little cross-reaction with LH. In a gonadal cell, hCG binds to its cell surface receptor (LH/CG-R), resulting in an increase in the concentration of intracellular cAMP (Wu et al., "Protein Engineering of Novel Constitutively

5 Active Hormone-Receptor Complex," *J Biol Chem* 271:31638-31642 (1996). hCG and hLH (human LH) are identical in structure except for the CTP of the β -subunit of hCG, which is highly glycosylated and has three serine-linked carbohydrate moieties (Cole et al., "The Structures of the Serine-Linked Sugar

10 Chains on Human Chorionic Gonadotropin," *Biochem Biophys Res Commun* 126 (1):333-339 (1985)). hLH and hCG, by virtue of their structural similarity, bind to the same lutropin receptor (LH-R) in the gonads. The LH-R is also a member of the G protein-coupled receptor (GPCR) family and contains a relatively large, highly glycosylated, N-terminal extracellular domain (ECD) known for high affinity ligand binding (Xie et al., "Extracellular Domain of

15 Lutropin/Choriogonadotropin Receptor Expressed in Transfected Cells Binds Choriogonadotropin with High Affinity," *J Biol Chem* 265(35):21411-21414 (1990) and Ji et al., "Exons 1-10 of the Rat LH Receptor Encode a High Affinity Hormone Binding Site and Exon 11 Encodes G-Protein Modulation and a

20 Potential Second Hormone Binding Site," *Endocrinology* 128:2648-2650 (1991)), a seven-transmembrane domain, and a short intracellular cytoplasmic tail (McFarland et al., "Lutropin-Choriogonadotropin Receptor: An Unusual Member of the G Protein-Coupled Receptor Family," *Science* 245(4917):494-499 (1989)).

The extracellular domain is characterized by a motif of imperfect leucine-rich repeats, which contributes largely to the high affinity hormone binding of the

25 receptor (Xie et al., "Extracellular Domain of Lutropin/Choriogonadotropin Receptor Expressed in Transfected Cells Binds Choriogonadotropin with High Affinity," *J Biol Chem* 265(35):21411-21414 (1990)). cDNAs of LH receptor have considerable interspecies homology among vertebrates (Ascoli et al., The

30 Lutropin/Choriogonadotropin Receptor, A 2002 Perspective," *Endocr Rev* 23(2):141-174 (2002)). hLH-R and hCG- β are also antigenic at the interspecies level (Ascoli et al., The Lutropin/Choriogonadotropin Receptor, A 2002 Perspective," *Endocr Rev* 23(2):141-174 (2002); Pal et al., "Active Immunization

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of Baboons (*Papio anubis*) with the Bovine LH Receptor,” *J Reprod Immunol* 21(2):163-174 (1992); Pal et al., “Biological Actions of Monoclonal Antibodies to Bovine Lutropin Receptor,” *J Reprod Immunol* 22(1):103 (1992); Remy et al., “Immunization Against Exon 1 Decapeptides From the

5 Lutropin/Choriogonadotropin Receptor or the Follitropin Receptor as Potential Male Contraceptive,” *J Reprod Immunol* 32(1):37-54 (1996); Singh et al., “Effect of Immunization with Lutropin-Receptor on the Ovarian Function of Rabbits,” *J Immunoassay* 16(1):1-16 (1995), Vaitukaitis et al., “A Radioimmunoassay Which Specifically Measures Human Chorionic Gonadotropin in the Presence of Human

10 Luteinizing Hormone,” *Am J Obstet Gynecol* 113(6):751-758 (1972)). Hence, hLH-R and hCG, and their functional epitopes, provide vital targets to be manipulated by genetic engineering to produce unique anti-fertility antigens.

[0005] Efficacies of antibodies to the hLH-R and hCG- β individually have been amply demonstrated in the regulation of gonadal function. There are a

15 number of reports that qualify LH-R as a potential anti-fertility antigen (Remy et al., “Immunization Against Exon 1 Decapeptides From the Lutropin/Choriogonadotropin Receptor or the Follitropin Receptor as Potential Male Contraceptive,” *J Reprod Immunol* 32(1):37-54 (1996); Saxena et al., “Modulation of Ovarian Function in Female Dogs Immunized With Bovine

20 Luteinizing Hormone Receptor,” *Reprod Domest Anim* 37(1):9-17 (2002), and Saxena et al., “Effect of Immunization with Bovine Luteinizing Hormone Receptor on Ovarian Function in Cats,” *Am J Vet Res* 64(3):292-298 (2003)). The injection of the recombinant mouse LH-R with hormone binding region to male mice induced immunity against the receptor. The data indicated that the specific

25 anti-gonadotropin receptor vaccination could potentially be used as a fertility control procedure in males (Remy et al., “Immunization Against Exon 1 Decapeptides From the Lutropin/Choriogonadotropin Receptor or the Follitropin Receptor as Potential Male Contraceptive,” *J Reprod Immunol* 32(1):37-54 (1996)). Previous studies showed that actively immunizing female dogs and cats

30 with highly purified bovine LH-R can produce antibodies to the LH-R, which suppresses progesterone synthesis due to the blockade of the gonadal receptor (Saxena et al., “Modulation of Ovarian Function in Female Dogs Immunized With Bovine Luteinizing Hormone Receptor,” *Reprod Domest Anim* 37(1):9-17 (2002)

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and Saxena et al., "Effect of Immunization with Bovine Luteinizing Hormone Receptor on Ovarian Function in Cats," *Am J Vet Res* 64(3):292-298 (2003)).

[0006] Elucidation of the human genome has provided an impetus for genetic engineering, gene therapy, and stem cell research. Recently, gene based
5 expression of functional recombinant proteins and their epitopes as well as antibodies against them have permitted the topographical mapping of receptors to identify active sites and understand the mechanism of ligand receptor binding. Synthesis of chimeric genes composed of desired functional epitopes of more than one gene to produce recombinant proteins has become possible (Sugahara et al.,
10 "Biosynthesis of a Biologically Active Single Chain Containing the Human Common α and Chorionic Gonadotropin β Subunits in Tandem," *Proc Natl Acad Sci USA* 92:2041-2045 (1995); Sugahara et al., "Expression of Biologically Active Fusion Genes Encoding the Common α Subunit and Either the CG β of FSH β Subunits: Role of a Linker Sequence," *Mol Cell Endocrinol* 125:71-77 (1996);
15 and Heikoop et al., "Structure-Based Design and Protein Engineering of Intersubunit Disulfide Bonds in Gonadotropins," *Nat Biotechnol* 15(7):658-662 (1997); Heikoop et al., "Evaluation of Subunit Truncation and the Nature of the Spacer for Single Chain Human Gonadotropins," *Eur J. Biochem* 245(3):656-662 (1997)). A single peptide chain containing α and β subunits of human chorionic
20 gonadotropin (hCG) in tandem has been shown to exhibit biological activity (Sugahara et al., "Biosynthesis of a Biologically Active Single Chain Containing the Human Common α and Chorionic Gonadotropin β Subunits in Tandem," *Proc Natl Acad Sci USA* 92:2041-2045 (1995)). Fusion genes encoding the common α -subunit of hCG and FSH- β subunit also expressed a biologically active protein
25 (Sugahara et al., "Expression of Biologically Active Fusion Genes Encoding the Common α Subunit and Either the CG β of FSH β Subunits: Role of a Linker Sequence," *Mol Cell Endocrinol* 125:71-77 (1996)). Narayan et al., "Yoked Complexes of Human Choriogonadotropin and the Lutropin Receptor: Evidence that Monomeric Individual Subunits are Inactive," *Mol Endocrinol* 16(12):2733-
30 2745 (2002), constructed two (hCG)-LHR complexes where the two hormone subunits and the receptor were engineered to form single polypeptide chains.

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[0007] Chemical conjugates to create functional chimeric proteins have been made to enhance their immunogenicity and/or to modulate metabolic clearance rates and biological activity (Klein et al., "Pharmacokinetics and Pharmacodynamics of Single-Chain Recombinant Human Follicle-Stimulating Hormone Containing the Human Chorionic Gonadotropin Carboxyterminal Peptide in the Rhesus Monkey," *Fertil Steril* 77(6):1248-1255 (2002)). Chimeric proteins have also been produced where the common human α -subunit of human glycoprotein hormone has been non-covalently linked to the hormone specific β -subunits of hCG and hFSH to express respective intact hormones, and their ability to interact with LH-hCG and FSH receptors has been examined (Campbell et al., "Conversion of Human Choriogonadotropin Into a Follitropin by Protein Engineering," *Proc Natl Acad Sci USA* 88(3):760-764 (1991)). The active sites of human FSH have been chemically modified to alter their activity by using an azidobenzoyl derivative of a glycopeptide isolated from the fetuin digest by photoactivation (Rathnam et al., "Conjugation of a Fetuin Glycopeptide to Human Follicle-Stimulating Hormone and its Subunits by Photoactivation," *Biochim Biophys Acta* 624(2):436-442 (1980)). Chemical reactions used for the addition, deletion, or replacement of functional epitopes have been fraught with non-specific side reactions and changes in the conformation and function of the proteins. However, the availability of nucleic acid sequences of expressed proteins has opened new avenues to synthesize nucleic acid constructs to express hybrids of two different proteins. This is achieved by the use of site-specific restriction enzymes and ligases to produce chimeric genes, which, in turn, can express the corresponding hybrid proteins. A single-chain construct containing hCG α and β subunits in tandem was fused with the N-terminus of receptor LH-R to investigate their structure-function relationship (Wu et al., "Protein Engineering of Novel Constitutively Active Hormone-Receptor Complex," *J Biol Chem* 271:31638-31642 (1996)).

[0008] What is needed now is a chimeric nucleic acid molecule that encodes a fusion protein having both a lutropin hormone receptor protein, or a fragment thereof, and a human chorionic gonadotropin protein or a fragment thereof, where the lutropin hormone receptor protein or fragment, and the human chorionic gonadotropin protein or fragment thereof, are immunogenic. Such a

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bifunctional, immunogenic protein would provide a unique antigen for immunocontraception and gonadal regulation in vertebrates, including humans. The present invention is directed to overcoming these and other deficiencies in the art.

5

SUMMARY OF THE INVENTION

[0009] The present invention relates to a chimeric nucleic acid molecule encoding a fusion protein having a human lutropin hormone receptor domain and a human chorionic gonadotropin- β domain. The chimeric nucleic acid molecule of the present invention encodes a fusion protein that includes a full length human lutropin hormone receptor or an antigenic fragment thereof, and a full length human chorionic gonadotropin- β or an antigenic fragment thereof.

[0010] The present invention also relates to an isolated human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein having a human lutropin hormone receptor domain and a human chorionic gonadotropin- β domain.

[0011] Another aspect of the present invention is an isolated antibody, or binding portion thereof, which recognizes the human lutropin hormone receptor domain and the human chorionic gonadotropin- β domain of the human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention.

[0012] Another aspect of the present invention is an isolated antibody, or binding portion thereof, which recognizes only the human lutropin hormone receptor domain of the human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention.

[0013] Another aspect of the present invention is an isolated antibody, or binding portion thereof, which recognizes only the human chorionic gonadotropin- β domain of the human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention.

[0014] The present invention also relates to an isolated antibody, or binding portion thereof, which recognizes a human lutropin hormone receptor protein or a fragment thereof.

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[0015] The present invention also relates to a composition having an antibody, or binding portion thereof, that recognizes the human lutropin hormone receptor domain and the human chorionic gonadotropin- β domain of the human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention, and a pharmaceutical carrier.

[0016] The present invention also relates to a composition having an antibody, or binding portion thereof, that recognizes only the human lutropin hormone receptor domain of the human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention, and a pharmaceutical carrier.

[0017] The present invention also relates to a composition having an antibody, or binding portion thereof, that recognizes only the human chorionic gonadotropin- β domain of the human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention, and a pharmaceutical carrier.

[0018] Another aspect of the present invention is a method of immunocontraception. This method involves administering an antibody that recognizes the human lutropin hormone receptor domain and the human chorionic gonadotropin- β domain of the human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention to a subject under conditions effective to provide immunocontraception to the subject.

[0019] Another method of immunocontraception is provided in the present invention. This method involves administering an antibody recognizing a human lutropin hormone receptor protein, or a fragment thereof, to a subject under conditions effective to provide immunocontraception to the subject.

[0020] Yet another method of immunocontraception provided by the present invention involves administering the human lutropin receptor/human chorionic gonadotropin- β fusion protein of the present invention to a subject under conditions effective to provide immunocontraception to the subject.

[0021] The present invention relates to yet another method of immunocontraception. This method involves administering the chimeric nucleic acid molecule of the present invention that encodes a human lutropin hormone

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receptor/human chorionic gonadotropin- β fusion protein to a subject under conditions effective to provide immunocontraception to the subject.

[0022] Another aspect of the present invention is a method of treating an androgen-excess-mediated disease condition in a subject. This method involves
5 administering an antibody, or a binding portion thereof, that recognizes the human lutropin hormone receptor domain and the human chorionic gonadotropin- β domain of the human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention, to a subject under conditions effective to treat the androgen-excess-mediated disease condition.

10 [0023] The present invention also relates to another method of treating an androgen-excess-mediated disease condition in a subject. This method involves administering an antibody, or a binding portion thereof, that recognizes the human lutropin hormone receptor domain, but not the human chorionic gonadotropin- β domain of the human lutropin hormone receptor/human chorionic gonadotropin- β
15 fusion protein of the present invention, to a subject under conditions effective to treat the androgen-excess-mediated disease condition.

[0024] The present invention also relates to nucleic acid constructs, expression vectors, and hosts that include a chimeric nucleic acid molecule encoding a fusion protein having a human lutropin hormone receptor domain and
20 a human chorionic gonadotropin- β domain.

[0025] The lutropin hormone receptor protein (hLH-R) and chorionic gonadotropin- β hormone (CG- β) are structurally and functionally similar and play an important role in reproduction via a common gonadal receptor (LH-R). A chimeric protein of LH-R conjugated with hCG- β could presumably become more
25 antigenic since it will present to the immune system as a heterologous antigen to facilitate antibody response in a subject. Thus, in females, the antibody to a bifunctional protein containing LH-R and hCG- β moieties would block the binding of LH to the receptor, whereas antibodies to hCG- β would neutralize hCG produced by the blastocyst and inhibit implantation. In males, antibodies to such
30 a bifunctional protein could be useful in blocking the LH receptor, leading to infertility. Thus, the present invention, which relates to a chimeric nucleic acid molecule encoding a hLH-R and hCG- β fusion protein, provides a unique antigen

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for immunocontraception and gonadal regulation in vertebrates, including humans.

BRIEF DESCRIPTION OF THE DRAWINGS

5 [0026] Figure 1 is a schematic diagram of the chimeric hLH-R-hCG- β nucleic acid molecule of the present invention. Full-length hCG- β with signal peptide is linked via two amino acids at the C-terminal of hLH-R. The N-terminus of hLH-R contains the signal peptide (SP) of hLH-R.

[0027] Figure 2 shows an agarose gel electrophoresis of nucleic acid
10 constructs before and after enzyme digestion of vector containing chimeric construct of full length cDNA of hLH-R and hCG- β . Lane 1: vector pBlueBac+hLH-R + hCG- β digested with *Bam*HI, *Bgl* II, and *Eco*RI. Lane 2: vector pBlueBac+hLH-R + hCG- β digested with *Bgl* II and *Eco*RI. Lane 3: vector pBlueBac+hLH-R + hCG- β without enzyme digestion. M: DNA size
15 markers.

[0028] Figures 3A-B are western blots of solubilized membrane proteins from Sf9 cells transfected with the hLH-R-hCG- β chimeric construct. Sf9 cells transfected by recombinant baculovirus were collected after 72 hrs of transfection. Sixty μ g of solubilized membrane proteins were separated on a 7.5% SDS-PAGE
20 under reducing conditions. Figure 3A shows blot probed with antibodies to LH-R. Figure 3B shows blot probed with antibodies to hCG- β . Lane 1: Membrane proteins from transfected Sf9 cells probed with antibodies. Lane 2: Membrane proteins from mock-transfected Sf9 cells probed with antibodies. M: Protein markers.

25 [0029] Figures 4A-B are graphs showing results of competitive binding assays determining the ability of the hLH-R-hCG- β fusion protein to bind to hCG ligand. Figure 4A shows the percent specific binding of biotinylated-hCG to recombinant chimeric protein hLH-R-hCG- β , expressed in 2×10^6 Sf9 insect cells. Figure 4B shows the percent specific binding of biotinylated-hCG to hLH-R
30 alone, expressed in 2×10^6 Sf9 insect cells. Total binding of biotinylated-hCG in the absence of unlabeled hCG was adjusted to 100%. Specific binding of

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biotinylated-hCG was determined from the displacement of bound biotinylated-hCG at increasing concentrations of unlabeled hCG. The data are shown as Mean $\pm$ SD of three independent experiments.

[0030] Figures 5A-B are graphs showing intracellular cyclic AMP stimulation expressed in 2×10^6 Sf9 insect cells as compared with cAMP in the culture medium. Figure 5A shows results of stimulation by the recombinant chimeric hLH-R-hCG- β protein. Figure 5B shows results of stimulation by LH-R protein alone. Dose-response curve of hCG-mediated increase in intracellular cAMP in Sf9 insect cells transfected with chimeric DNA construct as well as cAMP levels in the intercellular incubation medium. The data are shown as Means $\pm$ SD of three independent experiments.

[0031] Figure 6 is a schematic diagram of chimeric gene hLH-R-ECD-hCG- β -CTP. The extracellular domain (ECD) of hLH-R with the signal peptide (SP) at its N-terminus was fused to the N-terminus of hCG- β -CTP using Gly-Thr as the linker dipeptide.

[0032] Figure 7 shows the agarose gel electrophoresis of a chimeric DNA construct containing the extracellular domain of hLH-R and the hCG- β -CTP domain (hLH-R-ECD-hCG- β construct) after enzyme digestion of vector pBlueBac4.5 containing the hLH-R-ECD-hCG- β construct. Lane 1: recombinant DNA digested with *Bam*H I and *Kpn* I. Lane 2: recombinant DNA digested with *Bam*H I, *Kpn* I and *Eco*R I. M: DNA size markers.

[0033] Figure 8 shows the agarose electrophoresis separation of approximately 60 μ g of solubilized membrane proteins of Sf9 cells on 7.5% SDS-PAGE under reducing conditions. Lane 1: solubilized proteins from mock-transfected Sf9 cells. Lane 2: solubilized proteins from transfected Sf9 cells with hLH-R-ECD DNA construct. Lane 3: solubilized proteins from transfected Sf9 cells with chimeric DNA construct. M: Protein size markers (36 kDa, 48 kDa, 62 kDa and 79kDa).

[0034] Figures 9A-B are western blots of solubilized membrane proteins from Sf9 cells transfected with hLH-R-ECD-hCG- β CTP chimeric construct. Sf9 cells transfected by recombinant baculovirus were collected after 72 hrs of transfection. Sixty μ g of solubilized membrane proteins were separated on a 7.5%

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SDS-PAGE under reducing conditions. Figure 9A shows blot probed with antibodies to LH-R. Lane 1: solubilized proteins from mock-transfected Sf9 cells. Lane 2: solubilized proteins from Sf9 cells transfected with the hLH-R-ECD construct. Lane 3: solubilized protein from Sf9 cells transfected with the hLH-R-ECD/hCG-CTP chimeric DNA construct. Figure 9B shows blot probed with antibodies to hCG- β . Lane 1: solubilized proteins from Sf9 cells transfected with the hLH-R-ECD construct. Lane 2: solubilized protein from Sf9 cells transfected with the hLH-R-ECD/hCG-CTP chimeric DNA construct. Lane 3: Solubilized proteins from mock-transfected Sf9 cells. M: Protein size markers.

10 [0035] Figure 10 is a graph showing the percent binding of biotinylated-hCG to recombinant chimeric protein hLH-R-ECD-hCG- β expressed in 2×10^6 Sf9 insect cells. Total binding of biotinylated hCG in the absence of unlabeled hCG was adjusted to 100%. Binding of biotinylated-hCG was determined from the displacement of bound biotinylated-hCG at increasing concentrations of unlabeled hCG. Data are presented as Mean $\pm$ SD of three independent experiments.

[0036] Figures 11A-C are graphs showing the ability of the hLH-R-ECD-hCG- β -CTP recombinant chimeric protein to stimulate intracellular cyclic AMP, expressed in 2×10^6 Sf9 insect cells as compared with cAMP in the culture medium. Figure 11A shows the stimulation of cAMP production by the chimeric protein in the transfected Sf9 cells as well as in the incubation medium in the absence and presence of increasing quantities of hCG. Figure 11B shows the stimulation of cAMP production by the LH-R-ECD protein alone in the transfected Sf9 cells as well as in the incubation medium in the absence and presence of increasing quantities of hCG. Figure 11C shows the intracellular cAMP concentrations at different time intervals measured after Sf9 cells transfected with chimeric construct (O) and ECD-hLH-R alone ($\blacktriangle$) incubating with 0.1ng/ml hCG (C). The data are presented as Mean $\pm$ SD of three independent experiments.

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DETAILED DESCRIPTION OF THE INVENTION

[0037] The present invention relates to a chimeric nucleic acid molecule encoding a fusion protein having a human lutropin hormone receptor domain and a human chorionic gonadotropin- β domain. The fusion protein of the present invention includes a full length human lutropin hormone receptor or an antigenic fragment thereof, and a full length human chorionic gonadotropin- β or an antigenic fragment thereof, and includes additional amino acids suitable for linking together the human lutropin hormone receptor domain and the human chorionic gonadotropin- β domain.

10 [0038] An exemplary full length human lutropin hormone receptor suitable for use in the human lutropin hormone receptor/ human chorionic gonadotropin- β fusion protein of the present invention has the amino acid of SEQ ID NO:1, as follows:

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

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	Ser	His	Ala	Phe	Asn	Gly	Thr	Thr	Leu	Thr	Ser	Leu	Glu	Leu	Lys	Glu
			195					200					205			
5	Asn	Val	His	Leu	Glu	Lys	Met	His	Asn	Gly	Ala	Phe	Arg	Gly	Ala	Thr
		210					215					220				
	Gly	Pro	Lys	Thr	Leu	Asp	Ile	Ser	Ser	Thr	Lys	Leu	Gln	Ala	Leu	Pro
10		225				230					235					240
	Ser	Tyr	Gly	Leu	Glu	Ser	Ile	Gln	Arg	Leu	Ile	Ala	Thr	Ser	Ser	Tyr
					245					250					255	
15	Ser	Leu	Lys	Lys	Leu	Pro	Ser	Arg	Glu	Thr	Phe	Val	Asn	Leu	Leu	Glu
				260					265					270		
	Ala	Thr	Leu	Thr	Tyr	Pro	Ser	His	Cys	Cys	Ala	Phe	Arg	Asn	Leu	Pro
			275					280					285			
20	Thr	Lys	Glu	Gln	Asn	Phe	Ser	His	Ser	Ile	Ser	Glu	Asn	Phe	Ser	Lys
		290					295					300				
	Gln	Cys	Glu	Ser	Thr	Val	Arg	Lys	Val	Ser	Asn	Lys	Thr	Leu	Tyr	Ser
25		305				310					315					320
	Ser	Met	Leu	Ala	Glu	Ser	Glu	Leu	Ser	Gly	Trp	Asp	Tyr	Glu	Tyr	Gly
					325					330					335	
30	Phe	Cys	Leu	Pro	Lys	Thr	Pro	Arg	Cys	Ala	Pro	Glu	Pro	Asp	Ala	Phe
				340					345					350		
	Asn	Pro	Cys	Glu	Asp	Ile	Met	Gly	Tyr	Asp	Phe	Leu	Arg	Val	Leu	Ile
			355					360					365			
35	Trp	Leu	Ile	Asn	Ile	Leu	Ala	Ile	Met	Gly	Asn	Met	Thr	Val	Leu	Phe
		370					375					380				
	Val	Leu	Leu	Thr	Ser	Arg	Tyr	Lys	Leu	Thr	Val	Pro	Arg	Phe	Leu	Met
40		385				390					395					400
	Cys	Asn	Leu	Ser	Phe	Ala	Asp	Phe	Cys	Met	Gly	Leu	Tyr	Leu	Leu	Leu
					405					410					415	
45	Ile	Ala	Ser	Val	Asp	Ser	Gln	Thr	Lys	Gly	Gln	Tyr	Tyr	Asn	His	Ala
				420					425					430		
	Ile	Asp	Trp	Gln	Thr	Gly	Ser	Gly	Cys	Ser	Thr	Ala	Gly	Phe	Phe	Thr
			435					440					445			
50	Val	Phe	Ala	Ser	Glu	Leu	Ser	Val	Tyr	Thr	Leu	Thr	Val	Ile	Thr	Leu
		450					455					460				
	Glu	Arg	Trp	His	Thr	Ile	Thr	Tyr	Ala	Ile	His	Leu	Asp	Gln	Lys	Leu
55		465				470					475					480
	Arg	Leu	Arg	His	Ala	Ile	Leu	Ile	Met	Leu	Gly	Gly	Trp	Leu	Phe	Ser
					485					490					495	
60	Ser	Leu	Ile	Ala	Met	Leu	Pro	Leu	Val	Gly	Val	Ser	Asn	Tyr	Met	Lys
				500					505					510		

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	Val	Ser	Ile	Cys	Phe	Pro	Met	Asp	Val	Glu	Thr	Thr	Leu	Ser	Gln	Val	
			515					520					525				
5	Tyr	Ile	Leu	Thr	Ile	Leu	Ile	Leu	Asn	Val	Val	Ala	Phe	Phe	Ile	Ile	
		530					535					540					
	Cys	Ala	Cys	Tyr	Ile	Lys	Ile	Tyr	Phe	Ala	Val	Arg	Asn	Pro	Glu	Leu	
	545					550					555					560	
10	Met	Ala	Thr	Asn	Lys	Asp	Thr	Lys	Ile	Ala	Lys	Lys	Met	Ala	Ile	Leu	
					565					570					575		
	Ile	Phe	Thr	Asp	Phe	Thr	Cys	Met	Ala	Pro	Ile	Ser	Phe	Phe	Ala	Ile	
15				580					585					590			
	Ser	Ala	Ala	Phe	Lys	Val	Pro	Leu	Ile	Thr	Val	Thr	Asn	Ser	Lys	Val	
			595					600					605				
20	Leu	Leu	Val	Leu	Phe	Tyr	Pro	Ile	Asn	Ser	Cys	Ala	Asn	Pro	Phe	Leu	
		610					615					620					
	Tyr	Ala	Ile	Phe	Thr	Lys	Thr	Phe	Gln	Arg	Asp	Phe	Phe	Leu	Leu	Leu	
	625					630					635					640	
25	Ser	Lys	Phe	Gly	Cys	Cys	Lys	Arg	Arg	Ala	Glu	Leu	Tyr	Arg	Arg	Lys	
				645						650					655		
	Asp	Phe	Ser	Ala	Tyr	Thr	Ser	Asn	Cys	Lys	Asn	Gly	Phe	Thr	Gly	Ser	
30				660					665					670			
	Asn	Lys	Pro	Ser	Gln	Ser	Thr	Leu	Lys	Leu	Ser	Thr	Leu	His	Cys	Gln	
			675					680					685				
35	Gly	Thr	Ala	Leu	Leu	Asp	Lys	Thr	Arg	Tyr	Thr	Glu	Cys				
		690					695					700					

This full length human lutropin hormone receptor protein is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO:2, as follows:

	atgaagcagc	ggttctcggc	gctgcagctg	ctgaagctgc	tgctgctgct	gcagctgcag	60
	ccgccgctgc	cacgagcgct	gcgcgaggcg	ctctgccctg	agccctgcaa	ctgcgtgccc	120
	gacggcgccc	tgcgctgccc	cggcccccacg	gccggtctca	ctcgactatc	acttgcctac	180
	ctccctgtca	aagtgatccc	atctcaagct	ttcagaggac	ttaatgaggt	cataaaaatt	240
45	gaaatctctc	agattgattc	cctggaaagg	atagaagcta	atgcctttga	caacctcctc	300
	aatttgtctg	aaatactgat	ccagaacacc	aaaaatctga	gatacattga	gcccggagca	360
	tttataaatc	ttcccggatt	aaaatacttg	agcatctgta	acacaggcat	cagaaagttt	420
	ccagatgtta	cgaagggtctt	ctcctctgaa	tcaaatttca	ttctggaaat	ttgtgataac	480
	ttacacataa	ccaccatacc	aggaaatgct	tttcaaggga	tgaataatga	atctgtaaca	540
50	ctcaaactat	atggaaatgg	atttgaagaa	gtacaaagtc	atgcattcaa	tgggacgaca	600
	ctgacttcac	tggagctaaa	ggaaaacgta	catctggaga	agatgcacaa	tggagccttc	660
	cgtggggcca	cagggccgaa	aaccttggat	atttcttcca	ccaaattgca	ggccctgccc	720
	agctatggcc	tagagtccat	tcagaggcta	attgccacgt	catcctattc	tctaaaaaaa	780
	ttgccatcaa	gagaaacatt	tgtcaatctc	ctggaggcca	cgttgactta	ccccagccac	840
55	tgctgtgctt	ttagaaactt	gccaacaaaa	gaacagaatt	tttcacattc	catttctgaa	900
	aacttttcca	aacaatgtga	aagcacagta	aggaaagtga	gtaacaaaac	actttattct	960
	tccatgcttg	ctgagagtga	actgagtggc	tgggactatg	aatatggttt	ctgcttacct	1020
	aagacacccc	gatgtgctcc	tgaaccagat	gctttttaatc	cctgtgaaga	cattatgggc	1080

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5  tatgacttcc ttagggctcct gatttggctg attaatatc tagccatcat gggaaacatg 1140
   actgttcttt ttgttctcct gacaagtcgt tacaaactta cagtgcctcg ttttctcatg 1200
   tgcaatctct cctttgcaga cttttgcatg gggctctatc tgctgctcat agcctcagtt 1260
   gattcccaaa ccaagggcca gtactataac catgccatag actggcagac agggagtggg 1320
   tgcagcactg ctggcttttt cactgtattc gcaagtgaac tttctgtcta caccctcacc 1380
   gtcatcactc tagaaagatg gcacaccatc acctatgcta ttcacctgga ccaaagctg 1440
   cgattaagac atgccattct gattatgctt ggaggatggc tcttttcttc tctaattgct 1500
   atgttgcccc ttgtcgggtg cagcaattac atgaaggcca gtatttgctt ccccatggat 1560
   gtggaaacca ctctctcaca agtctatata ttaaccatcc tgattctcaa tgtggtggcc 1620
10 ttcttcataa tttgtgcttg ctacattaaa atttattttg cagttcgaaa cccagaatta 1680
   atggctacca ataaagatac aaagattgct aagaaaatgg caatcctcat cttcacctgat 1740
   ttcacctgca tggcacctat ctcttttttt gccatctcag ctgccttcaa agtacctctt 1800
   atcacagtaa ccaactctaa agttttactg gttctttttt atcccatcaa ttcttggtgcc 1860
   aatccatttc tgtatgcaat attcactaag acattccaaa gagatttctt tcttttgctg 1920
15 agcaaatttg gctgctgtaa acgtcgggct gaactttata gaaggaaaga tttttcagct 1980
   tacacctcca actgcaaaaa tggcttcact ggatcaaata agccttctca atccaccttg 2040
   aagttgtcca cattgcactg tcaaggtaga gctctcctag acaagactcg ctacacagag 2100
   tgt                                     2103

```

20 **[0039]** An exemplary full length human chorionic gonadotropin- β subunit suitable for use in a human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention has the amino acid of SEQ ID NO:3, as follows:

```

25 Met Glu Met Phe Gln Gly Leu Leu Leu Leu Leu Leu Leu Ser Met Gly
    1              5              10              15

   Gly Thr Trp Ala Ser Lys Glu Pro Leu Arg Pro Arg Cys Arg Pro Ile
        20              25              30

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

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

35

   Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg
    65              70              75              80

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

   Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu
    100             105             110

45 Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu
    115             120             125

   Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro
    130             135             140

   Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr
    145             150             155             160

55 Pro Ile Leu Pro Gln
    165

```

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This full length human chorionic gonadotropin- β subunit is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO:4, as follows:

```

5  atggagatgt tccaggggct gctgctgttg ctgctgctga gcatggggcgg gacatgggca 60
   tccaaggagc cgcttcggcc acggtgccgc cccatcaatg ccaccctggc tgtggagaag 120
   gagggctgcc ccgtgtgcat caccgtcaac accaccatct gtgccggcta ctgccccacc 180
   atgaccgcg tgctgcaggg ggtcctgccg gccctgcctc aggtgggtgtg caactaccgc 240
   gatgtgcgct tcgagtccat ccggctccct ggctgcccgc gcggcgtgaa ccccggtggtc 300
   tcctacgccg tggctctcag ctgtcaatgt gcactctgcc gccgcagcac cactgactgc 360
10 ggggggtccca aggaccaccc cttgacctgt gatgaccccc gcttccagga ctcctcttcc 420
   tcaaaggccc ctccccccag ccttccaagt ccatcccgac tcccggggcc ctcgacaccc 480
   ccgatcctcc cacaac                                     495

```

[0040] An exemplary fragment of the human lutropin hormone receptor domain suitable for use in a human lutropin hormone receptor/ human chorionic gonadotropin- β fusion protein is N-terminal extracellular domain (ECD) of the hLH-R, known for high affinity ligand binding. The ECD of hLH-R has the amino acid sequence of SEQ ID NO:5, as follows:

```

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

```

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	Ala	Phe	Asn	Gly	Thr	Thr	Leu	Thr	Ser	Leu	Glu	Leu	Lys	Glu	Asn	Val	
			195					200					205				
5	His	Leu	Glu	Lys	Met	His	Asn	Gly	Ala	Phe	Arg	Gly	Ala	Thr	Gly	Pro	
		210					215					220					
	Lys	Thr	Leu	Asp	Ile	Ser	Ser	Thr	Lys	Leu	Gln	Ala	Leu	Pro	Ser	Tyr	
	225					230					235					240	
10	Gly	Leu	Glu	Ser	Ile	Gln	Arg	Leu	Ile	Ala	Thr	Ser	Ser	Tyr	Ser	Leu	
					245					250					255		
	Lys	Lys	Leu	Pro	Ser	Arg	Glu	Thr	Phe	Val	Asn	Leu	Leu	Glu	Ala	Thr	
15				260					265					270			
	Leu	Thr	Tyr	Pro	Ser	His	Cys	Cys	Ala	Phe	Arg	Asn	Leu	Pro	Thr	Lys	
			275					280					285				
	Glu	Gln	Asn	Phe	Ser	His	Ser	Ile	Ser	Glu	Asn	Phe	Ser	Lys	Gln	Cys	
20		290					295					300					
	Glu	Ser	Thr	Val	Arg	Lys	Val	Ser	Asn	Lys	Thr	Leu	Tyr	Ser	Ser	Met	
	305					310					315					320	
25	Leu	Ala	Glu	Ser	Glu	Leu	Ser	Gly	Trp	Asp	Tyr	Glu	Tyr	Gly	Phe	Cys	
					325					330					335		
	Leu	Pro	Lys	Thr	Pro	Arg	Cys	Ala	Pro	Glu	Pro						
				340					345								
30																	

The hLH-R -is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO:6, as follows:

	atgaagcagc	ggttctcggc	gctgcagctg	ctgaagctgc	tgctgctgct	gcagccgccc	60
	ctgccacgag	cgctgcgcga	ggcgctctgc	cctgagccct	gcaactgcgt	gcccgcgccc	120
35	gccctgcgct	gccccggccc	cacggccggt	ctcactcgac	tatcacttgc	ctacctccct	180
	gtcaaagtga	tcccatctca	agctttcaga	ggacttaatg	aggtcataaa	aattgaaatc	240
	tctcagattg	attccctgga	aaggatagaa	gctaatgcct	ttgacaacct	cctcaatttg	300
	tctgaaatac	tgatccagaa	caccaaaaat	ctgagataca	ttgagcccgg	agcattttata	360
	aatcttcccc	gattaaaata	cttgagcatc	tgtaacacag	gcatcagaaa	gtttccagat	420
40	gttacgaagg	tcttctcctc	tgaatcaaat	ttcattctgg	aaatttgtga	taacttacac	480
	ataaccacca	taccaggaaa	tgcttttcaa	gggatgaata	atgaatctgt	aacactcaaa	540
	ctatatggaa	atggatttga	agaagtacaa	agtcatgcat	tcaatgggac	gacactgact	600
	tactgggagc	taaaggaaaa	cgtacatctg	gagaagatgc	acaatggagc	cttccgtggg	660
	gccacagggc	cgaaaacctt	ggatatattt	tccaccaa	at	gagagctat	720
45	ggcctagagt	ccattcagag	gctaattgcc	acgtcatcct	attctctaaa	aaaattgcca	780
	tcaagagaaa	catttgtcaa	tctcctggag	gccacgttga	cttaccaccag	ccactgctgt	840
	gcttttagaa	acttgccaac	aaaagaacag	aattttttcac	attccatttc	tgaaaacttt	900
	tccaaacaat	gtgaaagcac	agtaaggaaa	gtgagtaaca	aaacacttta	ttcttccatg	960
	cttgctgaga	gtgaactgag	tggtctgggac	tatgaatatg	gtttctgctt	acccaagaca	1020
50	ccccgatgtg	ctcctgaacc	a				1041

[0041] An exemplary fragment of the human chorionic gonadotropin- β subunit suitable for the human lutropin hormone receptor/ human chorionic

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gonadotropin- β fusion protein of the present invention has the amino acid sequence of SEQ ID NO:7, as follows:

```

5   Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala
    1      5      10      15
    Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp
      20      25      30
10  Thr Pro Ile Leu Pro Gln
    35

```

[0042] This fragment of the human chorionic gonadotropin- β subunit, which includes the carboxyl terminal peptide (CTP) of the human chorionic gonadotropin- β domain, is encoded by a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:8 as follows:

```

    ttgacctgtg atgacccccg cttccaggac tcctcttcct caaaggcccc tccccccagc   60
    cttccaagtc catcccgact cccggggccc tcggacaccc cgatcctccc acaa          114
20

```

[0043] Another exemplary full length human lutropin hormone receptor suitable for use in the hLH-R/hCG fusion protein of the present invention is a protein that is a variant of SEQ ID NO:1. This protein has an amino acid sequence of SEQ ID NO:9, as follows:

```

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

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	Ser	Ile	Cys	Asn	Thr	Gly	Ile	Arg	Lys	Phe	Pro	Asp	Val	Thr	Lys	Val	
	130						135					140					
5	Phe	Ser	Ser	Glu	Ser	Asn	Phe	Ile	Leu	Glu	Ile	Cys	Asp	Asn	Leu	His	
	145					150					155					160	
	Ile	Thr	Thr	Ile	Pro	Gly	Asn	Ala	Phe	Gln	Gly	Met	Asn	Asn	Glu	Ser	
					165					170					175		
10	Val	Thr	Leu	Lys	Leu	Tyr	Gly	Asn	Gly	Phe	Glu	Glu	Val	Gln	Ser	His	
				180					185					190			
	Ala	Phe	Asn	Gly	Thr	Thr	Leu	Thr	Ser	Leu	Glu	Leu	Lys	Glu	Asn	Val	
15			195					200					205				
	His	Leu	Glu	Lys	Met	His	Asn	Gly	Ala	Phe	Arg	Gly	Ala	Thr	Gly	Pro	
	210						215					220					
20	Lys	Thr	Leu	Asp	Ile	Ser	Ser	Thr	Lys	Leu	Gln	Ala	Leu	Pro	Ser	Tyr	
	225					230					235					240	
	Gly	Leu	Glu	Ser	Ile	Gln	Arg	Leu	Ile	Ala	Thr	Ser	Ser	Tyr	Ser	Leu	
					245					250					255		
25	Lys	Lys	Leu	Pro	Ser	Arg	Glu	Thr	Phe	Val	Asn	Leu	Leu	Glu	Ala	Thr	
				260					265					270			
	Leu	Thr	Tyr	Pro	Ser	His	Cys	Cys	Ala	Phe	Arg	Asn	Leu	Pro	Thr	Lys	
30			275					280					285				
	Glu	Gln	Asn	Phe	Ser	His	Ser	Ile	Ser	Glu	Asn	Phe	Ser	Lys	Gln	Cys	
		290					295					300					
35	Glu	Ser	Thr	Val	Arg	Lys	Val	Ser	Asn	Lys	Thr	Leu	Tyr	Ser	Ser	Met	
	305					310					315					320	
	Leu	Ala	Glu	Ser	Glu	Leu	Ser	Gly	Trp	Asp	Tyr	Glu	Tyr	Gly	Phe	Cys	
					325					330					335		
40	Leu	Pro	Lys	Thr	Pro	Arg	Cys	Ala	Pro	Glu	Pro	Asp	Ala	Phe	Asn	Pro	
				340					345					350			
	Cys	Glu	Asp	Ile	Met	Gly	Tyr	Asp	Phe	Leu	Arg	Val	Leu	Ile	Trp	Leu	
45			355					360					365				
	Ile	Asn	Ile	Leu	Ala	Ile	Met	Gly	Asn	Met	Thr	Val	Leu	Phe	Val	Leu	
		370					375					380					
50	Leu	Thr	Ser	Arg	Tyr	Lys	Leu	Thr	Val	Pro	Arg	Phe	Leu	Met	Cys	Asn	
	385					390					395					400	
	Leu	Ser	Phe	Ala	Asp	Phe	Cys	Met	Gly	Leu	Tyr	Leu	Leu	Leu	Ile	Ala	
				405						410					415		
55	Ser	Val	Asp	Ser	Gln	Thr	Lys	Gly	Gln	Tyr	Tyr	Asn	His	Ala	Ile	Asp	
				420				425						430			
	Trp	Gln	Thr	Gly	Ser	Gly	Cys	Ser	Thr	Ala	Gly	Phe	Phe	Thr	Val	Phe	
60			435				440						445				

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	Ala	Ser	Glu	Leu	Ser	Val	Tyr	Thr	Leu	Thr	Val	Ile	Thr	Leu	Glu	Arg
	450						455					460				
5	Trp	His	Thr	Ile	Thr	Tyr	Ala	Ile	His	Leu	Asp	Gln	Lys	Leu	Arg	Leu
	465					470					475					480
	Arg	His	Ala	Ile	Leu	Ile	Met	Leu	Gly	Gly	Trp	Leu	Phe	Ser	Ser	Leu
					485					490					495	
10	Ile	Ala	Met	Leu	Pro	Leu	Val	Gly	Val	Ser	Asn	Tyr	Met	Lys	Val	Ser
				500					505					510		
	Ile	Cys	Phe	Pro	Met	Asp	Val	Glu	Thr	Thr	Leu	Ser	Gln	Val	Tyr	Ile
15			515					520					525			
	Leu	Thr	Ile	Leu	Ile	Leu	Asn	Val	Val	Ala	Phe	Phe	Ile	Ile	Cys	Ala
		530					535					540				
20	Cys	Tyr	Ile	Lys	Ile	Tyr	Phe	Ala	Val	Arg	Asn	Pro	Glu	Leu	Met	Ala
	545					550					555					560
	Thr	Asn	Lys	Asp	Thr	Lys	Ile	Ala	Lys	Lys	Met	Ala	Ile	Leu	Ile	Phe
					565					570					575	
25	Thr	Asp	Phe	Thr	Cys	Met	Ala	Pro	Ile	Ser	Phe	Phe	Ala	Ile	Ser	Ala
				580					585					590		
	Ala	Phe	Lys	Val	Pro	Leu	Ile	Thr	Val	Thr	Asn	Ser	Lys	Val	Leu	Leu
30			595					600					605			
	Val	Leu	Phe	Tyr	Pro	Ile	Asn	Ser	Cys	Ala	Asn	Pro	Phe	Leu	Tyr	Ala
		610					615					620				
35	Ile	Phe	Thr	Lys	Thr	Phe	Gln	Arg	Asp	Phe	Phe	Leu	Leu	Leu	Ser	Lys
	625					630					635					640
	Phe	Gly	Cys	Cys	Lys	Arg	Arg	Ala	Glu	Leu	Tyr	Arg	Arg	Lys	Asp	Phe
					645					650					655	
40	Ser	Ala	Tyr	Thr	Ser	Asn	Cys	Lys	Asn	Gly	Phe	Thr	Gly	Ser	Asn	Lys
				660					665					670		
	Pro	Ser	Gln	Ser	Thr	Leu	Lys	Leu	Ser	Thr	Leu	His	Cys	Gln	Gly	Thr
45			675				680						685			
	Ala	Leu	Leu	Asp	Lys	Thr	Arg	Tyr	Thr	Glu	Cys					
		690					695									

50 This variant full-length hLH-R protein is encoded by a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:10, as follows:

55	atgaagcagc	ggttctcggc	gctgcagctg	ctgaagctgc	tgctgctgct	gcagccgccg	60
	ctgccacgag	cgctgcgcga	ggcgctctgc	cctgagccct	gcaactgcgt	gcccgcgcgc	120
	gccctgcgct	gccccggccc	cacggccggt	ctcactcgac	tatcacttgc	ctacctccct	180
	gtcaaagtga	tcccatctca	agctttcaga	ggacttaatg	aggtcataaa	aattgaaatc	240
	tctcagattg	attccctgga	aaggatagaa	gctaattgcct	ttgacaacct	cctcaatttg	300
	tctgaaatac	tgatccagaa	caccaaaaat	ctgagatata	ttgagcccgc	agcatttata	360

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aatcttcccg gattaaaata cttgagcatc tgtaacacag gcatcagaaa gtttccagat 420
gttacgaagg tcttctcctc tgaatcaa attcattctgg aaatttgtga taacttacac 480
ataaccacca taccaggaaa tgcttttcaa gggatgaata atgaatctgt aacactcaaa 540
ctatatggaa atggatttga agaagtacaa agtcatgcat tcaatgggac gacactgact 600
5 tcaactggagc taaaggaaaa cgtacatctg gagaagatgc acaatggagc cttccgtggg 660
gccacagggc cgaaaacctt ggatatttct tccaccaa at tgcaggccct gccgagctat 720
ggcctagagt ccattcagag gctaattgcc acgtcatcct attctctaaa aaaattgcca 780
tcaagagaaa catttgtcaa tctcctggag gccacgttga cttaccccag ccactgctgt 840
gcttttagaa acttgccaac aaaagaacag aatttttcac attccatttc tgaaaacttt 900
10 tccaaacaat gtgaaagcac agtaaggaaa gtgagtaaca aaacacttta ttcttccatg 960
cttgctgaga gtgaactgag tggctgggac tatgaatatg gtttctgctt acccaagaca 1020
ccccgatgtg ctctgaacc agatgctttt aatccctgtg aagacattat gggctatgac 1080
ttccttaggg tcctgatttg gctgattaat attctagcca tcatgggaaa catgactgtt 1140
ctttttgttc tcctgacaag tcgttacaaa cttacagtgc ctcgttttct catgtgcaat 1200
15 ctctcctttg cagacttttg catggggctc tatctgctgc tcatagcctc agttgattcc 1260
caaaccaagg gccagtacta taaccatgcc atagactggc agacagggag tgggtgcagc 1320
actgctggct ttttactgtt attcgcaagt gaactttctg tctacaccct caccgtcatc 1380
actctagaaa gatggcacac catcacctat gctattcacc tggaccaaaa gctgcgatta 1440
agacatgcca ttctgattat gcttgaggga tggctctttt cttctcta at tgctatgttg 1500
20 ccccttgctg gtgtcagcaa ttacatgaag gtcagtattt gcttccccat ggatgtggaa 1560
accactctct cacaagtcta tatattaacc atcctgatcc tcaatgtggg gcccttcttc 1620
ataatttgtg cttgctacat taaaatttat tttgcagttc gaaacccaga attaatggct 1680
accaataaag atacaaagat tgctaagaaa atggcaatcc tcatcttcac cgatttcacc 1740
tgcatggcac ctatctcttt ttttgccatc tcagctgcct tcaaagtacc tcttatcaca 1800
25 gtaaccaact ctaaagtttt actggttctt ttttatccca tcaattcttg tgccaatcca 1860
tttctgtatg caatattcac taagacattc caaagagatt tctttctttt gctgagcaaa 1920
tttggtgctg gtaaacgtcg ggctgaactt tatagaagga aagatttttc agcttacacc 1980
tccaactgca aaaatggctt cactggatca aataagcctt ctcaatccac cttgaagttg 2040
tccacattgc actgtcaagg tacagctctc ctagacaaga ctcgctacac agagtgt 2097
30

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This variant human lutropin hormone receptor (SEQ ID NO:10) differs from the human lutropin hormone receptor protein of SEQ ID NO:1 in lacking two amino acids, a leucine and a glycine, that are present in SEQ ID NO:1 at positions 19 and 20, respectively. SEQ ID NO:10 is missing the corresponding encoding

nucleotides that are present in SEQ ID NO:2 at positions 55-60. However, the variant protein shows no functional difference as compared with the receptor protein having SEQ ID NO:1 (Atger et al., "Structure of the Human Luteinizing Hormone-Choriogonadotropin Receptor Gene: Unusual Promoter and 5' Non-Coding Regions," *Mol Cell Endocrinol* 111(2):113-123 (1995), which is hereby incorporated by reference in its entirety).

[0044] Also suitable for making the human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention are proteins encoded by a nucleic acid molecule having at least 96% similarity, preferably 97%, most preferably 98% similarity to a nucleic acid molecule having SEQ ID Nos. 2, 4, 6, 8, or 10. The extent of similarity between two sequences can be based on percent sequence identity. "Identity" as used herein means the extent to which two (nucleotide or amino acid) sequences are invariant. Sequence

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“similarity” or “identity” in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. The similarity of two proteins (or nucleic acids) can be determined using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin et al., “Methods for Assessing the Statistical Significance of Molecular Sequence Features by Using General Scoring Schemes,” *Proc. Natl. Acad. Sci. USA* 87(6):2264-2268 (1990), modified as in Karlin et al., “Applications and Statistics for Multiple High-Scoring Segments in Molecular Sequences,” *Proc. Natl. Acad. Sci. USA* 90(12):5873-5877 (1993), which are hereby incorporated by reference in their entirety. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., *J. Mol. Biol.* 215:403-410 (1990), which is hereby incorporated by reference in its entirety. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., “Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs,” *Nucleic Acids Res.* 25:3389-3402 (1997), which is hereby incorporated by reference in its entirety. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Altschul et al., “Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs,” *Nucleic Acids Res.* 25:3389-3402 (1997), which is hereby incorporated by reference in its entirety). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. (See the NCBI website for more information.) Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the CGC sequence alignment software

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package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and
5 Robotti, *Comput. Appl. Biosci.* 10:3-5 (1994), which is hereby incorporated by reference in its entirety; and FASTA described in Pearson et al., "Improved tools for biological sequence comparison," *Proc Natl Acad Sci USA* 85:2444-8 (1988), which is hereby incorporated by reference in its entirety. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search. If ktup=2, similar
10 regions in the two sequences being compared are found by looking at pairs of aligned residues; if ktup=1, single aligned amino acids are examined. ktup can be set to 2 or 1 for protein sequences, or from 1 to 6 for DNA sequences. The default if ktup is not specified is 2 for proteins and 6 for DNA.

[0045] Also suitable for the preparation of the chimeras of the present
15 invention is any nucleic acid molecule capable of hybridizing to a nucleic acid molecule having SEQ ID Nos. 2, 4, 6, 8, or 10 under highly stringent conditions. The term "stringent conditions" as used herein refers to parameters which are familiar in the art. Nucleic acid hybridization parameters may be found in references which compile such methods, e.g. Sambrook et al., *Molecular Cloning:*
20 *A Laboratory Manual*, eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989) or *Current Protocols in Molecular Biology*, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995). Methods as taught by a manufacturer of hybridization supplies and reagents are also highly suitable for use in the present invention (e.g., Invitrogen,
25 Carlsbad, CA). More specifically, stringent conditions, as used herein, refers, for example, to hybridization at 56°-65° C in hybridization buffer having 4-5X SSC, 0.02% ficoll, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin, 0.5% SDS, 2 mM EDTA, where SSC is 0.1 5M sodium chloride/0.015M sodium citrate, pH 7; SDS is sodium dodecyl sulfate; and EDTA is ethylenediaminetetraacetic
30 acid. After hybridization, the membrane upon which the DNA is immobilized is washed in 2X SSC at room (ambient) temperature and then washed one or more times at 0.1X SSC/0.1% SDS, at temperatures from ambient temperature up to 68° C. There are other conditions, reagents, and so forth, which can be used, and

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would result in a similar degree of stringency. The precise conditions for any particular hybridization are left to those skilled in the art because there are variables involved in nucleic acid hybridizations beyond those of the specific nucleic acid molecules to be hybridized that affect the choice of hybridization conditions. These variables include: the substrate used for nucleic acid hybridization (e.g., charged vs. non-charged membrane); the detection method used (e.g., radioactive vs. chemiluminescent); and the source and concentration of the nucleic acid involved in the hybridization. All of these variables are routinely taken into account by those skilled in the art prior to undertaking a nucleic acid hybridization procedure. The skilled artisan will be familiar with such conditions, and thus they are not given here. It will be understood, however, that the skilled artisan will be able to manipulate the conditions in a manner to permit the clear identification of homologs and alleles of the hLH-R and hCG nucleic acids of the invention.

15 **[0046]** Suitable variant nucleic acid molecules having at least 98% similarity to SEQ ID Nos. 2, 4, 6, 8, and 10, or which are capable of hybridizing under stringent conditions as characterized herein, will encode a protein having a high amino acid identity to the native protein. Such nucleotide variants can be expected to encode a protein variant that is functionally equivalent to the human
20 lutropin hormone receptor and human chorionic gonadotropin- β proteins of the present invention having SEQ ID Nos. 1, 3, 5, 7 and 9. By "functionally equivalent" it is meant that the variant protein has substantially the same biological effect as the native protein of interest, in particular with regard to the antigenic properties of the protein.

25 **[0047]** An indication that two protein sequences are substantially identical is that one protein is immunologically reactive with antibodies raised against the second protein. A protein is substantially identical to a second protein, for example, where the two proteins differ only by a conservative substitution. Generally, sequence identity of an amino acid of the invention is at least 80%,
30 85% or 87%, alternatively at least 92%, 94%, or 96%, and preferably 98% sequence identity to the native proteins of the present invention (and their naturally occurring alleles).

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[0048] In one aspect of the present invention, the fusion protein of the present invention includes the full length human lutropin hormone receptor (SEQ ID NO:1) and the full length human chorionic gonadotropin- β subunit (SEQ ID NO: 3) joined to form a bifunctional chimeric fusion protein (chimera 1). A small peptide linker consisting of two amino acids, Arg-Ser, is located between the C-terminus of the hLH-R domain and the N-terminus of the hCG- β domain to form a contiguous hLH-R-hCG- β fusion protein. This fusion protein has the amino acid sequence of SEQ ID NO:11, as follows:

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

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	Ser	Tyr	Gly	Leu	Glu	Ser	Ile	Gln	Arg	Leu	Ile	Ala	Thr	Ser	Ser	Tyr
					245					250					255	
5	Ser	Leu	Lys	Lys	Leu	Pro	Ser	Arg	Glu	Thr	Phe	Val	Asn	Leu	Leu	Glu
				260					265					270		
	Ala	Thr	Leu	Thr	Tyr	Pro	Ser	His	Cys	Cys	Ala	Phe	Arg	Asn	Leu	Pro
			275					280					285			
10	Thr	Lys	Glu	Gln	Asn	Phe	Ser	His	Ser	Ile	Ser	Glu	Asn	Phe	Ser	Lys
		290					295					300				
	Gln	Cys	Glu	Ser	Thr	Val	Arg	Lys	Val	Ser	Asn	Lys	Thr	Leu	Tyr	Ser
15		305				310					315					320
	Ser	Met	Leu	Ala	Glu	Ser	Glu	Leu	Ser	Gly	Trp	Asp	Tyr	Glu	Tyr	Gly
					325					330					335	
	Phe	Cys	Leu	Pro	Lys	Thr	Pro	Arg	Cys	Ala	Pro	Glu	Pro	Asp	Ala	Phe
20				340					345					350		
	Asn	Pro	Cys	Glu	Asp	Ile	Met	Gly	Tyr	Asp	Phe	Leu	Arg	Val	Leu	Ile
			355					360					365			
25	Trp	Leu	Ile	Asn	Ile	Leu	Ala	Ile	Met	Gly	Asn	Met	Thr	Val	Leu	Phe
		370					375					380				
	Val	Leu	Leu	Thr	Ser	Arg	Tyr	Lys	Leu	Thr	Val	Pro	Arg	Phe	Leu	Met
30		385				390					395					400
	Cys	Asn	Leu	Ser	Phe	Ala	Asp	Phe	Cys	Met	Gly	Leu	Tyr	Leu	Leu	Leu
					405					410					415	
	Ile	Ala	Ser	Val	Asp	Ser	Gln	Thr	Lys	Gly	Gln	Tyr	Tyr	Asn	His	Ala
35				420					425					430		
	Ile	Asp	Trp	Gln	Thr	Gly	Ser	Gly	Cys	Ser	Thr	Ala	Gly	Phe	Phe	Thr
			435					440					445			
	Val	Phe	Ala	Ser	Glu	Leu	Ser	Val	Tyr	Thr	Leu	Thr	Val	Ile	Thr	Leu
40		450					455					460				
	Glu	Arg	Trp	His	Thr	Ile	Thr	Tyr	Ala	Ile	His	Leu	Asp	Gln	Lys	Leu
45		465				470					475					480
	Arg	Leu	Arg	His	Ala	Ile	Leu	Ile	Met	Leu	Gly	Gly	Trp	Leu	Phe	Ser
				485						490					495	
	Ser	Leu	Ile	Ala	Met	Leu	Pro	Leu	Val	Gly	Val	Ser	Asn	Tyr	Met	Lys
50				500					505					510		
	Val	Ser	Ile	Cys	Phe	Pro	Met	Asp	Val	Glu	Thr	Thr	Leu	Ser	Gln	Val
			515					520					525			
55	Tyr	Ile	Leu	Thr	Ile	Leu	Ile	Leu	Asn	Val	Val	Ala	Phe	Phe	Ile	Ile
		530					535					540				
	Cys	Ala	Cys	Tyr	Ile	Lys	Ile	Tyr	Phe	Ala	Val	Arg	Asn	Pro	Glu	Leu
60		545				550					555					560

[illegible]

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This human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention is encoded by a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:12, as follows:

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5
atgaagcagc ggttctcggc gctgcagctg ctgaagctgc tgctgctgct gcagctgcag 60
ccgccgctgc cagagcgctt gcgcgaggcg ctctgccctg agccctgcaa ctgcgtgccc 120
gacggcgccc tgcgctgccc cgccccacg gccggtctca ctcgactatc acttgccctac 180
ctccctgtca aagtgatccc atctcaagct ttcagaggac ttaatgaggt cataaaaaatt 240
10 gaaatctctc agattgattc cctggaaagg atagaagcta atgcctttga caacctcctc 300
aatttgctctg aaatactgat ccagaacacc aaaaatctga gatacattga gcccgagaca 360
tttataaatc ttcccggatt aaaatacttg agcatctgta acacaggcat cagaaagttt 420
ccagatgtta cgaagggtctt ctctcttgaa tcaaatattca ttctggaaat ttgtgataac 480
ttacacataa ccaccatacc aggaaatgct tttcaaggga tgaataatga atctgtaaca 540
15 ctcaaactat atggaaatgg atttgaagaa gtacaaagtc atgcattcaa tgggacgaca 600
ctgacttcac tggagctaaa ggaacacgta catctggaga agatgcacaa tggagccttc 660
cgtggggcca cagggccgaa aaccttggat atttcttcca ccaaattgca ggccctgccg 720
agctatggcc tagagtccat tcagaggcta attgccacgt catcctattc tctaaaaaaa 780
ttgccatcaa gagaaacatt tgtcaatctc ctggaggcca cgttgactta cccagccac 840
20 tgctgtgctt ttagaaactt gccaacaaaa gaacagaatt tttcacattc catttctgaa 900
aacttttcca aacaatgtga aagcacagta aggaaagtga gtaacaaaac actttattct 960
tccatgcttg ctgagagtga actgagtggc tgggactatg aatatggttt ctgcttacct 1020
aagacacccc gatgtgctcc tgaaccagat gcttttaatc cctgtgaaga cattatgggc 1080
tatgacttcc ttagggctct gatttggctg attaatattc tagccatcat gggaaacatg 1140
25 actgttcttt ttgttctcct gacaagtcgt taaaaactta cagtgcctcg ttttctcatg 1200
tgcaatctct cctttgcaga cttttgcatg gggctctatc tgctgctcat agcctcagtt 1260
gattcccaaa ccaagggcca gtactataac catgccatag actggcagac agggagtggg 1320
tgcagcactg ctggcttttt cactgtattc gcaagtgaac tttctgtcta caccctcacc 1380
gtcatcactc tagaaagatg gcacaccatc acctatgcta ttcacctgga ccaaaagctg 1440
30 cgattaagac atgccattct gattatgctt ggaggatggc tcttttcttc tctaattgct 1500
atgttgcccc ttgtcgggtg cagcaattac atgaaggta gtatttgctt ccccatggat 1560
gtggaaccca ctctctcaca agtctatata ttaaccatcc tgattctcaa tgtggtggcc 1620
ttcttcataa tttgtgcttg ctacattaaa atttattttg cagttcgaaa cccagaatta 1680
atggctacca ataaagatac aaagattgct aagaaaatgg caatcctcat cttcacccgat 1740
35 ttcacctgca tggcacctat ctcttttttt gccatctcag ctgccttcaa agtacctctt 1800
atcacagtaa ccaactctaa agttttactg gttctttttt atcccatcaa ttcttggtgcc 1860
aatccatttc tgtatgcaat attcactaag acattccaaa gagatttctt tcttttgctg 1920
agcaaatttg gctgctgtaa acgtcgggct gaactttata gaaggaaaga tttttcagct 1980
tacacctcca actgcaaaaa tggcttcact ggatcaaata agccttctca atccaccttg 2040
40 aagttgtcca cattgcactg tcaaggtaca gctctcctag acaagactcg ctacacagag 2100
tgtagatcta tggagatgtt ccaggggctg ctgctgttgc tgctgctgag catgggcggg 2160
acatgggcat ccaaggagcc gcttcggcca cggtgccgcc ccatcaatgc caccctggct 2220
gtggagaagg agggctgccc cgtgtgcate accgtcaaca ccaccatctg tgccggctac 2280
tgccccacca tgaccgcgct gctgcagggg gtccctgccg ccctgcctca ggtggtgtgc 2340
45 aactaccgcg atgtgcgctt cgagtcctac cggtccctg gctgcccgcg cggcgtgaac 2400
cccggtggtc cctacgccgt ggctctcagc tgtcaatgtg cactctgccg ccgcagcacc 2460
actgactgcg ggggtcccaa ggaccacccc ttgacctgtg atgacccccg cttccaggac 2520
tcctcttcct caaaggcccc tccccccagc cttccaagtc catcccgact cccggggccc 2580
50 tcggacaccc cgatcctccc acaa
2604

```

[0049] In another aspect of the present invention, the fusion protein of the present invention includes a full length human lutropin hormone receptor (SEQ ID NO: 1) and a suitable fragment of the full length human chorionic gonadotropin- β subunit (SEQ ID NO: 7). A small peptide linker consisting of two amino acids, Gly-Thr, is located between the C-terminus of the hLH-R domain and the N-

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terminus of the hCG- β domain to form a contiguous hLH-R-hCG- β fusion protein. This fusion protein (chimera 2) has the amino acid sequence of SEQ ID NO:13, as follows:

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

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						275																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
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		595				600						605					
		Val	Leu	Phe	Tyr	Pro	Ile	Asn	Ser	Cys	Ala	Asn	Pro	Phe	Leu	Tyr	Ala
		610						615					620				
5		Ile	Phe	Thr	Lys	Thr	Phe	Gln	Arg	Asp	Phe	Phe	Leu	Leu	Leu	Ser	Lys
		625					630					635					640
		Phe	Gly	Cys	Cys	Lys	Arg	Arg	Ala	Glu	Leu	Tyr	Arg	Arg	Lys	Asp	Phe
10						645					650					655	
		Ser	Ala	Tyr	Thr	Ser	Asn	Cys	Lys	Asn	Gly	Phe	Thr	Gly	Ser	Asn	Lys
					660					665					670		
15		Pro	Ser	Gln	Ser	Thr	Leu	Lys	Leu	Ser	Thr	Leu	His	Cys	Gln	Gly	Thr
				675					680					685			
		Ala	Leu	Leu	Asp	Lys	Thr	Arg	Tyr	Thr	Glu	Cys	Gly	Thr	Leu	Thr	Cys
20			690					695					700				
		Asp	Asp	Pro	Arg	Phe	Gln	Asp	Ser	Ser	Ser	Ser	Lys	Ala	Pro	Pro	Pro
		705					710					715					720
		Ser	Leu	Pro	Ser	Pro	Ser	Arg	Leu	Pro	Gly	Pro	Ser	Asp	Thr	Pro	Ile
25					725						730					735	
		Leu	Pro	Gln													

- 30 This human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention is encoded by the nucleic acid molecule having the nucleotide sequence of SEQ ID NO:14, as follows:

	atgaagcagc	ggttctcggc	gctgcagctg	ctgaagctgc	tgctgctgct	gcagccgccc	60
	ctgccacgag	cgctgcgcga	ggcgctctgc	cctgagccct	gcaactgcgt	gcccgcgccc	120
35	gccctgcgct	gccccggccc	cacggccggt	ctcactcgac	tatcacttgc	ctacctccct	180
	gtcaaagtga	tcccatctca	agctttcaga	ggacttaatg	aggtcataaa	aattgaaatc	240
	tctcagattg	attccctgga	aaggatagaa	gctaattgcct	ttgacaacct	cctcaatttg	300
	tctgaaatac	tgatccagaa	caccaaaaat	ctgagataca	ttgagcccgg	agcattttata	360
	aatcttcccg	gattaaaata	cttgagcatc	tgtaacacag	gcatcagaaa	gtttccagat	420
40	gttacgaagg	tcttctcctc	tgaatcaaat	ttcattctgg	aaatttgtga	taacttacac	480
	ataaccacca	taccaggaaa	tgcttttcaa	gggatgaata	atgaatctgt	aacactcaaa	540
	ctatatggaa	atggatttga	agaagtacaa	agtcatgcat	tcaatgggac	gacactgact	600
	tcactggagc	taaaggaaaa	cgtacatctg	gagaagatgc	acaatggagc	cttccgtggg	660
	gccacagggc	cgaaaacctt	ggatatttct	tccaccaaat	tgcaggccct	gccgagctat	720
45	ggcctagagt	ccattcagag	gctaattgcc	acgtcatcct	attctctaaa	aaaattgccca	780
	tcaagagaaa	catttgtcaa	tctcctggag	gccacgttga	cttaccaccag	ccactgctgt	840
	gcttttagaa	acttgccaac	aaaagaacag	aatttttcac	attccatttc	tgaaaacttt	900
	tccaaacaat	gtgaaagcac	agtaaggaaa	gtgagtaaca	aaacacttta	ttcttccatg	960
	cttgctgaga	gtgaactgag	tggctgggac	tatgaatatg	gtttctgctt	acccaagaca	1020
50	ccccgatgtg	ctcctgaacc	agatgctttt	aatccctgtg	aagacattat	gggctatgac	1080
	ttccttaggg	tcctgatttg	gctgattaat	attctagcca	tcattgggaa	catgactgtt	1140
	ctttttgttc	tcctgacaag	tcgttacaaa	cttacagtgc	ctcgttttct	catgtgcaat	1200
	ctctcctttg	cagacttttg	catggggctc	tatctgctgc	tcatagcctc	agttgattcc	1260
	caaaccaagg	gccagtacta	taaccatgcc	atagactggc	agacagggag	tgggtgcagc	1320
55	actgctggct	ttttcactgt	attcgcaagt	gaactttctg	tctacaccct	caccgtcatc	1380
	actctagaaa	gatggcacac	catcacctat	gctattcacc	tggaccaaaa	gctgcgatta	1440
	agacatgccca	ttctgattat	gcttggagga	tggctctttt	cttctctaat	tgctatgttg	1500
	ccccttgctg	gtgtcagcaa	ttacatgaag	gtcagtattt	gcttccccat	ggatgtggaa	1560

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accactctct cacaagtcta tatattaacc atcctgattc tcaatgtggt ggccttcttc 1620
ataatttgtg cttgctacat taaaatttat tttgcagttc gaaacccaga attaatggct 1680
accaataaag atacaaagat tgctaagaaa atggcaatcc tcatcttcac cgatttcacc 1740
tgcatggcac ctatctcttt ttttgccatc tcagctgcct tcaaagtacc tcttatcaca 1800
5 gtaaccaact ctaaagtttt actggttctt ttttatccca tcaattcttg tgccaatcca 1860
tttctgtatg caatattcac taagacattc caaagagatt tctttctttt gctgagcaaa 1920
tttggctgct gtaaacgctg ggctgaactt tatagaagga aagatttttc agcttacacc 1980
tccaactgca aaaatggctt cactggatca aataagcctt ctcaatccac cttgaagttg 2040
tccacattgc actgtcaagg tacagctctc ctagacaaga ctcgctacac agagtgtggt 2100
10 accttgacct gtgatgacct ccgcttccag gactcctctt cctcaaaggc ccctcccccc 2160
agccttccaa gtccatcccc actcccgggg ccctcggaca ccccgatcct cccacaa 2217

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[0050] The present invention also includes another chimeric human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein. This chimera has a fragment of the human lutropin hormone receptor (SEQ ID NO: 5) and a fragment of the human chorionic gonadotropin- β unit (SEQ ID NO: 7). A small peptide linker consisting of two amino acids, Gly-Thr, is located between the C-terminus of the hLH-R domain and the N-terminus of the hCG- β domain to form a contiguous hLH-R-hCG- β fusion protein (chimera 3). This fusion protein has the amino acid sequence of SEQ ID NO:15, as follows:

```

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

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	Val	Thr	Leu	Lys	Leu	Tyr	Gly	Asn	Gly	Phe	Glu	Glu	Val	Gln	Ser	His	
				180					185					190			
5	Ala	Phe	Asn	Gly	Thr	Thr	Leu	Thr	Ser	Leu	Glu	Leu	Lys	Glu	Asn	Val	
			195					200					205				
	His	Leu	Glu	Lys	Met	His	Asn	Gly	Ala	Phe	Arg	Gly	Ala	Thr	Gly	Pro	
		210					215					220					
10	Lys	Thr	Leu	Asp	Ile	Ser	Ser	Thr	Lys	Leu	Gln	Ala	Leu	Pro	Ser	Tyr	
	225					230					235					240	
	Gly	Leu	Glu	Ser	Ile	Gln	Arg	Leu	Ile	Ala	Thr	Ser	Ser	Tyr	Ser	Leu	
15					245					250					255		
	Lys	Lys	Leu	Pro	Ser	Arg	Glu	Thr	Phe	Val	Asn	Leu	Leu	Glu	Ala	Thr	
				260					265					270			
20	Leu	Thr	Tyr	Pro	Ser	His	Cys	Cys	Ala	Phe	Arg	Asn	Leu	Pro	Thr	Lys	
			275					280					285				
	Glu	Gln	Asn	Phe	Ser	His	Ser	Ile	Ser	Glu	Asn	Phe	Ser	Lys	Gln	Cys	
		290					295					300					
25	Glu	Ser	Thr	Val	Arg	Lys	Val	Ser	Asn	Lys	Thr	Leu	Tyr	Ser	Ser	Met	
	305					310					315					320	
	Leu	Ala	Glu	Ser	Glu	Leu	Ser	Gly	Trp	Asp	Tyr	Glu	Tyr	Gly	Phe	Cys	
30					325					330					335		
	Leu	Pro	Lys	Thr	Pro	Arg	Cys	Ala	Pro	Glu	Pro	Gly	Thr	Leu	Thr	Cys	
				340					345					350			
35	Asp	Asp	Pro	Arg	Phe	Gln	Asp	Ser	Ser	Ser	Ser	Lys	Ala	Pro	Pro	Pro	
			355					360					365				
	Ser	Leu	Pro	Ser	Pro	Ser	Arg	Leu	Pro	Gly	Pro	Ser	Asp	Thr	Pro	Ile	
		370					375					380					
40	Leu	Pro	Gln														
	385																

This hLH-R/hCG- β fusion protein of the present invention is encoded by the
 45 nucleic acid molecule having the nucleotide sequence of SEQ ID NO: 16, as
 follows:

	atgaagcagc	ggttctcggc	gctgcagctg	ctgaagctgc	tgctgctgct	gcagccgccc	60
	ctgccacgag	cgctgcgcga	ggcgctctgc	cctgagccct	gcaactgcgt	gcccgcgccc	120
	gccctgcgct	gcccgggccc	cacggccggt	ctcactcgac	tatcacttgc	ctacctccct	180
50	gtcaaagtga	tcccatctca	agctttcaga	ggacttaatg	aggtcataaa	aattgaaatc	240
	tctcagattg	attccctgga	aaggatagaa	gctaattgcct	ttgacaacct	cctcaatttg	300
	tctgaaatac	tgatccagaa	caccaaaaat	ctgagataca	ttgagcccgg	agcatttata	360
	aatcttcccg	gattaaaata	cttgagcatc	tgtaacacag	gcatcagaaa	gtttccagat	420
	gttacgaagg	tcttctcctc	tgaatcaaat	ttcattctgg	aaatttgtga	taacttacac	480
55	ataaccacca	taccaggaaa	tgcttttcaa	gggatgaata	atgaatctgt	aacactcaaa	540
	ctatatggaa	atggatttga	agaagtacaa	agtcatgcat	tcaatgggac	gacactgact	600
	tcaactggagc	taaaggaaaa	cgtacatctg	gagaagatgc	acaatggagc	cttccgtggg	660
	gccacagggc	cgaaaacctt	ggatatttct	tccaccaaata	tgcaggccct	gcccgcgccc	720

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5 ggcctagagt ccattcagag gctaattgcc acgtcatcct attctctaaa aaaattgccca 780
 tcaagagaaa catttgtcaa tctcctggag gccacgttga cttaccccag ccaactgctgt 840
 gcttttagaa acttgccaac aaaagaacag aattttttcac attccatttc tgaaaacttt 900
 tccaaacaat gtgaaagcac agtaaggaaa gtgagtaaca aaacacttta ttcttccatg 960
 cttgctgaga gtgaactgag tggctgggac tatgaatatg gtttctgctt acccaagaca 1020
 ccccgatgtg ctctgaacc aggtaccttg acctgtgatg acccccgtt ccaggactcc 1080
 tcttctcaa aggccctcc cccagcctt ccaagtccat cccgactccc ggggccctcg 1140
 gacaccccgga tcttcccaca a 1161

10

[0051] The present invention relates to yet another chimeric hLH-R/hCG- β fusion protein having a fragment of the human lutropin hormone receptor (SEQ ID NO: 5) and the full length human chorionic gonadotropin- β unit (SEQ ID NO: 3). A small peptide linker consisting of two amino acids, Gly-Thr, is located

15 between the C-terminus of the hLH-R domain and the N-terminus of the hCG- β domain to form a contiguous hLH-R-hCG- β fusion protein. This fusion protein has the amino acid sequence of SEQ ID NO: 17 (chimera 4), as follows:

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

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	Ala	Phe	Asn	Gly	Thr	Thr	Leu	Thr	Ser	Leu	Glu	Leu	Lys	Glu	Asn	Val
			195					200					205			
5	His	Leu	Glu	Lys	Met	His	Asn	Gly	Ala	Phe	Arg	Gly	Ala	Thr	Gly	Pro
		210					215					220				
	Lys	Thr	Leu	Asp	Ile	Ser	Ser	Thr	Lys	Leu	Gln	Ala	Leu	Pro	Ser	Tyr
10		225				230					235					240
	Gly	Leu	Glu	Ser	Ile	Gln	Arg	Leu	Ile	Ala	Thr	Ser	Ser	Tyr	Ser	Leu
					245					250					255	
	Lys	Lys	Leu	Pro	Ser	Arg	Glu	Thr	Phe	Val	Asn	Leu	Leu	Glu	Ala	Thr
15				260					265					270		
	Leu	Thr	Tyr	Pro	Ser	His	Cys	Cys	Ala	Phe	Arg	Asn	Leu	Pro	Thr	Lys
			275					280					285			
20	Glu	Gln	Asn	Phe	Ser	His	Ser	Ile	Ser	Glu	Asn	Phe	Ser	Lys	Gln	Cys
		290					295					300				
	Glu	Ser	Thr	Val	Arg	Lys	Val	Ser	Asn	Lys	Thr	Leu	Tyr	Ser	Ser	Met
25		305				310					315					320
	Leu	Ala	Glu	Ser	Glu	Leu	Ser	Gly	Trp	Asp	Tyr	Glu	Tyr	Gly	Phe	Cys
					325					330					335	
	Leu	Pro	Lys	Thr	Pro	Arg	Cys	Ala	Pro	Glu	Pro	Gly	Thr	Met	Glu	Met
30				340					345					350		
	Phe	Gln	Gly	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Ser	Met	Gly	Gly	Thr	Trp
			355					360					365			
35	Ala	Ser	Lys	Glu	Pro	Leu	Arg	Pro	Arg	Cys	Arg	Pro	Ile	Asn	Ala	Thr
		370					375					380				
	Leu	Ala	Val	Glu	Lys	Glu	Gly	Cys	Pro	Val	Cys	Ile	Thr	Val	Asn	Thr
40		385				390					395					400
	Thr	Ile	Cys	Ala	Gly	Tyr	Cys	Pro	Thr	Met	Thr	Arg	Val	Leu	Gln	Gly
					405					410					415	
	Val	Leu	Pro	Ala	Leu	Pro	Gln	Val	Val	Cys	Asn	Tyr	Arg	Asp	Val	Arg
45				420					425					430		
	Phe	Glu	Ser	Ile	Arg	Leu	Pro	Gly	Cys	Pro	Arg	Gly	Val	Asn	Pro	Val
			435					440					445			
50	Val	Ser	Tyr	Ala	Val	Ala	Leu	Ser	Cys	Gln	Cys	Ala	Leu	Cys	Arg	Arg
		450					455					460				
	Ser	Thr	Thr	Asp	Cys	Gly	Gly	Pro	Lys	Asp	His	Pro	Leu	Thr	Cys	Asp
55		465				470					475					480
	Asp	Pro	Arg	Phe	Gln	Asp	Ser	Ser	Ser	Ser	Lys	Ala	Pro	Pro	Pro	Ser
					485					490					495	
	Leu	Pro	Ser	Pro	Ser	Arg	Leu	Pro	Gly	Pro	Ser	Asp	Thr	Pro	Ile	Leu
60				500					505					510		

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Pro Gln

- 5 This chimeric hLH-R/hCG- β fusion protein of the present invention is encoded by a nucleic acid molecule having the nucleotide sequence of SEQ ID NO: 18, as follows:

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10 atgaagcagc ggttctcggc gctgcagctg ctgaagctgc tgctgctgct gcagccgccc 60
   ctgccacgag cgctgcgcga ggcgctctgc cctgagccct gcaactgcgt gcccgacggc 120
   gccctgcgct gccccggccc cacggccggt ctcactcgac tatcacttgc ctacctccct 180
   gtcaaagtga tcccatctca agcttttcaga ggacttaatg aggtcataaa aattgaaatc 240
   tctcagattg attccctgga aaggatagaa gctaatgcct ttgacaacct cctcaatttg 300
   tctgaaatac tgatccagaa caccaaaaat ctgagataca ttgagcccgg agcattttata 360
   aatcttcccg gattaaaata cttgagcatc tgtaacacag gcatcagaaa gtttccagat 420
15 gttacgaagg tcttctcctc tgaatcaaat ttcattctgg aaatttgtga taacttacac 480
   ataaccacca taccaggaaa tgcttttcaa gggatgaata atgaatctgt aacactcaaa 540
   ctatatggaa atggatttga agaagtacaa agtcatgcat tcaatgggac gacactgact 600
   tcaactggagc taaaggaaaa cgtacatctg gagaagatgc acaatggagc cttccgtggg 660
   gccacagggc cgaaaacctt ggatatttct tccaccaaat tgcaggccct gccgagctat 720
20 ggcctagagt ccattcagag gctaattgcc acgtcatcct attctctaaa aaaattgcca 780
   tcaagagaaa catattgtcaa tctcctggag gccacgttga cttaccccag ccaactgctgt 840
   gcttttagaa acttgccaac aaaagaacag aattttttcac attccatttc tgaaaacttt 900
   tccaaacaat gtgaaagcac agtaaggaaa gtgagtaaca aaacacttta ttcttccatg 960
   cttgctgaga gtgaactgag tggctgggac tatgaatatg gtttctgctt acccaagaca 1020
25 ccccgatgtg ctctgaacc aggtaccatg gagatgttcc aggggctgct gctgttgctg 1080
   ctgctgagca tgggcgggac atgggcatcc aaggagccgc ttcggccacg gtgccgcccc 1140
   atcaatgcca ccctggctgt ggagaaggag ggctgccccg tgtgcatcac cgtcaacacc 1200
   accatctgtg ccggctactg ccccaccatg acccgctgct tgcagggggg cctgccggcc 1260
   ctgcctcagg tgggtgtgcaa ctaccgcgat gtgcgcttcg agtccatccg gctccctggc 1320
30 tgcccgcgcg gcgtgaacc cgtggtctcc tacgccgtgg ctctcagctg tcaatgtgca 1380
   ctctgccgcc gcagcaccac tgactgcggg ggtcccaagg accaccctt gacctgtgat 1440
   gacccccgct tccaggactc ctcttcctca aaggccctc ccccagcct tccaagtcca 1500
   tcccgaactc cggggccctc ggacaccccg atcctcccac aa 1542

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- 35 In all aspects of the present invention shown above, the two domains of the chimeric hLH-R/hCG- β fusion protein are joined via a peptide linker. The linkers are used to facilitate manipulation of the two proteins in forming the chimera (as described in detail in Example 1), and are not meant to be limiting in either number or identity. Any other linkers, i.e., amino acids, or combinations thereof,
- 40 may be substituted in the fusion protein of the present invention, provided they do not interfere with the native properties of the two domains of the fusion protein, including the antigenic properties thereof.

[0052] The chimeric nucleic acid molecules encoding the fusion proteins of the present invention are prepared using well-known recombinant technology.

- 45 This involves, briefly, isolating the desired nucleic acid molecule encoding a full-length human lutropin hormone receptor, or a fragment thereof, and the desired nucleic acid molecule encoding a human chorionic gonadotropin- β subunit, or

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fragment thereof, and cloning the desired nucleic acid molecules into a single vector, and adding additional nucleotides encoding the desired amino acid linker sequence between the 3' end of the hLH-R coding sequence and the 5' end of the hCG- β subunit coding sequence. The preparation of the desired embodiment of the chimeric nucleic acid molecule of present invention is carried out using standard cloning procedures, such as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* Cold Springs Laboratory, Cold Springs Harbor, New York (1989), Methods in Enzymology, Vol. 152: *Guide to Molecular Cloning Techniques*, Berger and Kimmel, Eds., San Diego: Academic Press, Inc. (1987), *Current Protocols in Molecular Biology*, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995); *Plant Molecular Biology: A Laboratory Manual*, Clark, Ed., Springer-Verlag, Berlin (1997), which are hereby incorporated by reference in their entirety.

[0053] The nucleic acids of interest can also be amplified from nucleic acid samples using amplification techniques. For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the present invention and related genes directly from genomic DNA or cDNA libraries. PCR and other *in vitro* amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. The T4 gene 32 protein (Boehringer Mannheim, Indianapolis, IN) can be used to improve yield of long PCR products. PCR-based screening methods have been described. Alphey, L., "PCR-Based Method for Isolation of Full-Length Clones and Splice Variants from cDNA Libraries," *BioTechniques* 22(3):481-484 (1997) (which is hereby incorporated by reference in its entirety), describes a PCR-based method in which the longest cDNA is identified in the first step so that incomplete clones can be eliminated from study. Such methods are particularly effective in combination with a full-length cDNA construction methodology.

[0054] The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis using methods such as the phosphotriester method of Narang et al., "Improved Phosphotriester Method for the Synthesis of Gene Fragments," *Methods Enzymol* 68:90-98 (1979) (which is hereby

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incorporated by reference in its entirety), the phosphodiester method of Brown et al., "Chemical Synthesis and Cloning of a Tyrosine tRNA Gene," *Methods Enzymol* 68:109-51 (1979) (which is hereby incorporated by reference in its entirety), using an automated synthesizer, e.g., as described in Needham-
5 VanDevanter et al., "Characterization of an Adduct Between CC-1065 and a Defined Oligodeoxynucleotide Duplex," *Nucleic Acids Res* 12(15):6159-6168 (1984) (which is hereby incorporated by reference in their entirety), and the solid support method of U.S. Pat. No. 4,458,066 to Caruthers et al., (which is hereby incorporated by reference in its entirety). Chemical synthesis generally produces
10 a single stranded oligonucleotide. This may be converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill will recognize that while chemical synthesis of DNA is best employed for sequences of about 100 bases or less, longer sequences may be obtained by the ligation of
15 shorter sequences.

[0055] In one aspect of the present invention, a chimeric nucleic acid molecule encoding a hLH-R/hCG- β fusion protein is introduced into an expression system or expression vector of choice, creating a nucleic acid construct using conventional recombinant technology. Generally, this involves inserting the
20 chimeric nucleic acid molecule of the present invention into an expression system to which the molecule is heterologous (*i.e.*, not normally present). The heterologous nucleic acid molecule is inserted into the expression system or vector in proper sense (5'→3') orientation and correct reading frame. The vector contains the necessary 5' and 3' region elements for the proper transcription and
25 translation of the inserted protein-coding sequences. In preparing the nucleic acid construct of the present invention, the various nucleic acid molecules of the present invention may be inserted or substituted into a bacterial plasmid-vector. Any convenient plasmid may be employed, which will be characterized by having a bacterial replication system, a marker which allows for selection in a bacterium
30 and generally one or more unique, conveniently located restriction sites. Numerous plasmids, referred to as transformation vectors, are available for transformation. Suitable vectors include, but are not limited to, the following: viral vectors, such as lambda vector system gt11, gt WES.tB, Charon 4, and

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plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, CA, which is hereby incorporated by reference in its entirety), pQE, pIH821, pGEX, pET series (see F.W. Studier et al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," *Gene Expression Technology* vol. 185 (1990), which is hereby incorporated by reference in its entirety), and any derivatives thereof.

[0056] Certain "control elements" or "regulatory sequences" are also incorporated into the plasmid-vector constructs of the present invention. These include non-transcribed regions of the vector and 5' and 3' untranslated regions, which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and/or translation elements, including constitutive, inducible, and repressible promoters, as well as minimal 5' promoter elements may be used. Common 5' regulatory regions, including, but not limited to promoters, suitable for directing expression in mammalian cells include the SV40, MMTV, metallothionein-1, adenovirus Ela, CMV, immediate early, immunoglobulin heavy chain promoter and enhancer, and RSV-LTR promoters. The selection of a regulatory regions will be influenced by the host species selected so expression in the host is effected.

[0057] The nucleic construct of the present invention also includes an operable 3' regulatory region, selected from among those which are capable of providing correct transcription termination and polyadenylation of mRNA for expression in the host of choice. The 5' and 3' regulatory regions are operably linked to a DNA molecule, thereby allowing expression of the protein or polypeptide encoded by the DNA molecule of choice.

[0058] The nucleic acid molecule of the present invention, appropriate transcriptional and translational regulatory elements, and any additional desired components, including without limitation, enhancers, leader sequences, markers, reporter genes, etc., are cloned into the vector of choice using standard cloning procedures in the art, such as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* Cold Springs Laboratory, Cold Springs Harbor, New York

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(1989), and U.S. Patent No. 4,237,224 to Cohen and Boyer, which are hereby incorporated by reference in their entirety. The transcriptional and translational elements are operably linked to the nucleic acid molecule of the present invention or a fragment thereof, meaning that the resulting vector expresses the human
5 lutropin hormone receptor/ human chorionic gonadotropin- β fusion protein of the present invention when placed in a suitable host.

[0059] Once the chimeric nucleic acid molecule encoding the hLH-R/hCG- β fusion protein of the present invention has been cloned into an expression system, it is ready to be incorporated into a host. Accordingly, another
10 aspect of the present invention relates to a method of making a recombinant host. Basically, this method is carried out by transforming a host with a nucleic acid construct of the present invention under conditions effective to yield transcription of the nucleic acid molecule in the host. Such incorporation can be carried out in a variety of ways, depending upon the vector/host system. Recombinant
15 molecules can be introduced into a host via transformation, transduction, conjugation, mobilization, or electroporation. Preferably, the nucleic acid construct of the present invention is stably inserted into the genome of the recombinant host as a result of the transformation. Suitable hosts for the chimeric nucleic acid molecule of the present invention include, but are not limited to,
20 bacteria, virus, yeast, insect cells, and mammalian cells, including canine, feline, and human.

[0060] Transient expression in protoplasts allows quantitative studies of gene expression since the population of cells is very high (on the order of 10^6). To deliver DNA inside protoplasts, several methodologies have been proposed,
25 but the most common are electroporation (Neumann et al., "Gene Transfer into Mouse Lyoma Cells by Electroporation in High Electric Fields," *EMBO J.* 1: 841-45 (1982); Wong et al., "Electric Field Mediated Gene Transfer," *Biochem Biophys Res Commun* 30;107(2):584-7 (1982); Potter et al., "Enhancer-Dependent Expression of Human Kappa Immunoglobulin Genes Introduced into Mouse pre-
30 B Lymphocytes by Electroporation," *Proc. Natl. Acad. Sci. USA* 81: 7161-65 (1984), which are hereby incorporated by reference in their entirety) and polyethylene glycol (PEG) mediated DNA uptake, Sambrook et al., *Molecular Cloning: A Laboratory Manual* Chap. 16, 2d Edition, Cold Springs Laboratory,

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Cold Springs Harbor, New York (1989), which is hereby incorporated by reference in its entirety). During electroporation, the DNA is introduced into the cell by means of a reversible change in the permeability of the cell membrane due to exposure to an electric field. PEG transformation introduces the DNA by

5 changing the elasticity of the membranes. Unlike electroporation, PEG transformation does not require any special equipment. Another appropriate method of introducing the gene construct of the present invention into a host cell is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the chimeric gene (Fraley, et al.,

10 "Liposome-Mediated Delivery of Tobacco Mosaic Virus RNA Into Tobacco Protoplasts: A Sensitive Assay for Monitoring Liposome-Protoplast Interactions," *Proc Natl Acad Sci USA* 79:1859-63 (1982); Horsch et al., "A Simple and General Method for Transferring Genes into Plants," *Science* 227:1229-1231 (1985), which are hereby incorporated by reference in their entirety).

15 **[0061]** Stable transformants are preferable for the methods of the present invention, which can be achieved by using variations of the methods above as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* Chap. 16, Second Edition, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), *Methods in Enzymology*, Vol. 152: *Guide to Molecular Cloning*

20 *Techniques*, Berger and Kimmel, Eds., San Diego: Academic Press, Inc. (1987), *Current Protocols in Molecular Biology*, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995); which are hereby incorporated by reference in their entirety.

[0062] Typically, when a recombinant host is grown for the purpose

25 producing/expressing the desired recombinant protein, an antibiotic or other compound useful for selective growth of the transgenic cells only is added as a supplement to the media. The compound to be used will be dictated by the selectable marker element present in the plasmid with which the host cell was transformed. Suitable genes are those which confer resistance to gentamycin,

30 G418, hygromycin, streptomycin, spectinomycin, tetracycline, chloramphenicol, and the like. When the host is to be a mammalian cell, in particular a human cell, kanamycin is highly suitable. Similarly, "reporter genes," which encode enzymes providing for production of an identifiable compound identifiable, or other

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markers which indicate relevant information regarding the outcome of gene delivery, are suitable. For example, various luminescent or phosphorescent reporter genes are also appropriate, such that the presence of the heterologous gene may be ascertained visually.

5 [0063] An example of a marker suitable for the present invention is the green fluorescent protein (GFP) gene. The isolated nucleic acid molecule encoding a green fluorescent protein can be deoxyribonucleic acid (DNA) or ribonucleic acid (RNA, including messenger RNA or mRNA), genomic or recombinant, biologically isolated or synthetic. The DNA molecule can be a
10 cDNA molecule, which is a DNA copy of a messenger RNA (mRNA) encoding the GFP. In one embodiment, the GFP can be from *Aequorea victoria* (Prasher et al., "Primary Structure of the *Aequorea Victoria* Green-Fluorescent Protein," *Gene* 111(2):229-233 (1992); U.S. Patent No. 5,491,084 to Chalfie et al., which are hereby incorporated by reference in their entirety). A plasmid encoding the
15 GFP of *Aequorea victoria* is available from the ATCC as Accession No. 75547. Mutated forms of GFP that emit more strongly than the native protein, as well as forms of GFP amenable to stable translation in higher vertebrates, are commercially available from Clontech Laboratories, Inc. (Palo Alto, California) and can be used for the same purpose. The plasmid designated pT α 1-GFP β
20 (ATCC Accession No. 98299, which is hereby incorporated by reference in its entirety) includes a humanized form of GFP. Indeed, any nucleic acid molecule encoding a fluorescent form of GFP can be used in accordance with the subject invention. Standard techniques are then used to place the nucleic acid molecule encoding GFP under the control of the chosen cell specific promoter.

25 [0064] The selection marker employed will depend on the target species and/or host or packaging cell lines compatible with a chosen vector.

[0065] Following introduction into a host, the host is grown under conditions appropriate for the selection of hosts harboring the chimeric transgene. The expression of the hLH-R/hCG- β fusion protein of the present invention by
30 apparently transgenic hosts can be determined by carrying out screening techniques described herein below, and those hosts expressing the chimeric fusion protein are propagated and expanded under suitable culture conditions.

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[0066] The present invention also relates to an isolated recombinant hLH-R/hCG- β fusion protein. Recombinant human lutropin hormone receptor/ human chorionic gonadotropin- β fusion proteins of the present invention include, without limitation, the proteins having amino acid sequences of SEQ ID NO:11, 13, 15, and 17, shown herein above. The recombinant fusion protein of the present invention is secreted into the growth medium of transgenic host, for example, *E. coli*, harboring a nucleic acid molecule encoding a human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein. To isolate the desired protein, host carrying a chimeric transgene is propagated, collected, homogenized, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the desired protein of the present invention is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC or other chromatography techniques, for example, metal affinity chromatography. Alternative protein isolation methods known in the art may be used as suitable.

[0067] The present invention also encompasses chimeric hLH-R/hCG- β fusion proteins that are functionally equivalent variants of the proteins having SEQ ID NO:11, 13, 15, and 17. By "variant protein" is intended a protein derived from the native protein by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native protein deletion or addition of one or more amino acids at one or more sites in the native protein, or substitution of one or more amino acids at one or more sites in the native protein. Variant proteins encompassed by the present invention are biologically active, that is, they continue to possess the desired biological activity of the native protein. In particular, the variant protein will have the antigenic properties of the native proteins as described herein. Such variants may result from, for example, genetic polymorphism or from human manipulation. Biologically active variants of any hLH-R/hCG- β fusion protein of the invention described herein will have at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% and preferably about 98% sequence similarity to the amino acid sequence for a fusion protein described herein as determined by sequence alignment programs described elsewhere herein using default parameters. A biologically active variant

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of a protein of the invention may differ from that protein by as few as 1-15 amino acid residues, as few as 1-10, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

[0068] The proteins of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the proteins of the invention can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art (Kunkel, T.A., "Rapid and Efficient Site-Specific Mutagenesis Without Phenotypic Selection, *Proc Natl Acad Sci USA* 82(2):488-492 (1985); Kunkel et al., "Rapid and Efficient Site-Specific Mutagenesis Without Phenotypic Selection," *Methods Enzymol* 154:367-382 (1987); U.S. Pat. No. 4,873,192 to Kunkel; Walker and Gaastra, eds., *Techniques in Molecular Biology*, New York: MacMillan Publishing Company (1983) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff et al. (1978) *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.), herein incorporated by reference in its entirety. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferable. Variant nucleotide sequences and proteins also encompass sequences and proteins derived from a mutagenic and recombinogenic procedure such as DNA shuffling. With such a procedure, one or more different coding sequences of the invention can be manipulated to create a new sequence possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo*.

[0069] Thus, the chimeric nucleic acid molecules and amino acids of the invention include both the naturally occurring sequences as well as mutant forms. Likewise, the proteins of the invention encompass both naturally occurring proteins as well as variations and modified forms thereof. Obviously, the mutations that will be made in the DNA encoding the variant must not place the

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sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure (EP Patent Application Publication No. 75,444 to De Boer, which is hereby incorporated by reference in its entirety). The deletions, insertions, and substitutions of the protein sequences encompassed herein are not expected to produce radical changes in the characteristics of the protein. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. More details regarding routine assays to measure such activity are described in the Examples herein.

[0070] A common characteristic of any hLH-R/hCG- β fusion protein of the present invention will be the ability of the protein, when presented as an immunogen, to elicit production of an antibody specifically reactive to one or both protein domains of the hLH-R/hCG- β fusion protein of the present invention. The term "immunogen" is used herein for a substance which elicits the formation of antibodies as described herein above. The term "antigen" denotes a substance which couples with a matching antibody, thereby being bound and "neutralized" by the latter. In practice "antigens" and "immunogens" in a given context are often the same substances, thus, two terms are frequently used, including in the present specification, as synonyms. Likewise, "immunogenic" and "antigenic" are used interchangeably herein.

[0071] Furthermore, the proteins of the present invention will not bind to antisera raised against a polypeptide of the present invention which has been fully immunosorbed with the same polypeptide. Immunoassays for determining binding are well known to those of skill in the art. A preferred immunoassay is a competitive immunoassay, such as described below in the Examples. Thus, the proteins of the present invention can be employed as immunogens for constructing antibodies immunoreactive to one or both domains of the fusion protein of the present invention, or for such exemplary utilities as immunoassays or protein purification techniques.

[0072] Studies in animals have demonstrated that antibodies against LH-R suppress the production of progesterone in the female (Saxena et al., "Modulation of Ovarian Function in Female Dogs Immunized With Bovine Luteinizing

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Hormone Receptor,” *Reprod Domest Anim* 37(1):9-17 (2002) and Saxena et al., “Effect of Immunization with Bovine Luteinizing Hormone Receptor on Ovarian Function in Cats,” *Am J Vet Res* 64(3):292-298 (2003), which are hereby incorporated by reference in their entirety), and of testosterone in the male (Remy et al., “Immunization Against Exon 1 Decapeptides From the Lutropin/Choriogonadotropin Receptor or the Follitropin Receptor as Potential Male Contraceptive,” *J Reprod Immunol* 32(1):37-54 (1996), which is hereby incorporated by reference in its entirety), leading to infertility. Chimeras of hLH-R combined with hCG and their fragments have the advantage of presenting themselves as heterologous antigens and are likely to mount an enhanced host immune response. Therefore, the present invention also relates to an isolated antibody, or binding portions thereof, raised to the fusion protein(s) of the present invention or proteins having a high sequence identity (as described herein above) or encoded by a nucleic acid molecule having a high sequence identity to the nucleic acid molecules herein. Because the fusion protein of the present invention contains more than one antigen, the fusion protein will likely produce a variety of antibodies when used as an immunogen for antibody formation. In particular, antibodies may be raised that recognize the hLH-R domain, while not recognizing the hCG- β subunit domain; that recognize the hCG- β subunit domain, while not recognizing the hLH-R domain; and that recognize both the hLH-R domain and the hCG- β subunit domain. Each of these antibodies is useful in one or more aspects of the present invention.

[0073] Antibodies of the present invention include those which are capable of binding to the fusion protein of the present invention and inhibiting the activity of such a polypeptide or protein, either *in vitro* (in an assay) or *in vivo* (i.e., if injected into a subject as an immunogen). In addition, antibodies of the present invention may also bind to a native hLH-R and a hCG- β subunit *in vitro* and *in vivo*.

[0074] The antibodies of the present invention may be monoclonal or polyclonal. Monoclonal antibody production may be effected by techniques which are well-known in the art (*Monoclonal Antibodies – Production, Engineering and Clinical Applications*, Ritter et al., Eds. Cambridge University Press, Cambridge, UK (1995), which is hereby incorporated by reference in its

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entirety). Basically, the process involves first obtaining immune cells (lymphocytes) from the spleen of a mammal (e.g., mouse) which has been previously immunized with the antigen of interest either *in vivo* or *in vitro*. The antibody-secreting lymphocytes are then fused with (mouse) myeloma cells or transformed cells, which are capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. The resulting fused cells, or hybridomas, are cultured, and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned, and grown either *in vivo* or *in vitro* to produce large quantities of antibody. A description of the theoretical basis and practical methodology of fusing such cells is set forth in Kunkel et al., "Rapid and Efficient Site-Specific Mutagenesis Without Phenotypic Selection," *Methods Enzymol* 154:367-382 (1987), which is hereby incorporated by reference in its entirety.

[0075] Mammalian lymphocytes are immunized by *in vivo* immunization of the animal (e.g., a mouse) with the protein or polypeptide of the present invention. Such immunizations are repeated as necessary at intervals of up to several weeks to obtain a sufficient titer of antibodies. Following the last antigen boost, the animals are sacrificed and spleen cells removed.

[0076] Fusion with mammalian myeloma cells or other fusion partners capable of replicating indefinitely in cell culture is effected by standard and well-known techniques, for example, by using polyethylene glycol ("PEG") or other fusing agents (Kohler et al., "Derivation of Specific Antibody-Producing Tissue Culture and Tumor Lines by Cell Fusion," *Eur J Immunol* 6(7):511-519 (1976), which is hereby incorporated by reference in its entirety). This immortal cell line, which is preferably murine, but may also be derived from cells of other mammalian species, including without limitation, rats and humans, is selected to be deficient in enzymes necessary for the utilization of certain nutrients, to be capable of rapid growth, and to have good fusion capability. Many such cell lines are known to those skilled in the art, and others are regularly described.

[0077] Procedures for raising polyclonal antibodies are also well known. Typically, such antibodies can be raised by administering the protein or polypeptide of the present invention subcutaneously to New Zealand white rabbits which have first been bled to obtain pre-immune serum. The antigens can be

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injected at a total volume of 100 μ l per site at six different sites. Each injected material will contain synthetic surfactant adjuvant pluronic polyols, or pulverized acrylamide gel containing the protein or polypeptide after SDS-polyacrylamide gel electrophoresis. The rabbits are then bled approximately every two weeks
5 after the first injection and periodically boosted with the same antigen three times every six weeks. A sample of serum is then collected 10 days after each boost. Polyclonal antibodies are then recovered from the serum by affinity chromatography using the corresponding antigen to capture the antibody. Ultimately, the rabbits are euthenized with pentobarbital 150 mg/Kg IV. This and
10 other procedures for raising polyclonal antibodies are disclosed in Harlow, et. al., Eds., *Antibodies: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1988), which is hereby incorporated by reference in its entirety. A polyclonal antibody preparation may include antibodies of various specificity, i.e., antibodies that are reactive with the fusion protein *per se*, with the hLH-R domain,
15 or fragment thereof, or with the hCG- β domain, or fragment thereof, or with the proteins of both domains of the fusion protein.

[0078] It is also possible to use the anti-idiotypic technology to produce monoclonal antibodies that mimic an epitope. As used in this invention, "epitope" means any antigenic determinant on an antigen to which the paratope of an
20 antibody binds. Epitopic determinants usually consist of chemically active surface groupings of molecules, such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the
25 hypervariable region that is the image of the epitope bound by the first monoclonal antibody.

[0079] In addition to whole antibodies, the present invention encompasses binding portions of such antibodies. Such binding portions include Fab fragments, F(ab')₂ fragments, and Fv fragments. These antibody fragments can be
30 made by conventional procedures, such as proteolytic fragmentation procedures, as described in J. Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 98-118 N.Y. Academic Press (1983), and Harlow et al., *Antibodies: A Laboratory Manual* Cold Springs Harbor Laboratory, New York (1988), which

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are hereby incorporated by reference in their entirety, or other methods known in the art.

[0080] The present invention also relates to antibodies raised as described above, against the full length hHL-R proteins having SEQ ID NO:1 and SEQ ID NO:9, and antibodies raised against any fragments of the hLH-R domain, including, but not limited to the ECD (SEQ ID NO:5). This is meant to include any antibodies raised against variants of these proteins having an amino acid sequence that is at least 80%, 85% or 87%, alternatively at least 92%, 94%, or 96%, and preferably 98% sequence identity to the proteins having SEQ ID NO:1, 5, or 9 of the present invention.

[0081] The present invention also relates to a composition including the isolated fusion protein of the present invention having a human lutropin hormone receptor domain and a human chorionic gonadotropin- β domain, and a pharmaceutical carrier. This fusion protein includes a full length human lutropin hormone receptor domain or a fragment thereof, including, but not limited to those describe herein above, and a full length human chorionic gonadotropin- β domain or a fragment thereof, including, but not limited to, those describe herein above. A common characteristic of the fusion protein suitable for such a composition is the immunogenic capability of the protein. Thus, a suitable a hLH-R/hCG- β fusion protein is an isolated hLH-R and/or hCG- β fusion protein as described herein above that is capable of eliciting the formation of antibodies of the present invention as described herein above.

[0082] The immunogenic characteristic of the hLH-R/hCG- β fusion protein of the present invention is useful in stimulation of a host's immune response. By "immune response" is meant that antibodies are formed by the immune system in response to an immunogenic stimulus elicited by the immunogen. The antibodies will include antibodies more or less specific to the immunogenic determinants (or combination thereof) of the immunogen. When the immune system of an animal is challenged with an immunogen (e.g., by injection) to produce endogenous antibodies against an antigen having antigenic determinants corresponding to the immunogenic determinants of the immunogen, that is known as active immunization. Thus, the isolated or synthesized fusion protein of the present invention combined with a carrier may be used for the active

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immunization of a subject. Fusion proteins suitable for this aspect of the present invention include, without limitation, any of the hLH-R/hCG- β fusion proteins described herein above or a suitable variant thereof. The carrier in this aspect of the present invention includes any of a variety of known immunologic adjuvants or pharmaceutical additives that enable or enhance delivery of the fusion protein of the present invention to a subject.

[0083] Alternatively, the administration to a subject by injection of antibodies extraneously produced, for example, polyclonal or monoclonal antibodies prepared in a different animal (as described hereinabove) to produce resistance against antigens for which the antibodies are specific, is known as passive immunization. Thus, another aspect of the present invention is a composition having a pharmaceutical carrier and an antibody to any of the hLH-R/hCG- β fusion proteins as described herein, or a suitable variant thereof. A suitable antibody of this aspect of the present invention may be a monoclonal or polyclonal antibody or a binding portion thereof, prepared as described herein, and where the administration of the composition is as described herein below.

[0084] In addition, immunological preparations comprising antibodies to the hLH-R/hCG- β fusion protein of the present invention can be put to a variety of uses, including, without limitation, as immunoreagents for immunosorbitive processes and for quantitative and qualitative analytical tests, in particular, assays for diagnostic, pathological, forensic, and pharmacokinetic investigations involving hLH-R and hCG- β proteins and reproduction.

[0085] The present invention also relates to a method of treating an androgen-excess-mediated disease condition in a subject. This involves administering an antibody to the hLH-R/hCG- β fusion protein of the present invention to the subject under conditions effective to treat the androgen-excess-mediated disease condition. For females, an antibody recognizing both the h-LH-R and the hCG domains, i.e., antibody raised to chimera 1, 2, 3, and 4, as well as a mix of antibodies, where some of the antibodies recognize the h-LH-R domain and some recognize the hCG- β domain, are suitable for treatment of an androgen-excess-mediated condition. However, males do not produce hCG. Therefore, an antibody that recognizes the LH-R in males is sufficient for treatment of an androgen-excess mediated disease condition. However, if a male is immunized

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with an antibody that recognizes both the hLH-R and the hCG domains, the presence of the antibody to the hCG domain may act as a "mock domain," or a hapten, thereby enhancing the effect of the anti-hLH-R treatment. If an antibody is administered to males that includes a recognition site for hCG, it is preferable
5 that it be only the CTP fragment of the hCG- β - subunit. This includes, for example, antibodies to proteins having amino acid sequences of SEQ ID NO:11 and SEQ ID NO:13 of the present invention. Alternatively, antibodies that recognize only the hLH-R domain of the hLH-R/h hCG- β fusion protein of the present invention and antibodies raised to an hLH-R protein alone are also suitable
10 for administration to males in this aspect of the present invention.

[0086] Administration in this and all aspects of the present invention may be carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, intravaginally, by intranasal instillation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, or by
15 application to mucous membranes, such as, that of the nose, throat, and bronchial tubes. The antibody to the hLH-R/hCG- β fusion protein may be administered alone or with pharmaceutically or physiologically acceptable carriers, excipients, or stabilizers, and can be in solid or liquid form including tablets, capsules, powders, solutions, suspensions, or emulsions.

[0087] The solid unit dosage forms can be of the conventional type. The solid form can be a capsule, such as an ordinary gelatin type containing the antibodies or binding portions thereof of the present invention and a carrier, for example, lubricants and inert fillers such as, lactose, sucrose, or cornstarch. In another embodiment, these compounds are tableted with conventional tablet bases
25 such as lactose, sucrose, or cornstarch in combination with binders like acacia, cornstarch, or gelatin, disintegrating agents, such as cornstarch, potato starch, or alginic acid, and a lubricant, like stearic acid or magnesium stearate.

[0088] The antibodies to the hLH-R/hCG- β fusion protein of the present invention may also be administered in injectable dosages by solution or
30 suspension of these materials in a physiologically acceptable diluent with a pharmaceutical carrier. Such carriers include sterile liquids, such as water and oils, with or without the addition of a surfactant and other pharmaceutically and physiologically acceptable carrier, including adjuvants, excipients or stabilizers.

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Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols, such as propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions.

5 [0089] For use as aerosols, the antibodies of the present invention in solution or suspension may be packaged in a pressurized aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional adjuvants. The antibodies of the present invention also may be administered in a non-pressurized form such as in a nebulizer or atomizer.

[0090] In this aspect of the present invention the antibodies may be administered for treatment of various conditions known as androgen-excess syndromes, including, without limitation, polycystic ovarian syndrome, Klinefelter's Syndrome, and prostatic hyperplasia. Suitable subjects in this aspect of the present invention are any mammals, including canine, feline, and human, and may be either the male or the female of the species.

[0091] Another aspect of the present invention is a method of immunocontraception in a subject that involves administering an antibody to hLH-R/hCG- β fusion protein of the present invention to a subject under conditions effective to provide immunocontraception to the subject. Suitable for this aspect of the present invention are a mix of antibodies, where some of the antibodies recognize the hLH-R domain, and others recognize the hCG domains. When the subject is female, the preferable antibody of this aspect is an antibody capable of recognizing both the hLH-R domain and the /hCG- β domain of the fusion protein of the present invention. When the subject is a male, the antibody or antibodies administered are preferably those that recognize the hLH-R only, either the full-length domain (e.g., SEQ ID NO: 1 and 9) or a fragment thereof (e.g., SEQ ID NO:5), or an hLH-R/hCG hCG- β fusion protein, where the hLH-R is either full-length or a fragment thereof, and the hCG- β domain is the CTP fragment (e.g., SEQ ID NO:13, 15. Because males do not produce the chorionic gonadotropin hormone, immunocontraception can be conferred to males using an hLH-R antibody alone. When the antibody used in males also recognizes the CTP

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fragment of the hCG- β subunit, the CTP antibody serves as a hapten to enhance the immunocontraceptive affect of the hLH-R antibody. Administration of an antibodies in this aspect of the present invention is carried out as described above. Suitable subjects for this aspect of the present invention include those described
5 above.

[0092] The present invention also relates to a method of immunocontraception that involves administering the human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein of the present invention to a subject under conditions effective to provide immunocontraception to the
10 subject. Any of the hLH-R/hCG- β fusion proteins described herein that are immunogenic as described above are suitable in this aspect of the present invention. Administration of the fusion protein of the present invention is carried out as described above. Suitable subjects for this aspect of the present invention include those described above.

15 [0093] Yet another method of immunocontraception is provided in the present invention. This method involves administering the chimeric nucleic acid molecule of the present invention that encodes a human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein to a subject under conditions effective to provide immunocontraception to the subject. This is a type
20 of active immunization known as a "DNA vaccine." DNA vaccines, an alternative approach to standard immunization methods, induce both cellular and humoral immune responses in a subject. Due to advances in molecular biology techniques, DNA vaccines have become a reliable and major means to elicit immune responses in the past decade (Sasaki et al., "Adjuvant Formulations and
25 Delivery Systems for DNA Vaccines," *Methods* 31(3):243-254 (2003); Arnon et al., "Old and New Vaccine Approaches," *Int Immunopharmacol* 3(8):1195-1204 (2003); Garmony et al., "DNA Vaccines: Improving Expression of Antigens," *Genetic Vaccines and Therapy* 1(1):2 (2003); which are hereby incorporated by reference in their entirety). A DNA vaccine is used to induce an immune response
30 to an antigen protein expressed *in vivo*. It is based on the introduction of a purified DNA plasmid encoding for a chosen immunogenic protein or polypeptide sequence into subject. This involves, generally, the development of recombinant, or genetically engineered, live vector (virus or bacterium), capable of expressing

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the antigen and presenting the relevant protective antigen *in vivo* (Sasaki et al., "Adjuvant Formulations and Delivery Systems for DNA Vaccines," *Methods* 31(3):243-254 (2003), which is hereby incorporated by reference in its entirety).

[0094] DNA vaccines generally consist of plasmid vectors (derived from
5 bacteria) or viral vectors, that contain heterologous genes (transgenes) inserted under the control of a eukaryotic promoter, allowing expression of the gene product in mammalian cells (Garmony et al., "DNA Vaccines: Improving Expression of Antigens," *Genetic Vaccines and Therapy* 1(1):2 (2003), which is hereby incorporated by reference in its entirety). The basic requirements of a
10 plasmid DNA vector are a eukaryotic promoter, a cloning site, a polyadenylation sequence, a selectable marker, and a bacterial origin of replication. A strong promoter may be required for expression in mammalian cells. Suitable strong promoters for this aspect of the present invention include, without limitation, the cytomegalovirus (CMV) or simian virus 40 (SV40) promoters. Tissue-specific
15 promoters are also suitable. A cloning site downstream of the promoter is provided for the insertion of the desired heterologous nucleic acid molecule. Also included in the DNA vaccine plasmid of the present invention are a 3' polyadenylation sequence such as the bovine growth hormone (BGH) or SV40 polyadenylation sequence provides stabilization of mRNA transcripts, and is a
20 selectable marker, usually a bacterial resistance gene. For use in mammals, especially humans, kanamycin is highly suitable. Suitable nucleic acid molecules for this aspect of the present invention includes all of the chimeric nucleic acid molecules described herein above. The making of this plasmid vector is as described in greater detail herein above, or as described in Garmony et al., "DNA
25 Vaccines: Improving Expression of Antigens," *Genetic Vaccines and Therapy* 1(1):2 (2003); Sambrook et al., *Molecular Cloning: A Laboratory Manual* Cold Springs Laboratory, Cold Springs Harbor, New York (1989), *Methods in Enzymology*, Vol. 152: *Guide to Molecular Cloning Techniques*, Berger and Kimmel, Eds., San Diego: Academic Press, Inc. (1987), *Current Protocols in
30 Molecular Biology*, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995); which are hereby incorporated by reference in their entirety.

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[0095] Due to advances in recombinant technology, it is now possible to undertake a rational approach to DNA vaccine design (Garmony et al., "DNA Vaccines: Improving Expression of Antigens," *Genetic Vaccines and Therapy* 1(1):2 (2003), which is hereby incorporated by reference in its entirety). In addition to the basic vector preparation described hereinabove, strategies may include the incorporation of immunostimulatory sequences in the backbone of the plasmid, co-expression of stimulatory molecules, utilization of localization/secretory signals, and utilization of the appropriate delivery system (Garmony et al., "DNA Vaccines: Improving Expression of Antigens," *Genetic Vaccines and Therapy* 1(1):2 (2003), which is hereby incorporated by reference in its entirety).

[0096] The majority of DNA vaccines are administered parenterally (Hobson et al., "Mucosal Immunization with DNA Vaccines," *Methods* 31(3):217-224 (2003), which is hereby incorporated by reference in its entirety), however, all other methods of administration as described herein above are also suitable. In particular, the DNA vaccine may be administered in a formulation including microparticles, which allows the co-delivery of the antigen-producing plasmid and an appropriate immunostimulant combined with the microparticle (O'Hagan et al., "Microparticles as Vaccine Adjuvants and Delivery Systems," *Expert Rev Vaccines* 2(2):269-283 (2003), which is hereby incorporated by reference in its entirety); using a particle-mediated ("gene gun") or other epidermal delivery systems (Chen et al., "Needle-Free Epidermal Powder Immunization," *Expert Rev Vaccines* 1(3):265-276 (2002); Haynes, J.R., "Particle-Mediated DNA Vaccine Delivery to the Skin," *Expert Opin Biol Ther* 4(6):889-900 (2004), which are hereby incorporated by reference in their entirety); topical administration (Choi et al., "Topical Vaccination of DNA Antigens: Topical Delivery of DNA Antigens," *Skin Pharmacol Appl Skin Physiol* 16(5):271-282 (2003), which is hereby incorporated by reference in its entirety); delivery to mucosal membranes (O'Hagan et al., "Microparticles as Vaccine Adjuvants and Delivery Systems," *Expert Rev Vaccines* 2(2):269-283 (2003); Hobson et al., "Mucosal Immunization with DNA Vaccines," *Methods* 31(3):217-224 (2003), which are hereby incorporated by reference in their entirety); and any of the oral delivery systems now being designed and perfected

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particularly for the delivery of DNA vaccines (e.g., Sheu et al., "The Gene Pill and its Therapeutic Applications," *Curr Opin Mol Ther* 5(4):420-427 (2003), which is hereby incorporated by reference in its entirety). The DNA vaccines having the nucleic acid molecules of the present invention may also include an
5 adjuvant, either as a carrier for delivery or for immunostimulatory purposes, including, but not limited to those described above, as well as any others known in the art (Sasaki et al., "Adjuvant Formulations and Delivery Systems for DNA Vaccines," *Methods* 31(3):243-254 (2003), which is hereby incorporated by reference in its entirety). Suitable subjects, and suitable disease targets for this
10 aspect of the present invention include those described herein above.

EXAMPLES

Example 1 -- Synthesis of Chimeric hLH-R/ hCG- β Full-Length/Full-Length Nucleic Acid Construct

15 [0097] Since hLH-R and hCG- β have extensive structural and functional interspecies relationships, DNA sequence from human genome of hLH-R and hCG- β was used to prepare the chimeric nucleic acid molecule(s) of the present invention. To prepare chimera 1 of the present invention, a full-length hLH-R nucleic acid fragment containing 2103 bp (SEQ ID NO: 2) with signal peptide in
20 the vector pSG5-hLH-R, as the template, was amplified by PCR (Atger et al., "Structure of the Human Luteinizing Hormone-Choriogonadotropin Receptor Gene: Unusual Promoter and 5' Non-Coding Regions," *Mol Cell Endocrinol* 111(2):113-123 (1995), which is hereby incorporated by reference in its entirety). The PCR product was cloned into the transfer vector pBlueBac4.5/V5-His
25 (Invitrogen, Carlsbad, CA) using *Bam*HI and *Bgl* II restriction sites, which were inserted by PCR at 5' primer and 3' primer, respectively. Full-length hCG- β DNA with signal peptide containing 495 bp (SEQ ID NO:4) (Lobel et al., "Expression and Characterization of Recombinant Beta-Subunit hCG Homodimer," *Endocrin*
30 10(3):261-270 (1999), which is hereby incorporated by reference in its entirety) was also amplified by PCR, and two restriction enzyme sites, *Bgl* II and *Eco*RI, were introduced at 5' primer and 3' primer. The hCG- β was directly cloned into the pBlueBac4.5/V5-His vector, and then fused to hLH-R by subcloning into the

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pBlueBac4.5/V5-His+hLHR vector. Two amino acids (Arg and Ser) were inserted between the C-terminus of the hLH-R nucleotide sequence and the N-terminus of the hCG- β nucleotide sequence to form a contiguous BamHI-hLH-R–BglII-hCG- β -EcoRI fusion. The chimeric gene thus consisted of a total of 2604
5 bp ((SEQ ID NO: 12). The construct is shown schematically in Figure 1.

[0098] The recombinant clones were identified by restriction enzyme digestion of the nucleic acid molecule and sequenced from both 5' and 3' ends to ensure that no mutation occurred during the PCR amplification. After the clone was digested with *Bam*HI, *Bgl*II and *Eco*RI, the agarose gel electrophoresis
10 showed three bands of molecular size 5kb, 2kb and 0.5kb, shown in Figure 2, lane 1, corresponding to the vector pBlueBac4.5/V5-His, hLH-R and hCG- β respectively. In contrast, in Figure 2, lane 2, two bands of 7kb and 0.5kb were visible after the clone was digested with *Bgl* II and *Eco*RI corresponding to the hLH-R with vector and hCG- β . These results confirmed the DNA integrity of
15 hLH-R, hCG- β and the full-length/full length hLH-R/hCG- β chimera.

Example 2 – Expression of hLH-R/hCG- β Chimeric Nucleic Acid Molecule in Baculovirus System and Detection of Fusion Protein by Western Blots

20 [0099] The chimeric gene containing both full-length hLH-R and full-length hCG- β (SEQ ID NO:12) formed a single polypeptide chain consisting of 868 amino acids (SEQ ID NO:11) including two amino acids, Arg and Ser, as a linker between the C-terminus of the hLH-R and the N-terminus of hCG- β . The recombinant fusion protein was identified by western blots using polyclonal
25 antibody raised in the rabbit to LH receptor and antibody to hCG- β (Pal et al., “Active Immunization of Baboons (*Papio anubis*) with the Bovine LH Receptor,” *J Reprod Immunol* 21(2):163-174 (1992); Pal et al., “Biological Actions of Monoclonal Antibodies to Bovine Lutropin Receptor,” *J Reprod Immunol* 22(1):103 (1992) which are hereby incorporated by reference in their entirety).
30 Sf9 insect cells were grown in monolayers at 27 °C in Complete Grace's medium supplemented with 10% fetal calf serum and 10 mg/ml Gentamycin (Invitrogen, Carlsbad, CA). Individual recombinant pBlueBac4.5 transfer vectors were co-transfected with linearized Bac-N-BlueTM viral DNA (Invitrogen, Carlsbad, CA)

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into Sf9 insect cells in the presence of Cellfectin (Invitrogen, Carlsbad, CA). The recombinant baculovirus was purified by plaque assay. Blue putative recombinant plaques were transferred to 12-well microtiter plates and amplified in Sf9 cells. Approximately 2.5×10^6 cells were seeded in 60 mm dishes, and the virus was added with a multiplicity of infection (MOI) of 5.0 and incubated at 27°C for 72 hrs. Cell pellets and culture medium were collected after 72 hrs by centrifugation. The cell pellets were washed twice in PBS buffer (Bio-Rad, Hercules, CA) to remove the serum proteins. The cells were then solubilized by boiling in lysis buffer (1×10^6 cells per 100 μ l) as previously described (Dattatreya Murthy et al., "Isolation of the Luteinizing Hormone-Chorionic Gonadotropin Receptor in High Yield from Bovine Corpora Lutea Molecular Assembly and Oligomeric Nature," *J Biol Chem* 258(5):3140-3158 (1983) and Khan et al., "Purification and Properties of Human Chorionic Gonadotropin/Lutropin Receptor from Plasma-Membrane and Soluble Fractions of Bovine Corpora Lutea," *Biochem J* 197(1):7-22 (1981), which are hereby incorporated by reference in their entirety). The supernatant was boiled for 5 min. in electrophoresis sample buffer containing 62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS and 5% β -mercaptoethanol. The solubilized membrane proteins were analyzed by gel-electrophoresis in 7.5% SDS-polyacrylamide under reducing conditions for western blot analyses. Protein bands were transferred electrophoretically from the gel to the polyvinylidene difluoride (PVDF) membrane (Bio-Rad, Hercules, CA). The membrane was probed for the presence of both hLH-R and hCG- β using polyclonal antibodies. All blots were visualized by chemiluminescence via a secondary horseradish peroxidase-labeled anti-rabbit/ anti-mouse antibody according to the manufacturer's instructions (Bio-Rad, Hercules, CA).

[0100] As shown in Figure 3A, two bands with molecular weights of greater than 172 kD and 110 kD were detected using rabbit polyclonal antibody to LH-R (Pal et al., "Active Immunization of Baboons (*Papio anubis*) with the Bovine LH Receptor," *J Reprod Immunol* 21(2):163-174 (1992); Pal et al., "Biological Actions of Monoclonal Antibodies to Bovine Lutropin Receptor," *J Reprod Immunol* 22(1):103 (1992), which are hereby incorporated by reference in their entirety). Polyclonal antibody to hCG- β also detected the same chimeric

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protein by western blot, as shown in Figure 3B. As expected, exactly the same size bands were also shown when compared to the bands in Figure 3A, whereas these bands were absent in mock-transfected cells, indicating that the chimera protein was expressed in Sf9 cells and detected by antibodies against LH-R as well as hCG. In Figures 3A-B, the 110 kDa protein would represent the mature chimera with post-translation modification, and the other band with molecular weight greater than 172 kDa could be the aggregate of 110 kDa.

10 **Example 3 -- Functional Activity Assay for Ligand Binding Using Biotinylated-hCG**

[0101] A competitive binding assay was performed to determine whether the chimeric protein containing both full-length hLH-R and full-length hCG- β domains (SEQ ID NO:11) would bind to its ligand hCG. Highly purified 2.5 mg/ml hCG (CR-125, containing 11,500-12,000 IU/mg; NICHD, National Institutes of Health, Bethesda, MD) was biotinylated by incubating 10 mg/ml of animohexanoyl-biotin-N-hydroxysuccinimide ester (AH-BNHS) for 1 hr in 0.1 M bicarbonate buffer (pH 8.4) at room temperature (Paukku et al., "Persistence of Biological Activity of Biotinylated Human Chorionic Gonadotropin and its Use for Visualization of Rat Luteinizing Hormone Receptors in Tissue Sections," *J Histochem Cytochem* 46(9):993-998 (1998), which is hereby incorporated by reference in its entirety). The ratio of AH-BNHS to hCG was adjusted to 1:10 (w/w). The biotinylated-hCG was purified and identified by using anti-hCG and HRP-streptavidin conjugate according to the manufacturer's instructions (Zymed Labs, Inc., So. San Francisco, CA). After 24 hrs of transfection with recombinant baculovirus harboring the chimeric hLH-R/hCG- β gene (SEQ ID NO:12), Sf9 insect cells were incubated with 20 ng/ μ l of biotinylated-hCG, with increasing concentrations of unlabeled hCG as the competitor, for 24 hrs at 27 °C. The Sf9 cells infected with the recombinant baculovirus containing LH-R alone was set up as the control. After incubation the medium was aspirated and the cell pellets were washed with PBS buffer containing 0.5g/L BSA. Cells were then incubated for 24 hrs at 27°C with 0.8 μ g/ml of biotinylated-hCG per well with or without an excess of unlabeled hCG in a total volume of 2.5 ml of PBS buffer containing 0.5g/L BSA. Cell surface binding of hCG was determined as the biotin activity.

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For the determinations of the displacement of bound hCG, incubation conditions were identical except that increasing concentrations of purified hCG were used as the competitor. Specific binding of biotinylated-hCG was determined in triplicate for each independent transfection.

- 5 **[0102]** The biotinylated-hCG binding on the surface of Sf9 insect cells was examined by ELISA using horseradish peroxidase (HRP)-streptavidin conjugate (Zymed Labs, Inc., So. San Francisco, CA). As shown in Figure 4A, biotinylated-hCG binding to the Sf9 cells was displaced with increasing amounts of unlabeled hCG, indicating that hCG bound specifically to the hLH-R-hCG- β
- 10 chimeric fusion protein and biotinylated-hCG preserved its ability to bind specifically to LH-R. As shown in Figure 4B, the control with hLH-R alone also specifically bound to biotinylated-hCG as demonstrated by the displacement of binding in the presence of increasing concentration of unlabeled hCG.

15 **Example 4 -- Intracellular cAMP Stimulation by hLH-R-hCG- β**
 Recombinant Fusion Protein

- [0103]** The chimeric hLH-R.hCG- β -CTP protein was further examined for its ability to stimulate the cAMP production in the transfected Sf9 cells as well as in the incubation medium in the absence and presence of increasing quantities of
- 20 hCG. The Sf9 cells were plated in a 12-well plate and washed twice by serum-free medium containing 0.8 mM isobutylmethylxanthine and 0.1% BSA. Sixteen to 18 hrs after transfection with the chimeric hLH-R/hCG- β nucleic acid molecule (SEQ ID NO:12), the cells were incubated in the same medium for 15 min at 27 °C. Increasing concentrations of hCG were added, and incubation was continued
- 25 for 4 hrs at 27 °C. The incubation medium and cell pellets were collected by centrifugation. Cells were lysed in 0.1N HCl. The supernatants and the incubation medium were collected for cAMP assay using cAMP kit according to the manufacturer's instructions (BioMol Research Inc., Plymouth Meeting, PA).

- [0104]** Figure 5A shows the ability of the chimeric protein hLH-R-hCG- β
- 30 to stimulate the cAMP produced in the transfected Sf9 cells as well as in the incubation medium in the absence and presence of increasing quantities of hCG. In addition, the Sf9 cells infected with the recombinant baculovirus containing LH-R alone was set up as a control, shown in Figure 5B. The Sf9 cells expressing

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chimeric hLH-R-hCG- β exhibited an increase in cAMP levels in a dose-dependent pattern and reached up to 59.1 pmol/L, which was approximately a 6-fold increase over the baseline level in the absence of hCG. In the absence of hCG, the intracellular cAMP level was also compared in the transfected and mock-

5 transfected Sf9 cells to determine whether hCG- β at the C-terminal end of the chimeric protein would have bound to the N-terminus of the hLH-R in the chimeric protein. The cAMP concentration in transfected Sf9 cells was 13.2 ± 1.61 pmol/L, compared to 10.09 ± 2.38 pmol/L in mock-transfected cells, indicating no significant difference ($p > 0.05$), and suggesting that the production of

10 cAMP was not stimulated by hCG- β component of the chimeric protein. The level of cAMP in mock-transfected cells also did not change in the presence of increasing quantities of hCG. Furthermore, the cAMP in the incubation medium from infected cells showed no difference compared to mock-infected cells. Compared to the hLH-R, the chimera showed significant increase in intracellular

15 cAMP when the concentration of hCG was only 0.01 ng/ml.

Example 5 -- Determination of hCG- β in Recombinant Protein by hLH-R/hCG- β Chimeric Nucleic Acid Molecule

[0105] Expression of hCG- β protein by the chimera 1 gene was

20 determined in the solubilized membrane fractions of transfected Sf9 cells. Approximately 2.5×10^6 cells were seeded in 60 mm dishes. The recombinant baculovirus containing chimeric nucleic acid molecule was added with a multiplicity of infection (MOI) of 5.0 and incubated at 27 °C. After 72 hrs, cell pellets were collected by centrifugation. Solubilized membrane proteins were

25 analyzed for total protein by using DC Protein Assay kit (Bio-Rad, Hercules, CA). hCG- β was determined in the soluble membrane fraction by using the ADVIA Centaur Total hCG and hCG- β assay by Automated Chemiluminescence System (ACS) 180-SE. As shown in Table 1, below, all the samples contained a similar amount of protein, including the mock-transfected cells. hCG was not detectable

30 in the mock-transfected Sf9 cells (see Table 1), whereas in the infected Sf9 cells, the concentration of the expressed hCG- β was 267.76 ± 7.6 IU hCG/L. These

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results attest to the expression of a bifunctional protein containing both hLH-R and hCG- β activities by the hLH-R-hCG- β chimera.

Table 1

5 Total protein and hCG concentration in solubilized membrane fractions of Sf9 insect cells transfected with hLH-R-hCG- β chimeric construct.

Solubilized	Total protein	Total hCG & hCG- β *
Membrane protein	mg/ml	IU/L
Normal Sf9 cells	5.16 $\pm$ 0.82	ND
Infected Sf9 cells	6.10 $\pm$ 3.87	267.76 $\pm$ 7.6 (3)

[0106] In the present invention, a unique chimeric nucleic acid construct of full-length hLH-R and full-length hCG- β (chimera 1) was designed and synthesized. This construct was expressed in Sf9 insect cells to produce a bifunctional protein displaying both LH-R and hCG- β activities, and functional properties such as ligand binding ability and intracellular cAMP stimulation. Western blot analyses of membrane solubilized fractions revealed that the protein expressed in Sf9 cells was detected by the antibody to LH-R as well as the antibody to hCG- β , indicating that the recombinant protein contained antigenic sites of both LH-R and hCG- β .

[0107] The recombinant chimeric hLH-R-hCG- β protein recognized its ligand, as shown by the specific binding of hCG to the chimeric protein. This also suggests that the hCG- β component of the chimeric protein did not interfere with the binding of exogenous hCG to LH-R. The hCG- β alone does not bind to the LH-R. In the yoked fusion protein expressed in COS-7 cells as described by Wu et al (Wu et al., "Protein Engineering of Novel Constitutively Active Hormone-Receptor Complex," *J Biol Chem* 271:31638-31642 (1996), which is hereby incorporated by reference in its entirety), the α and β subunits of hCG were attached to the N-terminal ligand binding domain of the receptor, and exogenous hCG probably could not bind to the yoked LH-R.

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[0108] The stimulation of intracellular cAMP by hCG was examined in order to investigate whether the binding of hCG to the chimera resulted in biological function. As shown in Figure 5A, the intracellular cAMP concentration increased, with increasing concentrations of hCG in a dose-response manner, while cAMP levels in culture medium did not change regardless of the concentration of hCG, which indicated that cAMP was produced inside Sf9 cells. To further confirm the stimulation of cAMP only by exogenous hCG, and not by the intramolecular hCG- β , the cAMP production in both mock-transfected as well as transfected Sf9 cells was determined in the absence of exogenous hCG. Levels of intracellular cAMP in both mock as well as transfected Sf9 cells did not show any significant difference, further indicating that the intracellular hCG- β of the chimeric protein did not have any effect on the stimulation of cAMP indicating that intracellular hCG- β did not bind to the hLH-R component of the chimeric protein. It is well known that hCG- β alone does not bind to the receptor.

15

Example 6 -- Synthesis of Chimeric hLH-R Extracellular Domain/hCG- β Carboxy Terminal Peptide Nucleic Acid Construct

[0109] Restriction enzymes were purchased from New England BioLabs, Inc. (Beverly, MA). Oligonucleotides used for PCR amplification and sequencing were synthesized by Invitrogen (Carlsbad, CA). FBS was purchased from Gibco (Invitrogen, Carlsbad, CA). DNA Polymerase, T4 Ligase, *E. Coli* strain DH5 α competent cells, *pBlueBac4.5/V5-His*, linearized Bac-N-BlueTM viral DNA, Cellfectin, gentamycin, and Grace's insect cell medium were purchased from Invitrogen (Invitrogen, Carlsbad, CA). Amino hexanoyl-biotin-N-hydroxysuccinimide ester (AH-BNHS) was obtained from Zymed Labs Inc. (San Francisco, CA). Polyvinylidene difluoride (PVDF) membrane, chemiluminescence kit, PBS Buffer and TBS buffer were obtained from Bio-Rad (Hercules, CA). hCG (CR-125, containing 11,500-12,000 IU/mg) was provided by NICHD, National Institutes of Health (Bethesda, MD). Isobutylmethylxanthine was obtained from Calbiochem (San Diego, CA). cAMP kits were purchased from BioMol Research Inc. (Plymouth Meeting, PA). Antibody to LH receptor and antibody to hCG- β were raised in rabbits (Pal et al., "Biological Actions of Monoclonal Antibodies to Bovine Lutropin Receptor," *J*

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Reprod Immunol 22(1):103 (1992), which is hereby incorporated by reference in its entirety).

[0110] The cDNA encoding the hLH-R-ECD containing 1041 bp (SEQ ID NO:6) with signal peptide of 20 amino acids in the vector pBluescript-hLH-R (Minegishi et al., "Cloning and Sequencing of Human LH/hCG Receptor cDNA," *Biochem Biophys Res Commun* 201:1057 (1994), which is hereby incorporated by reference in its entirety) was amplified by PCR. The PCR product was cloned into the transfer vector pBlueBac4.5/V5-His using *BamH* I and *Kpn* I restriction sites, which were inserted by PCR at 5' primer and 3' primer, respectively. The cDNA of hCG- β -CTP containing 114 bp (SEQ ID NO:8) was also amplified by PCR (Lobel et al., "Expression and Characterization of Recombinant Beta-Subunit hCG Homodimer," *Endocrin* 10(3):261-270 (1999), which is hereby incorporated by reference in its entirety), and fused to hLH-R-ECD by subcloning into expression vector pBlueBac4.5/V5-His containing ECD of hLH-R with *Kpn* I and *EcoR* I. Two amino acids (Gly and Thr) were inserted between the C-terminus of the hLH-R-ECD and the N-terminus of the hCG- β -CTP to form a contiguous *BamH* I-hLH-R-ECD-*Kpn* I-hCG- β -CTP-*EcoR* I fusion. The chimeric gene thus consisted of a total of 1161 bp (SEQ ID NO:16). This construct is shown schematically in Figure 6. Ligation reactants were transformed into competent DH5 α cells. The recombinant clones were identified by restriction enzyme digestions of the DNA as well as by DNA sequencing, to ensure that no mutations were introduced during the amplification process. The positive clones were identified by restriction enzyme digestion of DNA followed by agarose gel electrophoresis, which showed three bands of molecular size 5kb, 1kb, and 0.1kb corresponding to the vector pBlueBac4.5/V5-His, hLH-R-ECD and hCG- β -CTP, respectively, as shown in Figure 7. The DNA sequence of the construct showed that no mutation occurred during the PCR amplification. These results confirmed the DNA integrity hLH-R-ECD-hCG- β -CTP chimera.

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Example 7 -- Expression of hLH-R-ECD-hCG- β -CTP Nucleic Acid Molecule in Baculovirus System and Detection of Fusion Protein by Western Blots

[0111] The chimeric gene containing both the extracellular domain of hLH-R and CTP of hCG- β formed a single polypeptide chain consisting of 387 amino acids (SEQ ID NO:15) with two amino acids, Gly and Thr, as a linker between the C-terminus of the hLH-R-ECD and the N-terminus of hCG- β -CTP. Individual recombinant pBlueBac4.5 transfer vectors containing both the chimeric DNA and hLH-R-ECD alone were co-transfected with linearized Bac N-BlueTM viral DNA into Sf9 insect cells in the presence of Cellfectin. Sf9 insect cells were grown in monolayers at 27 °C in Complete Grace's medium, supplemented with 10% fetal calf serum and 10 μ g/ml gentamycin. The recombinant baculovirus was purified by the plaque assay. Blue putative recombinant plaques were transferred to 12-well microtiter plates and amplified in Sf9 cells to make high titer viral solution. Approximately 2.5×10^6 Sf9 cells were seeded in 60 mm dishes, and the virus was added with a multiplicity of infection (MOI) of 5.0 and incubated at 27°C for 72 hr. Cell pellets and culture medium were collected after 72 hr and separated by centrifugation. Solubilized fractions were analyzed in a 7.5 % SDS-PAGE. Samples were resolved in the 7.5% polyacrylamide gels under reducing conditions, and the gel was stained with Coomassie Brilliant Blue R 250.

[0112] For western blot analysis, cell pellets were washed twice in PBS buffer to remove the serum proteins. The cells were then solubilized by boiling in the lysis buffer (1×10^6 cells per 100 μ l) as previously described (Hao et al., "Expression of A Recombinant Bifunctional Protein from a Chimera of Human Lutropin Receptor and Human Chorionic Gonadotropin β -Subunit," *Journal of Reproductive Immunology* 63:123-135 (2004), which is hereby incorporated by reference in its entirety). The supernatant was then boiled for 5 min. in electrophoresis sample buffer containing 62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS and 5% β -1 mercaptoethanol. The solubilized proteins were analyzed by gel-electrophoresis in a 7.5% SDS-polyacrylamide under reducing conditions. Proteins were then transferred electrophoretically from the gel to the polyvinylidene difluoride (PVDF) membrane after blocking with 5% milk proteins dissolved in TBS buffer. The membranes were probed for the presence

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of both hLH-R and hCG- β by incubating separately with antibody to LH-R and with antibody to hCG- β (Pal et al., "Biological Actions of Monoclonal Antibodies to Bovine Lutropin Receptor," *J Reprod Immunol* 22(1):87-103 (1992), which is hereby incorporated by reference in its entirety). The antibody-bound protein

5 bands in the membrane were detected using chemiluminescence via a secondary horseradish peroxidase-labeled anti-rabbit/ anti-mouse antibody according to the manufacturer's instructions. Protein bands on the membrane were visualized by exposure to X-ray film.

[0113] As shown in Figure 8, the SDS-PAGE showed three bands of

10 molecular weights of about 36 kDa, 50 kDa and 75 kDa. As shown in Figure 9A, three bands with molecular weights of about 36 kDa, 50 kDa and 75 kDa were detected using antibody to LH-R. Antibody to hCG- β also detected the same molecular weight chimeric proteins by western blots, shown in Figure 9B, similar to the bands in Figure 9A, whereas these bands were absent in mock-transfected

15 cells, indicating that the chimeric protein was expressed in Sf9 cells and was detected by antibody against LH-R as well as by the antibody against hCG- β . The 50 kDa protein would probably represent the mature chimera with post translation modification. The protein bands of molecular weight of approximately, 35 kDa and 75 kDa probably represent the oligomeric forms of the chimera. The culture

20 medium showed no protein bands in western blots, indicating that the recombinant proteins were not secreted.

Example 8– Functional Activity Assay for Ligand Binding Using Biotinylated hCG

25 [0114] Highly purified, 2.5 mg/ml, hCG (CR-125, containing 11,500-12,000 IU/mg was incubated with 10 mg/ml of aminohexanoyl-biotin-N-hydroxysuccinimide ester (AH BNHS) for 1 hr in 0.1 M bicarbonate buffer (pH 8.4) at room temperature (Paukku et al., "Persistence of Biological Activity of Biotinylated Human Chorionic Gonadotropin and its Use for Visualization of Rat

30 Luteinizing Hormone Receptors in Tissue Sections," *J Histochem Cytochem* 46(9):993-998 (1998), which is hereby incorporated by reference in its entirety). The ratio of AH-BNHS to hCG was adjusted to 1:10 (w/w). The biotinylated-

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hCG was purified and identified by using anti-hCG and HRP-streptavidin conjugate according to the manufacturer's instructions.

[0115] Twenty four hours after transfection in 12-well plates, competitive ligand binding assays were carried out on intact transfected Sf9 cells expressing chimera, as well as hLH-R-ECD alone as the control. The Sf9 cells were rinsed with PBS buffer containing 0.5 g/L BSA. The cells were then incubated for 24 hr at 27°C with 0.8 µg/ml of biotinylated-hCG per well with or without an excess of unlabeled hCG, in a total volume of 2.5 ml of PBS buffer containing 0.5g/L BSA. The cell surface binding of hCG was determined as the biotin activity according to the manufacturer's instructions. For the determinations of the displacement of bound hCG, incubation conditions were identical except that increasing concentrations of purified hCG were used as the competitor. Binding of biotinylated-hCG was determined in triplicate for each independent transfection.

15 **Example 9 -- Determination of Ligand-Induced Intracellular cAMP
Production by hLH-R-ECD/hCG-β-CTP Recombinant Fusion
Protein**

[0116] The chimeric protein hLH-R-ECD-hCG-β-CTP was further examined for its ability to stimulate the cAMP production in the transfected Sf9 cells as well as in the incubation medium in the absence and presence of increasing quantities of hCG. The Sf9 cells were seeded in a 12-well plate. Sixteen to 18 hr after transfection with the hLH-R-ECD-hCG-β-CTP chimeric construct, the cells were washed twice by serum-free medium containing 0.8 mM isobutylmethylxanthine and 0.1% BSA and incubated in the same medium for 15 min at 27°C. Increasing concentrations of hCG were added, and incubation was continued for 4 hr. at 27°C. The incubation medium and cell pellets were collected by centrifugation. The production of cAMP was also measured at different time intervals. Cells were lysed in 0.1N HCl. The lysis supernatants and the incubation medium were collected for cAMP assay using cAMP kit according to the manufacturer's instructions.

[0117] The Sf9 cells expressing chimeric hLH-R-ECD-hCG-β-CTP exhibited an increase in cAMP levels in a dose-dependent pattern, as shown in Figure 11A, while hLH-R-ECD did not stimulate the production of intracellular

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cAMP, as shown in Figure 11B. . In the absence of hCG, the intracellular cAMP level was also compared in the transfected and mock-transfected Sf9 cells to determine whether hCG- β -CTP at the C-terminal end of the chimeric protein would have interacted with the N-terminus of hLH-R-ECD in the chimeric protein. The results showed little difference between transfected and mock-transfected cells in the absence of hCG. cAMP level in mock-transfected cells also did not change in the presence of increasing quantities of hCG. Furthermore, the cAMP in the incubation medium from infected cells showed no difference compared with mock-infected cells. As shown in Figure 11C, the production of cAMP in Sf9 cells transfected with chimeric DNA construct showed maximum stimulation at one hour of incubation with hCG, which was similar to the pattern of LH-R when incubated with hCG (Igarashi et al., "Functional Expression or Recombinant Human Luteinizing Hormone/Human Choriogonadotropin Receptor," *Biochem Biophys Res Commun* 201:248-256 (1994), which is hereby incorporated by reference in its entirety). There was no stimulation of cAMP production in Sf9 cells transfected with hLH-R-ECD alone.

Example 10 -- Measurement of hCG - β CTP in Recombinant Protein

[0118] Approximately 2.5×10^6 cells were seeded in 60 mm dishes. The recombinant baculovirus containing chimeric hLH-R-ECD-hCG- β -CTP nucleic acid molecule was added with a multiplicity of infection (MOI) of 5.0 and incubated at 27 °C. After 72 hr, cell pellets were collected by centrifugation. Solubilized proteins were analyzed for total protein by using DC Protein Assay kit. hCG β -CTP was determined in the soluble membrane fraction by using the ADVIA Centaur. Total hCG and hCG- β assay using two a site specific antibody by Automated Chemiluminescence System (ACS) 180-SE.

Example 11 -- Affinity of hCG Binding to Chimeric hLH-R-ECD-hCG- β -CTP

[0119] A competitive binding assay was performed to determine whether hCG would bind to the chimeric protein containing both hLH-R-ECD and hCG- β -CTP. After 24 hr of transfection with recombinant baculovirus, Sf9 insect cells were incubated for 24 hr at 27°C with 20 ng/ μ l of biotinylated-hCG and

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increasing concentrations of unlabeled hCG as the competitor. The Sf9 cells infected with the recombinant baculovirus containing ECD of LH-R alone was set up as the control. The binding of biotinylated-hCG on the surface of the Sf9 insect cells was examined by ELISA using horseradish peroxidase (HRP)-streptavidin conjugate. Unlabeled hCG inhibited the binding of biotinylated-hCG to the chimeric protein, as shown in Figure 10, but not to the hLH-R-ECD. This suggests that the expressed hLH-R-ECD was trapped inside the cells while chimeric protein was located on the membrane. The results indicated that hCG bound specifically to the hLH-R-ECD-hCG- β -CTP chimeric protein and that the biotinylated-hCG preserved its ability to bind specifically to LH-R.

Example 12-- Expression of Chimeric hLH-R Extracellular Domain/hCG- β Carboxy Terminal Peptide Nucleic Acid Construct

[0120] Expression of hCG- β -CTP protein by the chimeric hLH-R-ECD-hCG- β -CTP nucleic acid molecule was further examined for its ability to stimulate the cAMP production in the solubilized fractions of transfected Sf9 cells, as well as in the incubation medium in the absence and presence of increasing quantities of hCG. As shown in Table 2, below, the chimera protein hLH-R-ECD-hCG- β -CTP contained 355 ± 2.5 mIU hCG/ml, but hCG was not detectable in Sf9 cells transfected with hLH-R-ECD alone as well as in the mock-transfected solubilized Sf9 cells. These results further attested that the hCG- β -CTP, with only 38 amino acid chain, still had the antigenic site, which was recognized by antibodies to hCG, and confirmed the expression of a bifunctional protein containing both hLH-R and hCG- β activities by the hLH-R-ECD-hCG- β -CTP chimera.

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Table 2

Total protein and hCG concentration in solubilized fractions of Sf9* insect cells transfected with DNA constructs.

Solubilized Proteins	Total protein mg/ml	Total hCG & hCG-β** mIU/mL	hCG /protein mIU/mg
Mock-transfected Sf9 cells	5.16 ± 0.82	ND	N/A
hLH-R-ECD	8.1 ± 0.09	ND	N/A
hLH-R-ECD-hCG-β-CTP	13.9 ± 0.303	355 ± 2.5	25.5

5 * Sf9 cells transfected with recombinant baculovirus were collected after 72 hr. of transfection with two DNA chimera constructs. Solubilized proteins were analyzed for total protein and total hCG and hCG- β concentration.

10 **[0121]** An interspecies usefulness of the present invention is readily visualized because of the structural homology, functional similarity, and immunological cross-reactivity of LH-R of other vertebrates with human LH-R (Ascoli et al., The Lutropin/Choriogonadotropin Receptor, A 2002 Perspective,” *Endocr Rev* 23(2):141-174 (2002); Pal et al., “Active Immunization of Baboons

15 (*Papio anubis*) with the Bovine LH Receptor,” *J Reprod Immunol* 21(2):163-174 (1992); Pal et al., “Biological Actions of Monoclonal Antibodies to Bovine Lutropin Receptor,” *J Reprod Immunol* 22(1):103 (1992); Remy et al., “Immunization Against Exon 1 Decapeptides From the Lutropin/Choriogonadotropin Receptor or the Follitropin Receptor as Potential

20 Male Contraceptive,” *J Reprod Immunol* 32(1):37-54 (1996); Singh et al., “Effect of Immunization with Lutropin-Receptor on the Ovarian Function of Rabbits,” *J Immunoassay* 16(1):1-16 (1995), which are hereby incorporated by reference in their entirety). Female dogs and cats, actively immunized with highly purified bovine LH-R, produced antibodies to the LH-receptor and suppressed

25 progesterone synthesis, apparently due to the blockade of the gonadal receptor (Saxena et al., “Modulation of Ovarian Function in Female Dogs Immunized With Bovine Luteinizing Hormone Receptor,” *Reprod Domest Anim* 37(1):9-17 (2002) and Saxena et al., “Effect of Immunization with Bovine Luteinizing Hormone Receptor on Ovarian Function in Cats,” *Am J Vet Res* 64(3):292-298 (2003),

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which are hereby incorporated by reference in their entirety). Endogenous antibodies against hCG- β alone have been shown to inhibit conception (Gupta et al., "Contraceptive Vaccines," *Adv Contracept Deliv Syst* 10(3-4):255-65 (1994); Heikoop et al., "Evaluation of Subunit Truncation and the Nature of the Spacer for Single Chain Human Gonadotropins," *Eur J Biochem* 245:656-662 (1997) and Stevens, V.C., "Use of Synthetic Peptides as Immunogens for Developing a Vaccine Against Human Chorionic Gonadotropin," *Ciba Found Symp* 119:200-25 (1986), which are hereby incorporated by reference in their entirety). Moreover, studies in dogs, cats, and baboon (Saxena et al., "Modulation of Ovarian Function in Female Dogs Immunized With Bovine Luteinizing Hormone Receptor," *Reprod Domest Anim* 37(1):9-17 (2002); Saxena et al., "Effect of Immunization with Bovine Luteinizing Hormone Receptor on Ovarian Function in Cats," *Am J Vet Res* 64(3):292-298 (2003); and Pal et al., "Active Immunization of Baboons (*Papio anubis*) with the Bovine LH Receptor," *J Reprod Immunol* 21(2):163-174 (1992); Pal et al., "Biological Actions of Monoclonal Antibodies to Bovine Lutropin Receptor," *J Reprod Immunol* 22(1):103 (1992), which are hereby incorporated by reference in their entirety) indicate that the infertile ovarian and behavioral profiles during immunization against LH-R alone were reversible, however, it remains to be seen if the effect of immunization against the LHR-hCG- β chimera would also be reversible. Hence, the antibodies to a bifunctional protein containing LH-R and hCG- β moieties, as described here, would be expected to block the binding of LH to the receptor. The antibodies to the hCG- β would specifically neutralize chorionic gonadotropin (CG)-like material produced by the blastocyst at the time of implantation and inhibit conception (Saxena, B.B., "Measurement and Clinical Significance of Preimplantation Blastocyst Gonadotropins," *J Reprod Fertil Suppl* 37:115-119 (1989), which is hereby incorporated by reference in its entirety).

[0122] Even though LH-R alone is antigenic at the interspecies level, a chimeric construct of LH-R and hCG- β could presumably become more antigenic since it will present to the immune system as a heterologous antigen and facilitate antibody response (Vaitukaitis et al., "A Radioimmunoassay Which Specifically Measures Human Chorionic Gonadotropin in the Presence of Human Luteinizing

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Hormone,” *Am J Obstet Gynecol* 113(6):751-758 (1972); Ross, G.T., “Clinical Relevance of Research on the Structure of Human Chorionic Gonadotropin,” *Am J Obstet Gynecol* 129(7):795-808 (1977); Vaitukaitis et al., “Gonadotropins and Their Subunits: Basic and Clinical Studies,” *Recent Prog Horm Res* 32:289-331 (1976); and Vaitukaitis, J.L., “Radioimmunoassay of Human Choriogonadotropin,” *Clin Chem* 31(10):1749-1754 (1985), which are hereby incorporated by reference in their entirety). Thus, the recombinant bifunctional LH-R and hCG- β protein provide a potentially new antigen to be used for the immuno-regulation of the gonadal function as postulated earlier (Saxena et al., “New Approaches in Fertility Regulation,” *J Obstet Gynaecol* 4(Suppl 1):S16-22 (1984), which is hereby incorporated by reference in its entirety). The effects of antibodies to the hLH-R-hCG- β chimera on non-gonadal hCG and LH-R function remains to be elucidated. Antibodies to hLH-R or chimeras with hCG- β may also assist in further understanding the mechanism of ligand receptor interaction at the molecular levels (Fralish et al., “Structural Analysis of Yoked Chorionic Gonadotropin-Luteinizing Hormone Receptor Ectodomain Complexes by Circular Dichroic Spectroscopy,” *Mol Endocrinol* 17(7):1192-1202 (2003), which is hereby incorporated by reference in its entirety).

[0123] The present invention discloses the synthesis of a chimeric DNA construct of full-length of hLH-receptor and hCG- β and its expression in Sf9 cells to produce a bifunctional protein. Recombinant protein was recognized by antibodies to LHR as well as anti-hCG- β in western blots, thus indicating the preservation of immunological epitopes for both hLH-R and hCG- β in the chimera. Specific ligand binding of recombinant hLH-R component was demonstrated by the displacement of bound labeled hCG at increasing concentrations of unlabeled hCG, indicating that the presence of hCG- β component of the chimera did not interfere with the binding of hCG to LH-R. hCG- β was also present in the recombinant chimeric protein as shown by a specific hCG- β chemiluminescence assay. Treatment of transfected Sf9 cells with hCG induced dose-dependent increase in the stimulation of intracellular cAMP production, which showed that the ligand binding had functional activity. These results demonstrate that the chimeric DNA construct of hLH-R-hCG- β expressed

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a bifunctional protein containing both hLH-R and hCG- β activities, which could provide a unique potential antigen for immunocontraception in vertebrates.

[0124] The present invention also describe the synthesis of a DNA construct of hLH-R-ECD fused with hCG- β -CTP, and the expression of a
5 chimeric bifunctional protein, which would produce bifunctional antibodies with dual effects. Antibodies directed to the ECD of LH-R would specifically block the high affinity binding of the ligand to the receptor; whereas the antibodies to hCG- β -CTP moiety would specifically neutralize hCG produced at the time of implantation (Saxena, B.B., "Measurement and Clinical Significance of
10 Preimplantation Blastocyst Gonadotropins," *J Reprod Fertil Suppl* 37:115-119 (1989), which is hereby incorporated by reference in its entirety) and consequently suppress the production of progesterone by the corpus luteum. The bifunctional antibodies would thereby inhibit fertility. Antibodies specific to the C-terminal peptide of hCG- β would have little cross-reaction with pituitary LH. Thus, hLH-
15 R-ECD-hCG- β -CTP chimeric protein provides a unique antigen for immuno-regulation of gonadal function among vertebrates in both female and male.

[0125] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing
20 from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS
COMPREND PLUS D'UN TOME.**

CECI EST LE TOME __1__ DE __2__

NOTE: Pour les tomes additionels, veuillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

THIS IS VOLUME __1__ OF __2__

NOTE: For additional volumes please contact the Canadian Patent Office.

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WHAT IS CLAIMED:

1. A chimeric nucleic acid molecule encoding a fusion protein comprising a human lutropin hormone receptor domain and a human chorionic gonadotropin- β domain.
5
2. The chimeric nucleic acid molecule according to claim 1, wherein the nucleic acid molecule encodes a fusion protein comprising a full-length human lutropin hormone receptor domain and a full-length human chorionic gonadotropin- β domain.
10
3. The chimeric nucleic acid molecule according to claim 2, wherein the nucleic acid molecule encodes a fusion protein further comprising a peptide linker between the full-length human lutropin hormone receptor domain and the full-length human chorionic gonadotropin- β domain.
15
4. The chimeric nucleic acid molecule according to claim 3, wherein the peptide linker comprises arginine and serine.
- 20 5. The chimeric nucleic acid molecule according to claim 4, wherein the nucleic acid molecule either 1) has a nucleotide sequence of SEQ ID NO:12; 2) encodes a protein having an amino acid sequence of SEQ ID NO:11; or 3) hybridizes to the nucleic acid molecule having SEQ ID NO:12 under highly stringent conditions characterized as hybridization for at least 1 hour at 65° C in
25 5X SSC, 0.02% ficoll, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin, 0.5% SDS, 2 mM EDTA, a wash in 2X SSC at room temperature and one or more washes at 0.1X SSC/0.1% SDS at 68° C.
6. The chimeric nucleic acid molecule according to claim 5,
30 wherein the nucleic acid molecule has a nucleotide sequence of SEQ ID NO:12.

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7. The chimeric nucleic acid molecule according to claim 5, wherein the nucleic acid molecule encodes a protein having an amino acid sequence of SEQ ID NO:11.

5 8. The chimeric nucleic acid molecule according to claim 5, wherein the nucleic acid molecule hybridizes to the nucleic acid molecule having SEQ ID NO:12 under highly stringent conditions characterized as hybridization for at least 1 hour at 65° C in 5X SSC, 0.02% ficoll, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin, 0.5% SDS, 2 mM EDTA, a wash in 2X SSC at
10 room temperature and one or more washes at 0.1X SSC/0.1% SDS at 68° C.

9. The chimeric nucleic acid molecule according to claim 1, wherein the nucleic acid molecule encodes a fusion protein comprising a full-length human lutropin hormone receptor domain and a fragment of a human
15 chorionic gonadotropin- β domain.

10. The chimeric nucleic acid molecule according to claim 9, wherein the fragment of the human chorionic gonadotropin- β domain is the carboxyl terminal peptide of the human chorionic gonadotropin- β protein.
20

11. The chimeric nucleic acid molecule according to claim 10, wherein the nucleic acid molecule encodes a fusion protein further comprising a peptide linker between the full-length human lutropin hormone receptor domain and the carboxyl terminal peptide of the human chorionic gonadotropin- β domain.
25

12. The chimeric nucleic acid molecule according to claim 11, wherein the peptide linker comprises glycine and threonine.

13. The chimeric molecule according to claim 12, wherein the
30 nucleic acid molecule either 1) has a nucleotide sequence of SEQ ID NO:14; 2) encodes a protein having an amino acid sequence of SEQ ID NO:13; or 3) hybridizes to the nucleic acid molecule having SEQ ID NO:14 under highly stringent conditions characterized as hybridization for at least 1 hour at 65° C in

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5X SSC, 0.02% ficoll, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin, 0.5% SDS, 2 mM EDTA, a wash in 2X SSC at room temperature and one or more washes at 0.1X SSC/0.1% SDS at 68° C.

5 14. The chimeric nucleic acid molecule according to claim 13, wherein the chimeric nucleic acid molecule has a nucleotide sequence of SEQ ID NO: 14.

10 15. The chimeric nucleic acid molecule according to claim 13, wherein the nucleic acid molecule encodes a protein having an amino acid sequence of SEQ ID NO: 13.

15 16. The chimeric nucleic acid molecule according to claim 13, wherein the nucleic acid molecule hybridizes to the nucleic acid molecule having SEQ ID NO:14 under highly stringent conditions characterized as hybridization for at least 1 hour at 65° C in 5X SSC, 0.02% ficoll, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin, 0.5% SDS, 2 mM EDTA, a wash in 2X SSC at room temperature and one or more washes at 0.1X SSC/0.1% SDS at 68° C.

20 17. The chimeric nucleic acid molecule according to claim 1, wherein the nucleic acid molecule encodes a fusion protein comprising a fragment of a human lutropin hormone receptor protein and a fragment of a human chorionic gonadotropin- β protein.

25 18. The chimeric nucleic acid molecule according to claim 17, wherein the fragment of the human lutropin hormone receptor protein is the extracellular ligand binding domain of the human lutropin hormone receptor and the fragment of the human chorionic gonadotropin- β protein is the carboxyl terminal peptide of the human chorionic gonadotropin- β protein.

30 19. The chimeric nucleic acid molecule according to claim 18, wherein the nucleic acid molecule encodes a fusion protein further comprising a peptide linker between the extracellular ligand binding domain of the human

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lutropin hormone receptor and the carboxyl terminal peptide of the human chorionic gonadotropin- β protein.

20. The chimeric nucleic acid molecule according to claim 19,
5 wherein the peptide linker comprises glycine and threonine.

21. The chimeric molecule according to claim 20, wherein the nucleic acid molecule either 1) has a nucleotide sequence of SEQ ID NO:16; 2) encodes a protein having an amino acid sequence of SEQ ID NO:15; or 3)
10 hybridizes to the nucleic acid molecule having SEQ ID NO:16 under highly stringent conditions characterized as hybridization for at least 1 hour at 65° C in 5X SSC, 0.02% ficoll, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin, 0.5% SDS, 2 mM EDTA, a wash in 2X SSC at room temperature and one or more washes at 0.1X SSC/0.1% SDS at 68° C.

15

22. The chimeric nucleic acid molecule according to claim 21, wherein the nucleic acid molecule has a nucleotide sequence of SEQ ID NO: 16.

23. The chimeric nucleic acid molecule according to claim 21,
20 wherein the nucleic acid molecule encodes a fusion protein having an amino acid sequence of SEQ ID NO: 15.

24. The chimeric nucleic acid molecule according to claim 21, wherein the nucleic acid molecule hybridizes to the nucleic acid molecule having
25 SEQ ID NO:16 under highly stringent conditions characterized as hybridization for at least 1 hour at 65° C in 5X SSC, 0.02% ficoll, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin, 0.5% SDS, 2 mM EDTA, a wash in 2X SSC at room temperature and one or more washes at 0.1X SSC/0.1% SDS at 68° C.

25. The chimeric nucleic acid molecule according to claim 1,
30 wherein the nucleic acid molecule encodes a fusion protein comprising a fragment of a human lutropin hormone receptor protein domain and a full-length human chorionic gonadotropin- β domain.

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26. The chimeric nucleic acid molecule according to claim 25, wherein the fragment of the human lutropin hormone receptor protein is the extracellular ligand binding domain of the human lutropin hormone receptor.

5

27. The chimeric nucleic acid molecule according to claim 26, wherein the nucleic acid molecule encodes a fusion protein further comprising a peptide linker between the fragment of a human lutropin hormone receptor domain and the full-length human chorionic gonadotropin- β domain.

10

28. The chimeric nucleic acid molecule according to claim 27, wherein the peptide linker comprises glycine and threonine.

29. The chimeric molecule according to claim 28, wherein the nucleic acid molecule either 1) has a nucleotide sequence of SEQ ID NO:18; 2) encodes a protein having an amino acid sequence of SEQ ID NO:17; or 3) hybridizes to the nucleic acid molecule having SEQ ID NO:18 under highly stringent conditions characterized as hybridization for at least 1 hour at 65° C in 5X SSC, 0.02% ficoll, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin, 0.5% SDS, 2 mM EDTA, a wash in 2X SSC at room temperature and one or more washes at 0.1X SSC/0.1% SDS at 68° C.

15
20

30. The chimeric nucleic acid molecule according to claim 29, wherein the nucleic acid molecule has a nucleotide sequence of SEQ ID NO: 18.

25

31. The chimeric nucleic acid molecule according to claim 29, wherein the nucleic acid molecule encodes a protein having an amino acid sequence of SEQ ID NO: 17.

30

32. The chimeric nucleic acid molecule according to claim 29, wherein the nucleic acid molecule hybridizes to the nucleic acid molecule having SEQ ID NO:18 under highly stringent conditions characterized as hybridization for at least 1 hour at 65° C in 5X SSC, 0.02% ficoll, 0.02% polyvinyl pyrrolidone,

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0.02% bovine serum albumin, 0.5% SDS, 2 mM EDTA, a wash in 2X SSC at room temperature and one or more washes at 0.1X SSC/0.1% SDS at 68° C.

5 33. A nucleic acid construct comprising:
 the chimeric nucleic acid molecule according to claim 1,
and
 transcriptional and translational regulatory nucleotide
sequences linked to the nucleic acid molecule to allow expression of the chimeric
nucleic acid molecule.

10

 34. A host comprising the chimeric nucleic acid molecule
according to claim 1.

15 35. The host according to claim 34, wherein the host is selected
from the group consisting of a bacterial cell, a virus, a yeast cell, an insect cell,
and a mammalian cell.

 36. The host according to claim 35, wherein the host is an
insect cell.

20

 37. The host according to claim 35, wherein the host is a
mammalian cell.

25 38. The host according to claim 37, wherein the mammalian
cell is selected from the group consisting of a canine cell, a feline cell, and a
human cell.

30 39. The host according to claim 34, wherein the host expresses
the human lutropin hormone receptor/human chorionic gonadotropin- β fusion
protein encoded by the nucleic acid molecule and exhibits bifunctional activity
comprising human lutropin hormone receptor activity and human chorionic
gonadotropin- β activity.

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40. An isolated human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein comprising:

a human lutropin hormone receptor domain and
a human chorionic gonadotropin- β domain.

5

41. The isolated human lutropin hormone receptor/human chorionic gonadotropin- β fusion protein according to claim 40, wherein the fusion protein comprises a full-length human lutropin hormone receptor domain and a full-length human chorionic gonadotropin- β domain.

10

42. The isolated fusion protein according to claim 41, wherein the protein further comprises peptide linker between the human lutropin hormone receptor domain and the human chorionic gonadotropin- β domain.

15

43. The chimeric nucleic acid molecule according to claim 42, wherein the peptide linker comprises arginine and serine.

20

44. The isolated fusion protein according to claim 43, wherein the protein has an amino acid sequence of SEQ ID NO: 11.

45. The isolated fusion protein according to claim 40, wherein the fusion protein comprises a full-length human lutropin hormone receptor domain and a fragment of a human chorionic gonadotropin- β domain.

25

46. The isolated fusion protein according to claim 45, wherein the fragment of the human chorionic gonadotropin- β protein is the carboxyl terminal peptide of the human chorionic gonadotropin- β protein.

30

47. The isolated fusion protein according to claim 46, wherein the fusion protein further comprises a peptide linker between the full-length human lutropin hormone receptor domain and the carboxyl terminal peptide of the human chorionic gonadotropin- β domain.

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48. The isolated fusion protein according to claim 47, wherein the peptide linker comprises glycine and threonine.

49. The isolated fusion protein according to claim 48, wherein
5 the protein has an amino acid sequence of SEQ ID NO: 13.

50. The isolated fusion protein according to claim 40, wherein the fusion protein comprises a fragment of a human lutropin hormone receptor protein and a fragment of a human chorionic gonadotropin- β protein.

10

51. The isolated fusion protein according to claim 50, wherein the fragment of the human lutropin hormone receptor protein is the extracellular ligand binding domain of the human lutropin hormone receptor and the fragment of the human chorionic gonadotropin- β protein is the carboxyl terminal peptide of
15 the human chorionic gonadotropin- β protein.

52. The isolated fusion protein according to claim 51, wherein the protein further comprises a peptide linker between the extracellular ligand binding domain of the human lutropin hormone receptor and the carboxyl terminal
20 peptide of the human chorionic gonadotropin- β domain.

53 The isolated fusion protein according to claim 52, wherein the peptide linker comprises glycine and threonine.

54. The isolated fusion protein according to claim 53, wherein
25 the fusion protein has an amino acid sequence of SEQ ID NO: 15.

55. The isolated fusion protein according to claim 40, wherein the fusion protein comprises a fragment of a human lutropin hormone receptor
30 protein domain and a full-length human chorionic gonadotropin- β domain.

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56. The isolated fusion protein according to claim 55, wherein the fragment of the human lutropin hormone receptor protein is the extracellular ligand binding domain of the human lutropin hormone receptor.

5 57. The isolated fusion protein according to claim 56, wherein the fusion protein further comprises a peptide linker between the fragment of a human lutropin hormone receptor domain and the full-length human chorionic gonadotropin- β domain.

10 58. The isolated fusion protein according to claim 57, wherein the peptide linker comprises the amino acids glycine and threonine.

59. The isolated fusion protein according to claim 58, wherein fusion protein has an amino acid sequence of SEQ ID NO: 17.

15 60. The isolated fusion protein according to claim 40, wherein the fusion protein exhibits bifunctional activity comprising human lutropin hormone receptor activity and human chorionic gonadotropin- β activity.

20 61. An isolated antibody, or binding portion thereof, which recognizes the human lutropin hormone receptor domain and the human chorionic gonadotropin- β domain of the fusion protein according to claim 40.

25 62. The isolated antibody according to claim 61, wherein the antibody recognizes a full-length human lutropin hormone receptor domain and a full-length human chorionic gonadotropin- β domain.

30 63. The isolated antibody according to claim 61, wherein the antibody recognizes a full-length human lutropin hormone receptor domain and a fragment of the human chorionic gonadotropin- β domain.

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64. The isolated antibody according to claim 61, wherein the antibody recognizes a fragment of the human lutropin hormone receptor protein and a fragment of the human chorionic gonadotropin- β protein.
- 5 65. The isolated antibody according to claim 61, wherein the antibody recognizes a fragment of the human lutropin hormone receptor protein domain and a full-length human chorionic gonadotropin- β domain.
- 10 66. The isolated antibody according to claim 61, wherein the antibody is selected from the group consisting of a polyclonal antibody and a monoclonal antibody.
- 15 67. An isolated antibody, or binding portion thereof, which recognizes the human lutropin hormone receptor domain of the fusion protein according to claim 40 while not recognizing the human chorionic gonadotropin- β domain.
- 20 68. An isolated antibody, or binding portion thereof, which recognizes the human chorionic gonadotropin- β domain of the fusion protein according to claim 40 while not recognizing the human lutropin hormone-receptor domain.
- 25 69. An isolated antibody, or binding portion thereof, which binds to a human lutropin hormone receptor protein or a fragment thereof, wherein the human lutropin hormone receptor protein or a fragment thereof is selected from the group having an amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 9.
- 30 70. A composition comprising:
the isolated fusion protein according to claim 40 and
a pharmaceutical carrier.

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71. A composition comprising a pharmaceutical carrier and the antibody according to claim 61.

72. A composition comprising a pharmaceutical carrier and the
5 antibody according to claim 67.

73. A composition comprising a pharmaceutical carrier and the antibody according to claim 68.

10 74. A composition comprising a pharmaceutical carrier and the antibody according to claim 69.

75. A method of immunocontraception comprising:
administering the antibody according to claim 61 to a
15 subject under conditions effective to provide immunocontraception to the subject.

76. The method according to claim 75, wherein said administering is carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, intravaginally, or by application to mucous
20 membrane.

77. The method according to claim 75, wherein the subject is a mammal.

25 78. The method according to claim 77, wherein the mammal is selected from the group consisting of a canine, a feline, and a human.

79. The method according to claim 78, wherein the mammal is a human.

30

80. The method according to claim 75, wherein the subject is female.

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- 5
81. A method of immunocontraception comprising:
administering the antibody according to any of claims 63,
64, 67, 68, or claim 69 to a subject under conditions effective to provide
immunocontraception to the subject.
- 10
82. The method according to claim 81, wherein said
administering is carried out orally, parenterally, subcutaneously, intravenously,
intramuscularly, intraperitoneally, intravaginally, or by application to mucous
membrane.
- 15
83. The method according to claim 81, wherein the subject is a
mammal.
84. The method according to claim 83, wherein the mammal is
selected from the group consisting of a canine, a feline, and a human.
- 20
85. The method according to claim 84, wherein the mammal is
a human.
- 25
86. The method according to claim 81, wherein the subject is
male.
87. A method of immunocontraception comprising:
administering the human lutropin hormone receptor/human
chorionic gonadotropin- β fusion protein according to claim 40 to a subject under
conditions effective to provide immunocontraception to the subject.
- 30
88. The method according to claim 87, wherein the fusion
protein is selected from the group consisting of fusion proteins having the amino
acid sequence of SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID
NO: 17.

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89. The method according to claim 87, wherein said administering is carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, intravaginally, or by application to mucous membrane.

5

90. The method according to claim 87, wherein the subject is a mammal.

91. The method according to claim 90, wherein the mammal is
10 selected from the group consisting of a canine, a feline, and a human.

92. The method according to claim 87, wherein the mammal is a human.

15 93. The method according to claim 87, wherein the subject is female.

94. A method of immunocontraception in a subject comprising:
administering the chimeric nucleic acid molecule according
20 to claim 1 to a subject under conditions effective to provide immunocontraception to the subject.

95. The method according to claim 94, wherein said administering is carried out orally, parenterally, subcutaneously, intravenously,
25 intramuscularly, intraperitoneally, intravaginally, or by application to mucous membrane.

96. The method according to claim 95, wherein the subject is a mammal.

30

97. The method according to claim 96, wherein the mammal is selected from the group consisting of a canine, a feline, and a human.

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98. The method according to claim 97, wherein the mammal is a human.

99. A method of treating an androgen-excess-mediated disease condition in a subject comprising:
administering the antibody according to claim 61 to the subject under conditions effective to treat the androgen-excess-mediated disease condition.

100. The method according to claim 99, wherein the disease condition is selected from the group consisting of polycystic ovarian syndrome, Klinefelter's Syndrome, and prostatic hyperplasia.

101. The method according to claim 99, wherein said administering is carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, intravaginally, or by application to mucous membrane.

102. The method according to claim 99, wherein the subject is a mammal.

103. The method according to claim 102, wherein the mammal is a human.

104. The method according to claim 99, wherein the subject is female.

105. A method of treating an androgen-excess-mediated disease condition in a subject comprising:
administering the antibody according to any one of claims 63, 64, 67, 68, or 69 to the subject under conditions effective to treat the androgen-excess-mediated disease condition.

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106. The method according to claim 105, wherein the disease condition is selected from the group consisting of polycystic ovarian syndrome, Klinefelter's Syndrome, and prostatic hyperplasia.

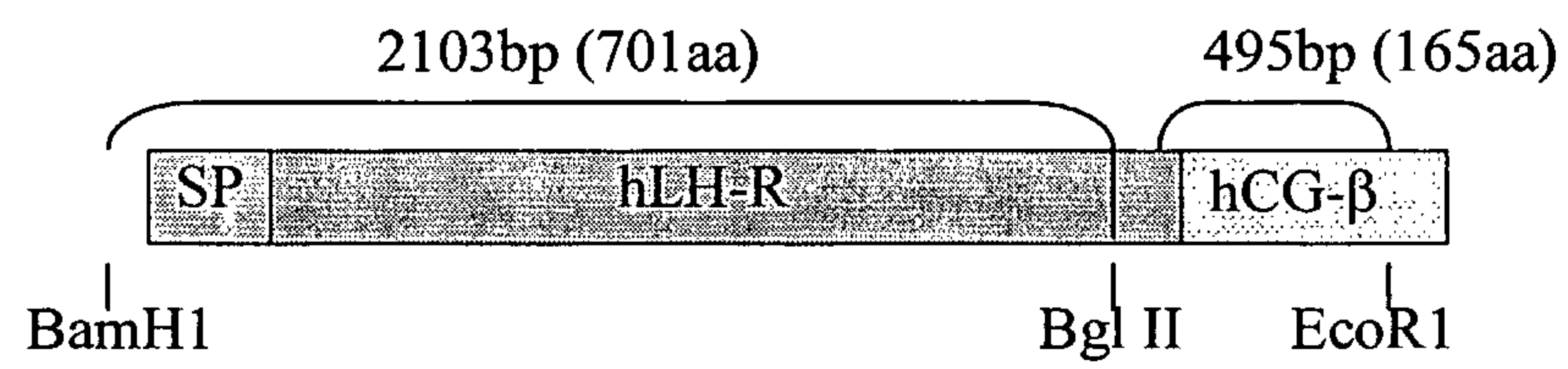
5 107. The method according to claim 105, wherein said administering is carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, intravaginally, or by application to mucous membrane.

10 108. The method according to claim 105, wherein the subject is a mammal.

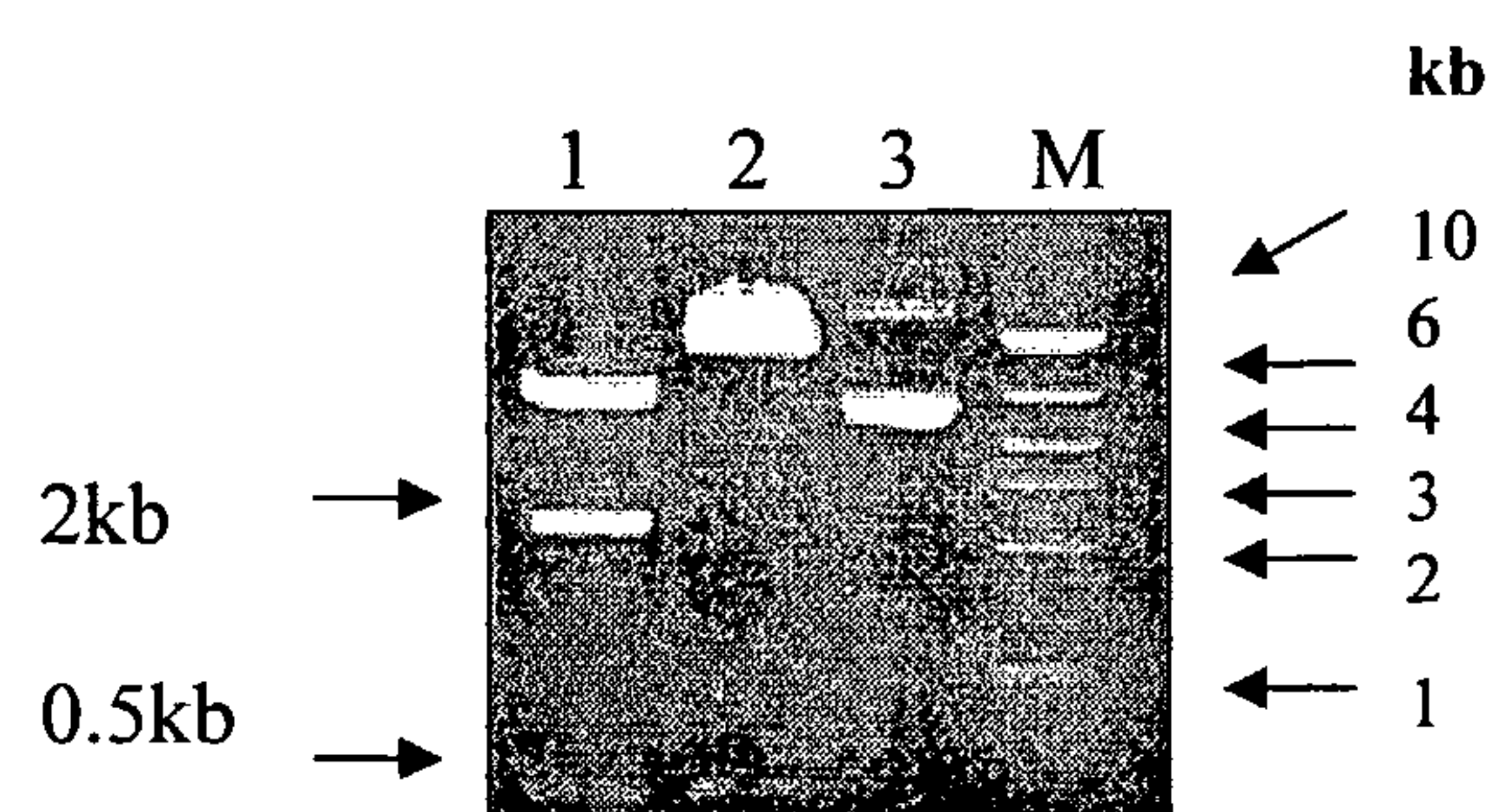
109. The method according to claim 105, wherein the mammal is a human.

15 110. The method according to claim 105, wherein the subject is male.

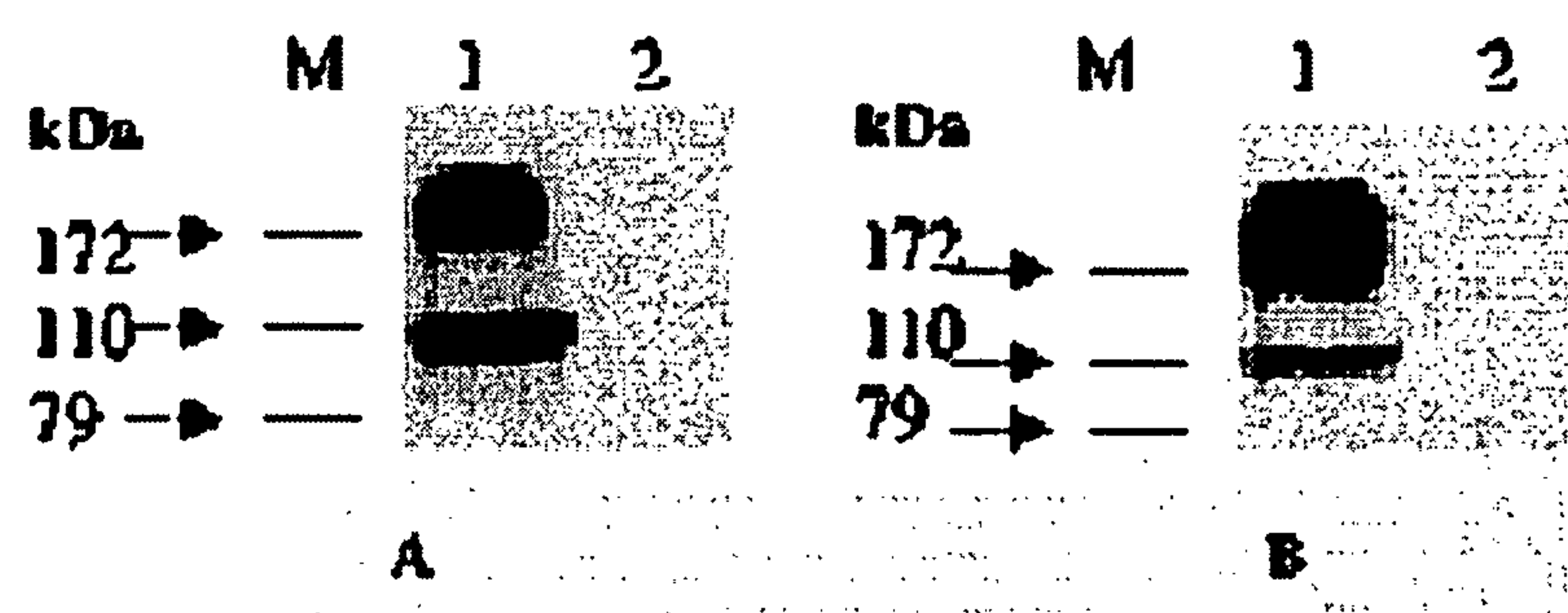
1/15

***FIG. 1***

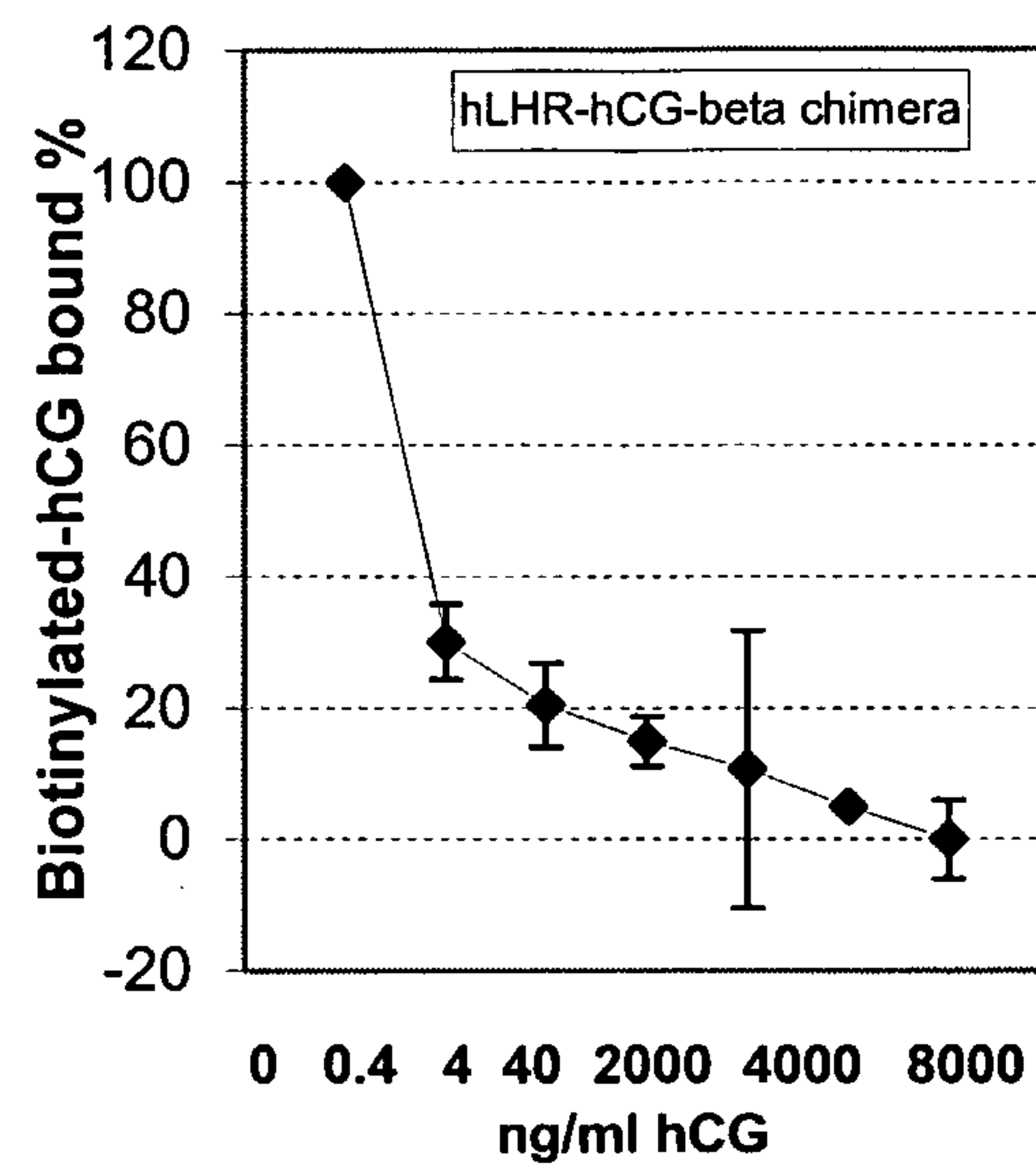
2/15

**FIG. 2**

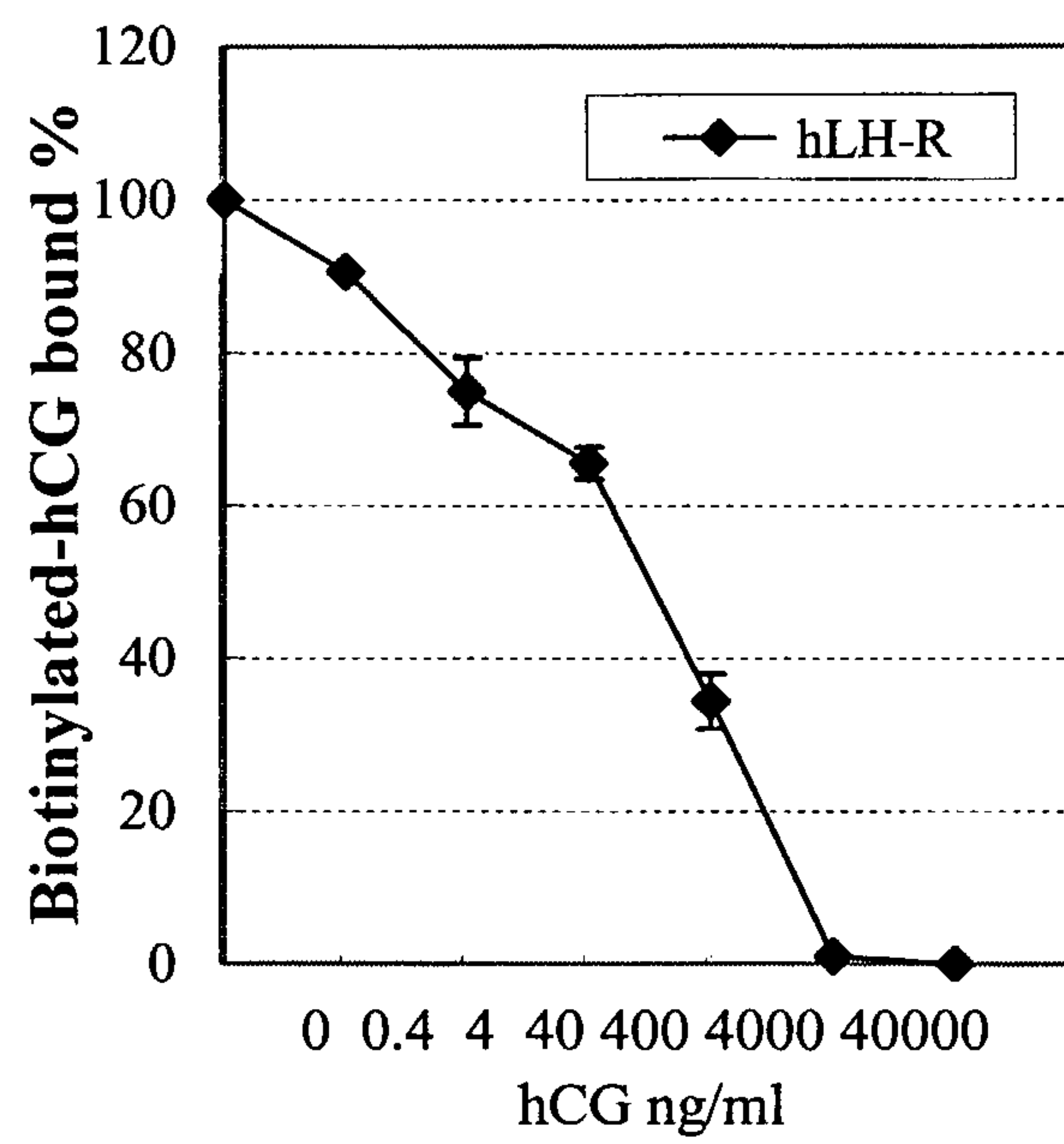
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**FIG. 3**

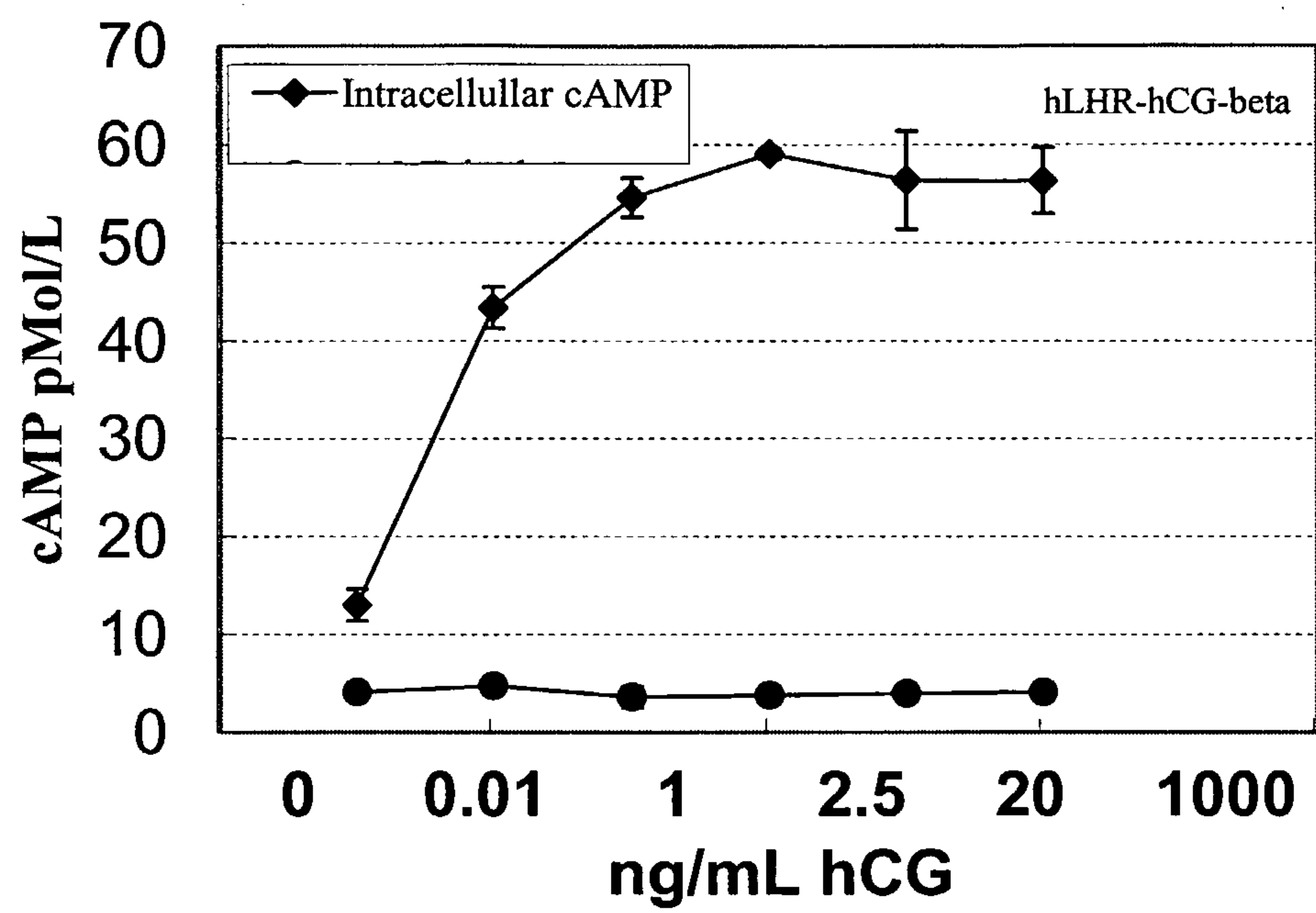
4/15

**FIG. 4A**

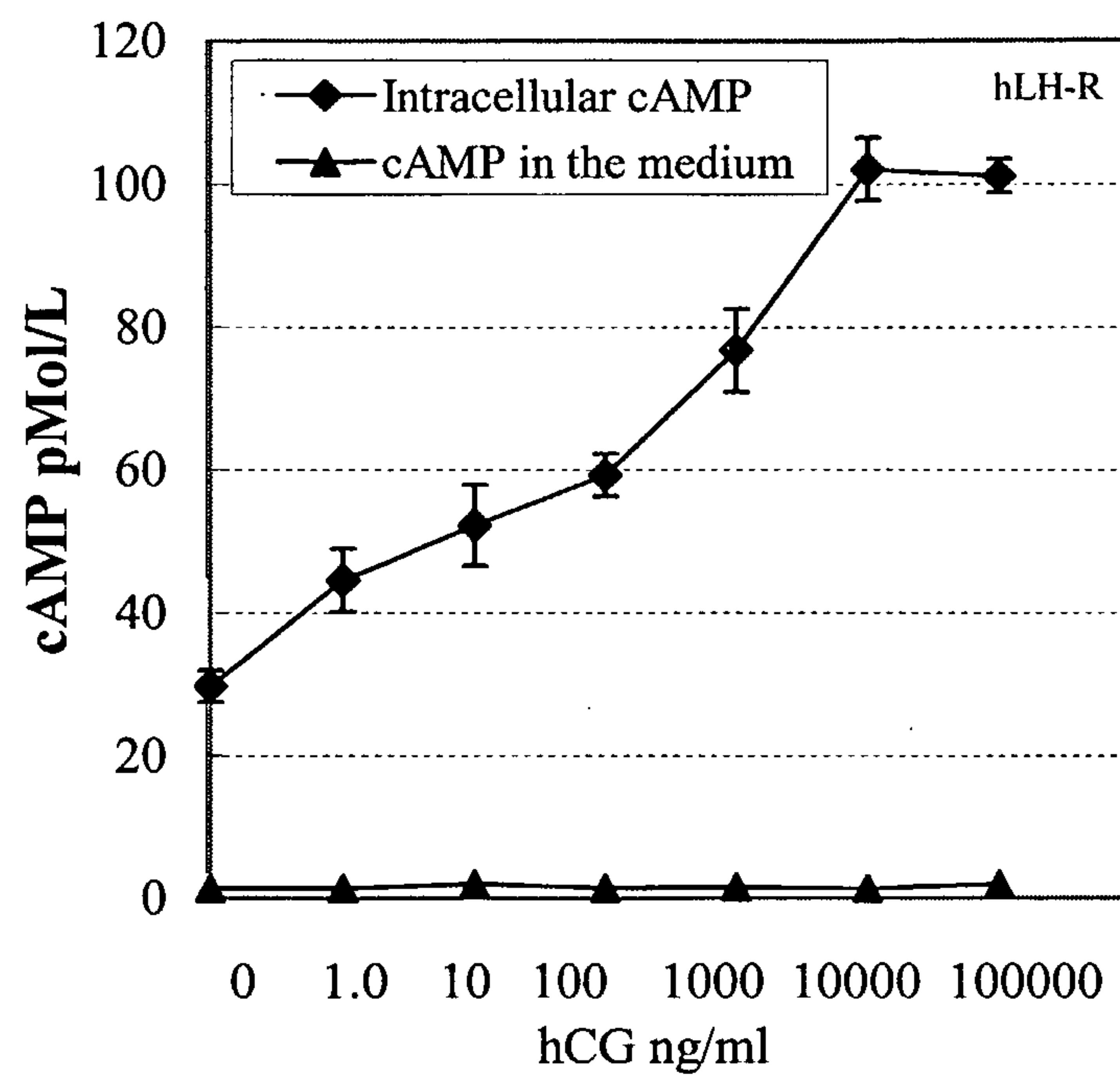
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**FIG. 4B**

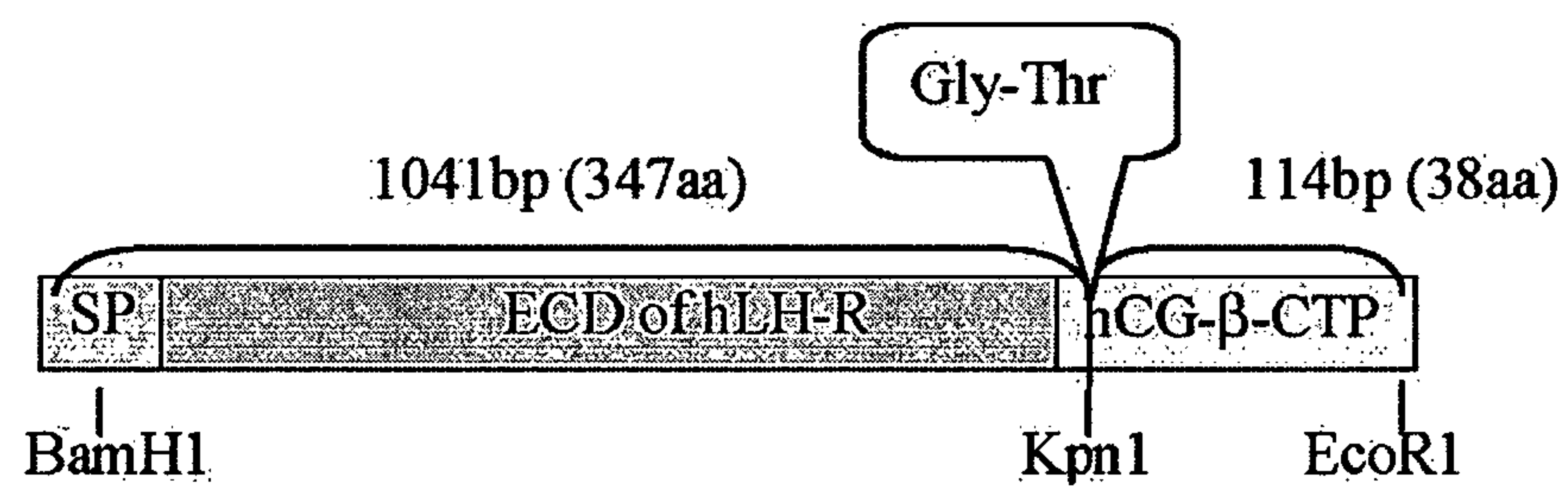
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**FIG. 5A**

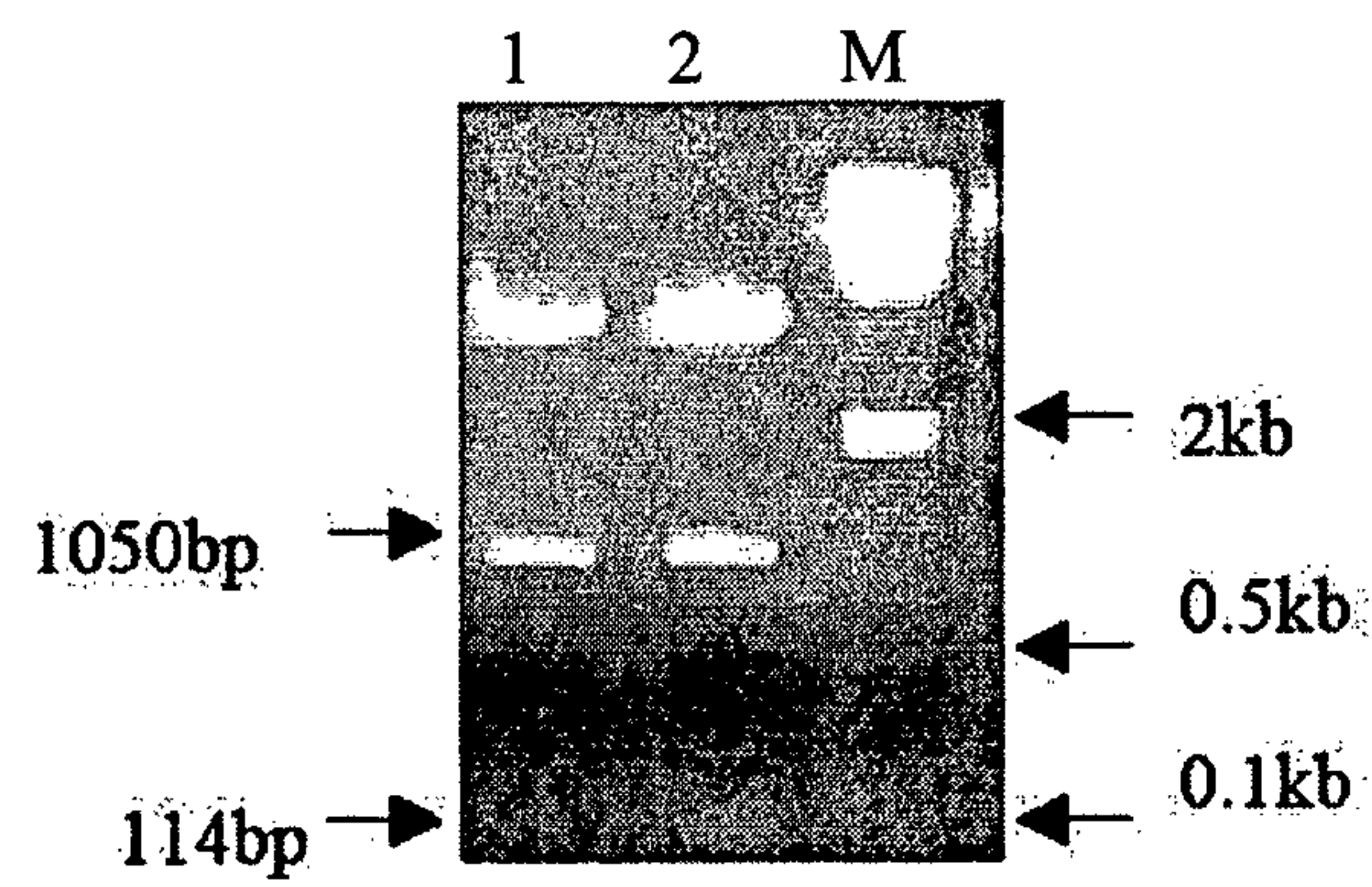
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**FIG. 5B**

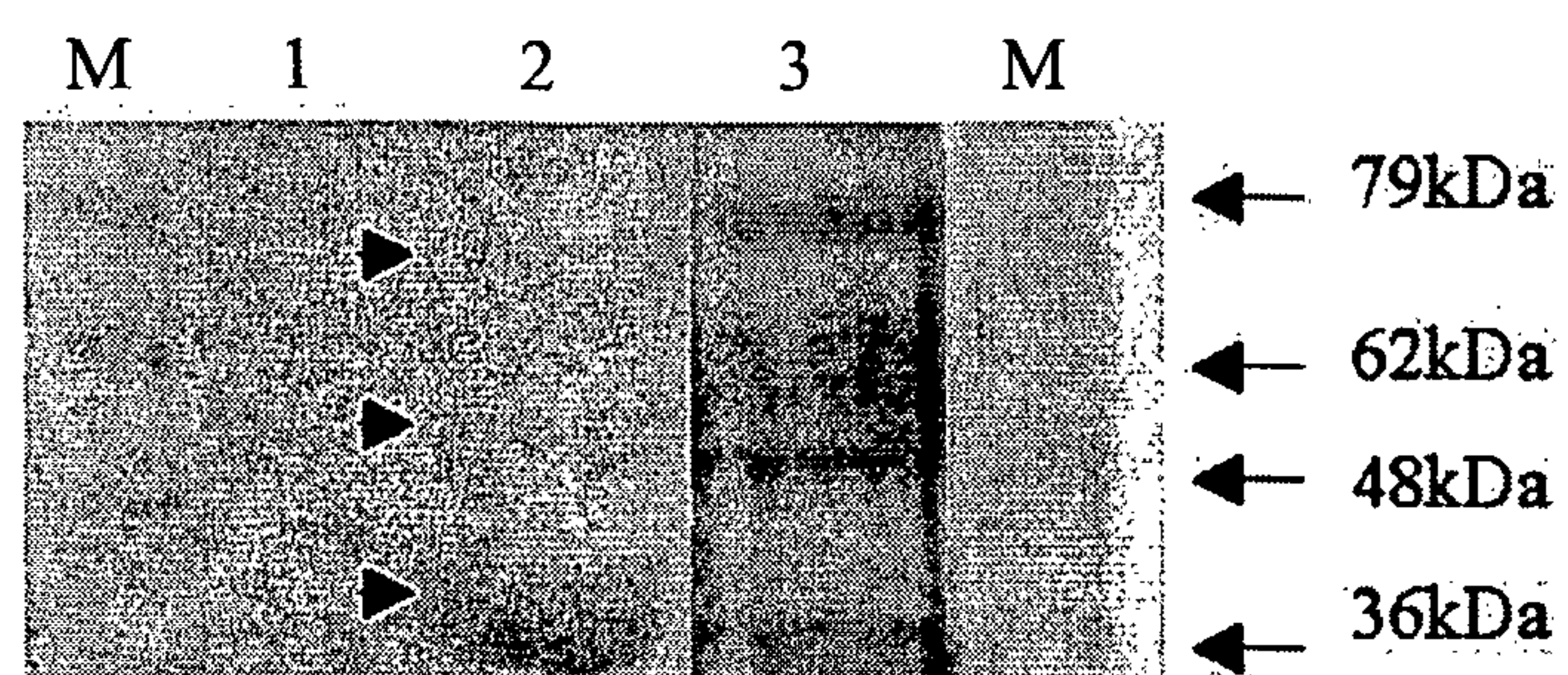
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**FIG. 6**

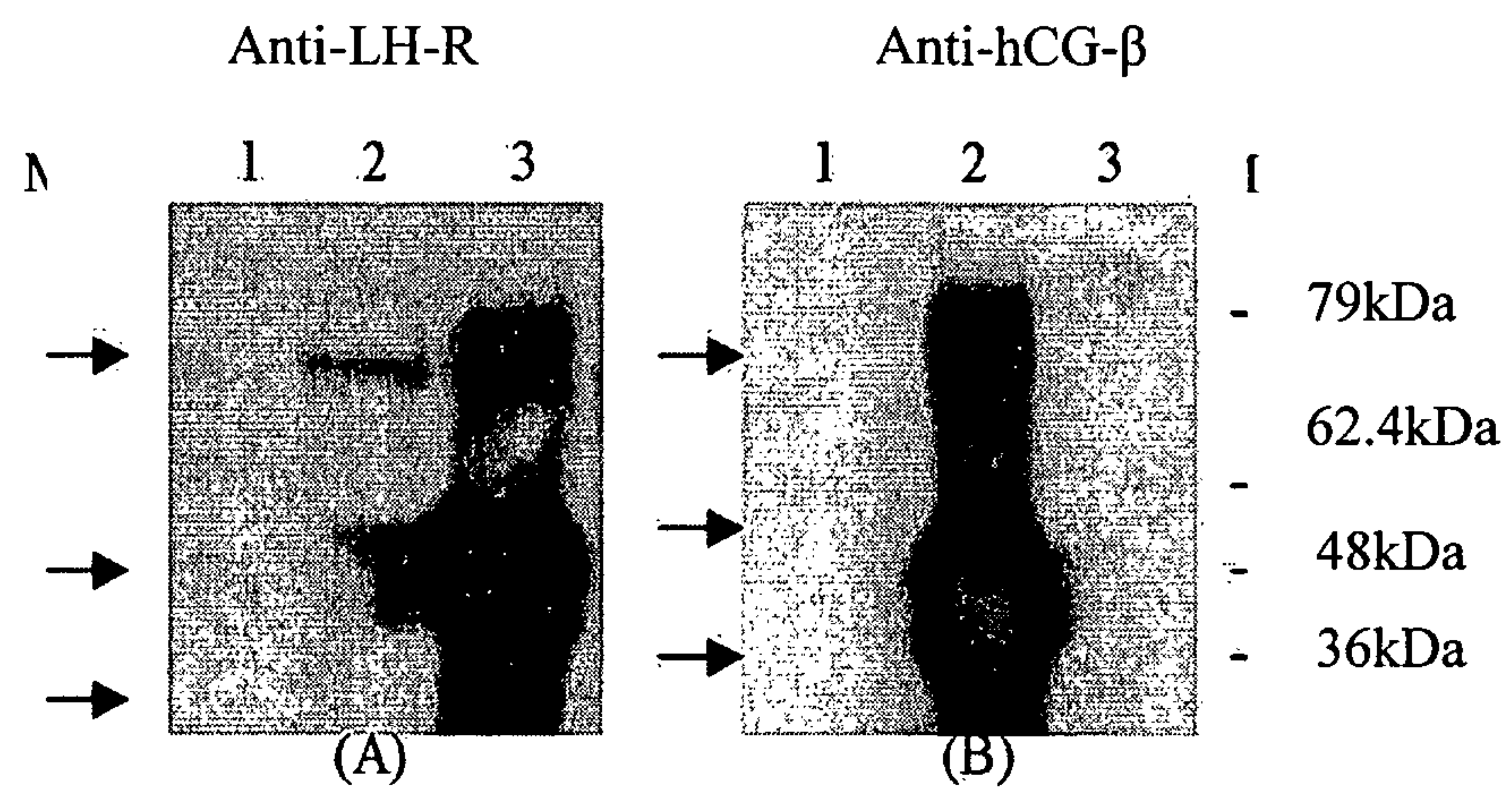
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**FIG. 7**

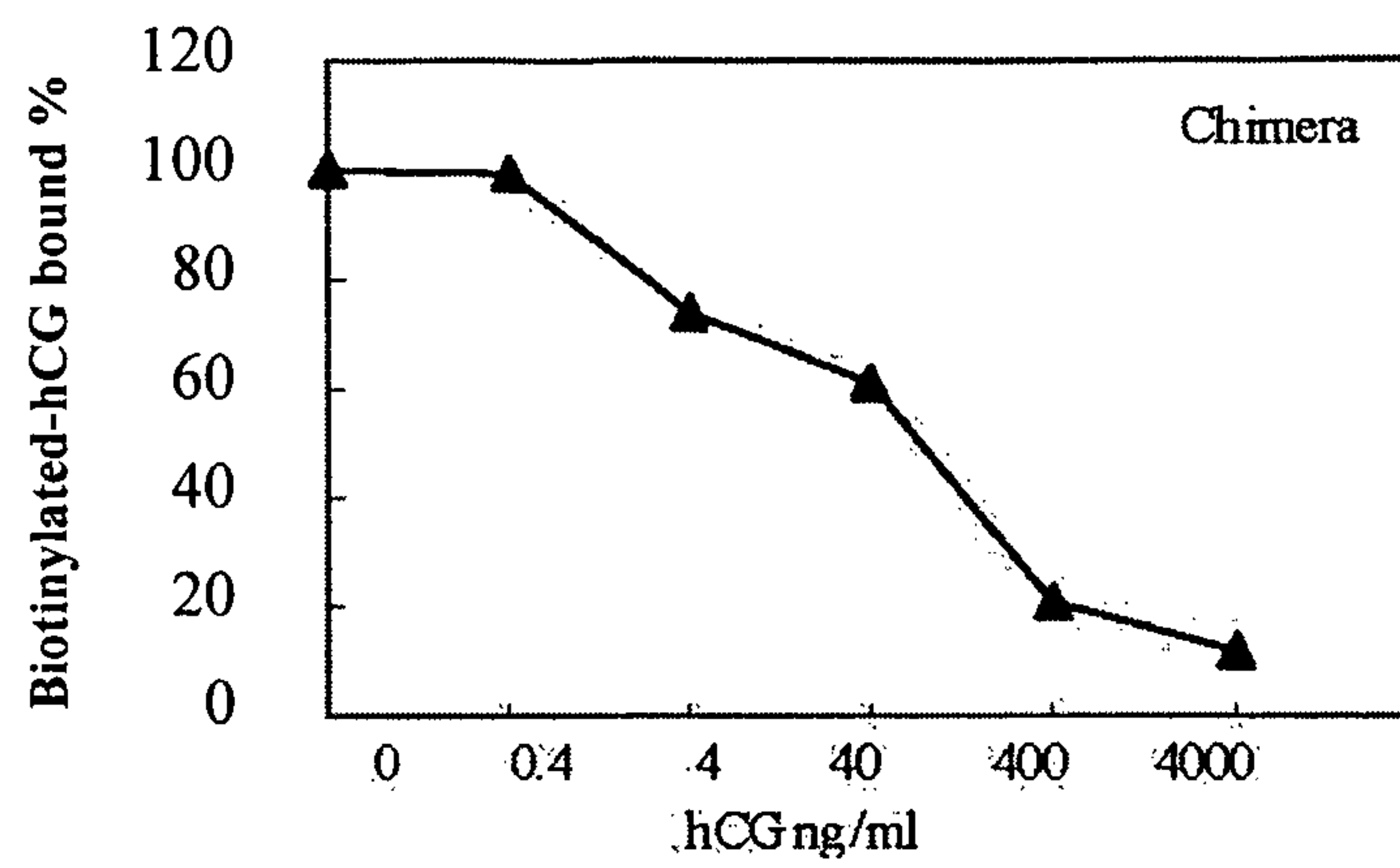
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**FIG. 8**

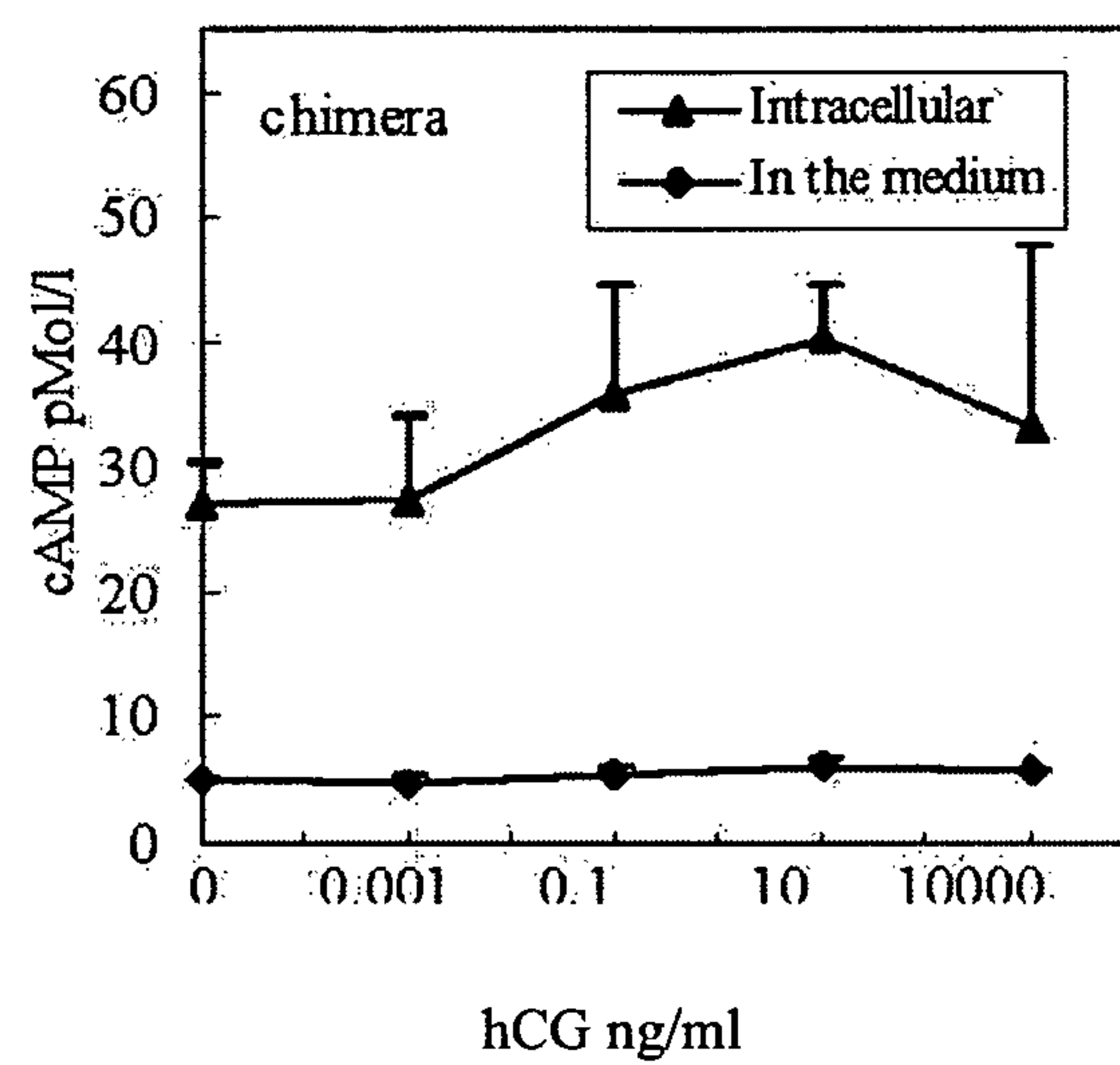
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**FIG. 9**

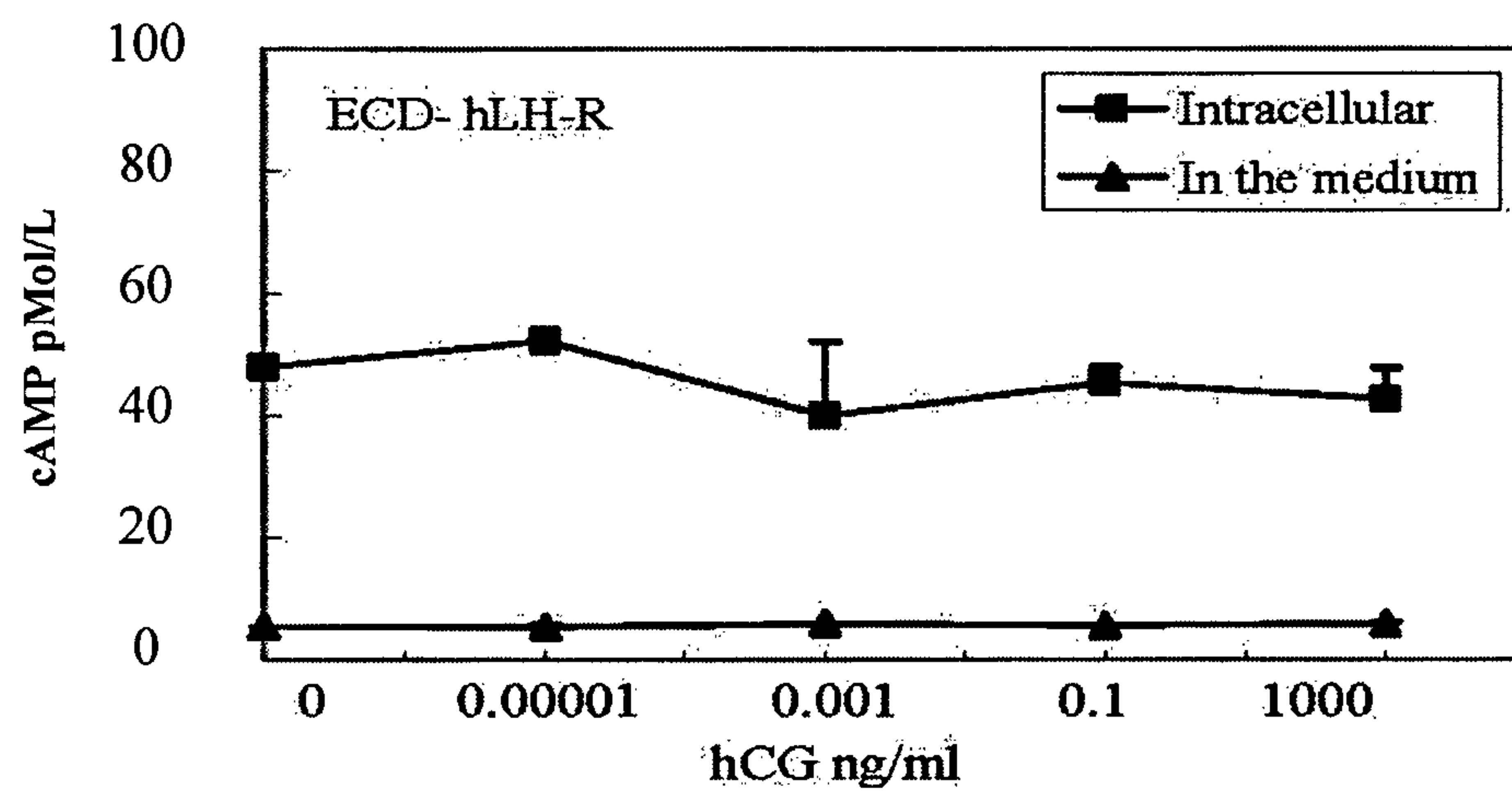
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**FIG. 10**

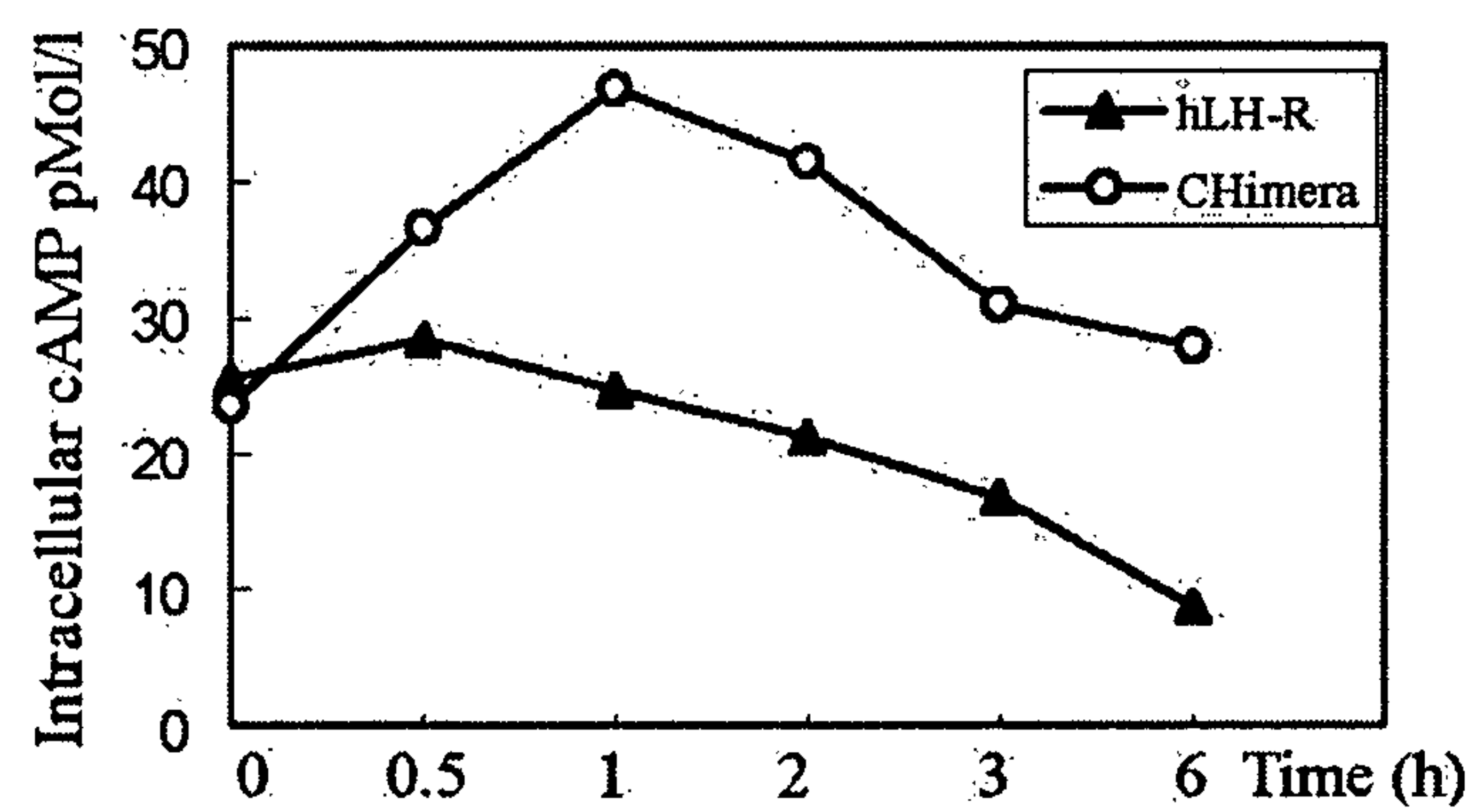
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**FIG. 11A**

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**FIG. 11B**

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**FIG. 11C**