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(57) Abstract

This invention relates generally to a method for treating the infertility associated with improper nutrition in a mammal in need of such treatment by administration of a therapeutically effective amount of a leptin receptor agonist.

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TREATMENT OF INFERTILITY WITH LEPTIN RECEPTOR LIGANDS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to a method for treating the infertility associated with improper nutrition in a mammal in need of such treatment by administration of a therapeutically effective amount of a leptin receptor agonist.

2. Description of the Related Art

Improper nutrition (both undernutrition and obesity) can lead to fertility problems: delay in onset of puberty, abnormal menstrual cycles, and sterility in females. In obese males, fertility is also greatly reduced. It has been reported that leptin levels in obese patients are higher than in lean patients and that starvation reduces leptin levels in blood. F. Chehab (Chehab, F.F. *et al.* (1996) Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nature Genetics,* **12**:318-320) showed that the sterility in *ob/ob* mice can be corrected by treatment with human leptin.

The consistent finding that leptin levels relate to gender and puberty, even with correction for percentage of body fat, suggests a rule that is not limited to the lipostat (Hassink, S.G., *et al.* (1996) Serum leptin in children with obesity: Relationship to gender and development. *Pediatrics* **98**:201-203). The logic of having a central hormone of the lipostat involved in gonadal development and puberty is suggested by the relationship between the attainment of required body weight and the onset of puberty (Kennedy, G.C., *et al.* (1963) Body weight and food intake as initiating factors for puberty in the rat. *J. Physiol.* **166**:408-418; Frisch, R.E. *et al.* (1974) Menstrual cycles: Fatness as a determinant of minimum

weight or height necessary for maintenance or onset. *Science*, **185**:949-951; Frisch, R.E. (1980) Pubertal adipose tissue: Is it necessary for normal sexual maturation? Evidence from the rat and human female. *Fed. Proc.*, **39**:2395-2400). Body weight or fat are more closely related to puberty than age (Frisch, R.E. *et al.* (1972) Weight at menarche: Similarity for well nourished and undernourished girls at differing ages and evidence for historical constancy. *Pediatrics*, **50**:445-450), and low body weight or fat are known to delay puberty (Van der Spuy, Z. (1985) Nutrition and reproduction. *Clin. Obstet. Gynæcol.* **12**:579-604). The *ob/ob* and *db/db* mice are infertile even though their body weight is abnormally high. Leptin administration to deficient *ob/ob* mice restores fertility (Chehab, F.F. *et al.* (1996) Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nature Genetics,* **12**:318-3207). Leptin administration to starved mice tends to reverse the effects of starvation on testosterone and LH levels (Ahima, R.S. *et al.* (1996) Leptin is a major mediator of the neuroendocrine response to fasting. *Submitted*). Additional evidence for a role of leptin in the hypothalamic-pituitary-gonadal axis (HPG) derives from the distribution of the long form of OB-R that is abundant in the gonads as well as the hypothalamus (Lee, G.H. *et al.* (1996) Abnormal splicing of the leptin receptor in diabetic mice. *Nature* **379**:632-635). The role of neuropeptide Y (NPY) in leptin action also suggests possible involvement in the HPG (Weigle, D.S., *et al.* (1995) Recombinant ob protein reduces feeding and body weight in the *ob/ob* mouse. *J. Clin. Invest.* **96**:2065-2070). NPY has long been known to alter luteinizing hormone -releasing hormone (LHRH) release and is known to have receptors in the region of the hypothalamus containing LHRH neurons.

These and other data led Ahima et al. to investigate the effects of leptin on the onset of puberty in starved lean mice (Ahima, R.S., *et al.* (1996) Leptin accelerates the onset of puberty in normal female mice.

Submitted). They found that leptin administered subcutaneously at doses that did not alter weight gain induced an earlier onset of vaginal opening, estrus, and cycling. No relationship was seen, however, between leptin plasma levels and vaginal opening or estradiol.

SUMMARY OF THE INVENTION

Accordingly, a major object of the present invention is to provide a method of treating the infertility associated with improper nutrition. Leptin used as an adjunct in weight loss programs would not only aid the weight loss itself but could prevent fertility problems that were induced by a rapid drop in leptin levels.

The use of leptin receptor ligands represents an opportunity for therapy in conditions associated with either abnormally low or high adiposity, or relative leptin deficiency. For example, infertility in subjects with low adiposity may be restored by exogenous leptin administration. Delayed puberty may be treated with a leptin regimen.

This invention, therefore, discloses the utility of leptin, leptin mimetics, or novel leptin analogs to treat fertility disorders and delayed puberty in either obese or lean subjects. Alternatively, leptin antagonists or neutralizing antileptin antibodies may be used to treat precocious puberty.

With the foregoing and other objects, advantages and features of the invention that will become hereinafter apparent, the nature of the invention may be more clearly understood by reference to the following detailed description of the preferred embodiments of the invention and to the appended claims.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

This invention arose from a desire of the inventors to provide a safe, effective treatment for infertility, in particular a treatment which would prevent or inhibit the infertility associated with improper nutrition in a mammal in need of such treatment.

For purposes of the present invention, as disclosed and claimed herein, the following terms and abbreviations are defined as follows:

Base pair (bp) -- refers to DNA or RNA. The abbreviations A,C,G, and T correspond to the 5'-monophosphate forms of the nucleotides (deoxy)adenine, (deoxy)cytidine, (deoxy)guanine, and (deoxy)thymine, respectively, when they occur in DNA molecules. The abbreviations U,C,G, and T correspond to the 5'-monophosphate forms of the nucleosides uracil, cytidine, guanine, and thymine, respectively when they occur in RNA molecules. In double stranded DNA, base pair may refer to a partnership of A with T or C with G. In a DNA/RNA heteroduplex, base pair may refer to a partnership of T with U or C with G.

Chelating Peptide -- An amino acid sequence capable of complexing with a multivalent metal ion.

DNA -- Deoxyribonucleic acid.

EDTA -- an abbreviation for ethylenediamine tetraacetic acid.

ED50 -- an abbreviation for half-maximal value.

FAB-MS -- an abbreviation for fast atom bombardment mass spectrometry.

Hypothalamic-Pituitary-Adrenal-Adipose Axis (HPAAA): A physiological regulatory system wherein each of the named elements (the hypothalamus, the pituitary gland, the adrenal glands, and adipose tissue) release chemicals that regulate the activity of the others. For example, CRH released by the hypothalamus stimulates pituitary secretion of ACTH, that in turn stimulates adrenal secretion of glucocorticoids, which in turn

modulates adipose tissue leptin release, that finally acts back on the hypothalamus.

Immunoreactive Protein(s) -- a term used to collectively describe antibodies, fragments of antibodies capable of binding antigens of a similar nature as the parent antibody molecule from which they are derived, and single chain polypeptide binding molecules as described in PCT Application No. PCT/US 87/02208, International Publication No. WO 88/01649.

mRNA -- messenger RNA.

MWCO -- an abbreviation for molecular weight cut-off.

Modulating -- stimulation, potentiation, or inhibition of the activity of a receptor or system.

Plasmid -- an extrachromosomal self-replicating genetic element.

PMSF -- an abbreviation for phenylmethylsulfonyl fluoride.

Reading frame -- the nucleotide sequence from which translation occurs "read" in triplets by the translational apparatus of tRNA, ribosomes and associated factors, each triplet corresponding to a particular amino acid. Because each triplet is distinct and of the same length, the coding sequence must be a multiple of three. A base pair insertion or deletion (termed a frameshift mutation) may result in two different proteins being coded for by the same DNA segment. To insure against this, the triplet codons corresponding to the desired polypeptide must be aligned in multiples of three from the initiation codon, i.e. the correct "reading frame" must be maintained. In the creation of fusion proteins containing a chelating peptide, the reading frame of the DNA sequence encoding the structural protein must be maintained in the DNA sequence encoding the chelating peptide.

Receptor agonist -- any compound that binds to a receptor and triggers the action of the receptor (usually an intracellular signalling event

or, in the case of receptors that form transmembrane channel, the opening or closing of the channel).

Receptor antagonist -- any compound that binds to a receptor and blocks the action of the receptor (usually by out-competing the endogenous agonist for binding sites on the receptor).

Receptor ligand -- any compound that binds to a receptor.

Recombinant DNA Cloning Vector -- any autonomously replicating agent including, but not limited to, plasmids and phages, comprising a DNA molecule to which one or more additional DNA segments can or have been added.

Recombinant DNA Expression Vector -- any recombinant DNA cloning vector in which a promoter has been incorporated.

Replicon -- A DNA sequence that controls and allows for autonomous replication of a plasmid or other vector.

RNA -- ribonucleic acid.

RP-HPLC -- an abbreviation for reversed-phase high performance liquid chromatography.

Transcription -- the process whereby information contained in a nucleotide sequence of DNA is transferred to a complementary RNA sequence.

Translation -- the process whereby the genetic information of messenger RNA is used to specify and direct the synthesis of a polypeptide chain.

Tris -- an abbreviation for tris-(hydroxymethyl)aminomethane.

Treating -- describes the management and care of a patient for the purpose of combating the disease, condition, or disorder and includes the administration of a leptin receptor ligand of the present invention to prevent the onset of the symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition, or disorder. Treating obesity, for example, includes the inhibition of food

intake, the inhibition of weight gain, and inducing weight loss in patients in need thereof.

Vector -- a replicon used for the transformation of cells in gene manipulation bearing polynucleotide sequences corresponding to appropriate protein molecules which, when combined with appropriate control sequences, confer specific properties on the host cell to be transformed. Plasmids, viruses, and bacteriophage are suitable vectors, since they are replicons in their own right. Artificial vectors are constructed by cutting and joining DNA molecules from different sources using restriction enzymes and ligases. vectors include Recombinant DNA cloning vectors and Recombinant DNA expression vectors.

X-gal -- an abbreviation for 5-bromo-4-chloro-3-idolyl beta-D-galactoside.

Leptin receptor agonists may be used to treat fertility disorders and delayed puberty. Alternatively, leptin antagonists, alone or combined with specific antibodies to leptin, may be used to treat precocious puberty. In addition to the full mature protein, proteins include a leader sequence such as Met-Asp or Met-Arg, or altered primary sequence to achieve higher physical stability.

The inventors have found that administration of leptin receptor ligands, in particular leptin receptor agonists or leptin receptor antagonists, effectively may be used to treat the infertility associated with improper nutrition. The phrases "receptor ligands", "receptor agonists", and "receptor antagonists" used herein are understood to refer to pharmacologically active compounds, and to salts thereof. Preferred leptin receptor agonists for use in the present invention include endogenous leptin (i.e., endogenous OB protein - the protein produced from the obesity gene following transcription and translation and deletion of introns, translation to a protein and processing to the mature protein with secretory

signal peptide removed, e.g., from the N-terminal valine-proline to the C-terminal cysteine of the mature protein). The mouse OB protein and human OB protein are published in Zhang *et al., Nature* **372**:425-432 (1994). The rat OB protein is published in Murakami et al., *Biochem. Biophys. Res. Com.* **209**:944-952 (1995). The porcine and bovine OB genes and proteins are disclosed in EP 0 743 321, the contents of which are incorporated by reference. Various primate OB genes and proteins are disclosed in U.S. Application Serial No.08/710,483, the contents of which are incorporated by reference. Also preferred for use in the present invention are leptin analogs, preferably leptin analogs having one or more amino acid substitution, more preferably less than five and most preferably less than three substitutions. Particularly preferred leptin analogs for use in the present invention include proteins disclosed by Basinski et al., in WO 96/23515 and WO 96/23517 (the contents of which are incorporated by reference), of the Formula (I):

SEQ ID NO: 1

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

or pharmaceutically acceptable salts thereof, wherein:

Xaa at position 28 is Gln or absent;

said protein having at least one of the following substitutions:

Gln at position 4 is replaced with Glu;

Gln at position 7 is replaced with Glu;

Asn at position 22 is replaced with Gln or Asp;

Thr at position 27 is replaced with Ala;

Xaa at position 28 is replaced with Glu;

Gln at position 34 is replaced with Glu;

Met at position 54 is replaced with methionine sulfoxide, Leu, Ile, Val, Ala, or Gly;

Gln at position 56 is replaced with Glu;

Gln at position 62 is replaced with Glu;

Gln at position 63 is replaced with Glu;

Met at position 68 is replaced with methionine sulfoxide, Leu, Ile, Val, Ala, or Gly;

Asn at position 72 is replaced with Gln, Glu, or Asp;

Gln at position 75 is replaced with Glu;

Ser at position 77 is replaced with Ala;

Asn at position 78 is replaced with Gln or Asp;

Asn at position 82 is replaced with Gln or Asp;

His at position 97 is replaced with Gln, Asn, Ala, Gly, Ser, or Pro;

Trp at position 100 is replaced with Ala, Glu, Asp, Asn, Met, Ile, Phe, Tyr, Ser, Thr, Gly, Gln, Val, or Leu;

Ala at position 101 is replaced with Ser, Asn, Gly, His, Pro, Thr, or Val;

Ser at position 102 is replaced with Arg;

Gly at position 103 is replaced with Ala;

Glu at position 105 is replaced with Gln;

Thr at position 106 is replaced with Lys or Ser;

Leu at position 107 is replaced with Pro;

Asp at position 108 is replaced with Glu;

Gly at position 111 is replaced with Asp;

Gly at position 118 is replaced with Leu;

Gln at position 130 is replaced with Glu;

Gln at position 134 is replaced with Glu;

Met at position 136 is replaced with methionine sulfoxide, Leu, Ile, Val, Ala, or Gly;

Trp at position 138 is replaced with Ala, Glu, Asp, Asn, Met, Ile, Phe, Tyr, Ser, Thr, Gly, Gln, Val, or Leu; or

Gln at position 139 is replaced with Glu.

In addition, the leptin receptor ligands of the present invention are optionally substituted with a functional group. Any art-recognized functional group which does not eliminate or significantly reduce the compound's ability to bind to leptin receptors are contemplated, including, but not limited to, ester, amide, acid, amine, alcohol, ether, thioether, etc. Solvates, e.g., hydrates of the compounds useful in the methods of the present invention, are also included within the scope of the present invention. Methods of solvation to produce such solvates are generally known in the art.

Pharmaceutical salts of the leptin receptor agonists and antagonists suitable for administration by a variety of routes are known in the art and need not be described herein in detail. Examples of pharmaceutically acceptable salts of the leptin receptor ligands and derivatives thereof according to the invention, include base salts, e.g., derived from an appropriate base. Pharmaceutically acceptable salts of an acid group or an amino group include, but are not limited to, salts of organic carboxylic

acids such as acetic, lactic, tartaric, malic, isothionic, and lactobionic acids; organic sulfonic acids such as methanesulfonic, ethanesulfonic, benzenesulfonic and *p*-tolylsulfonic acids, and inorganic acids such as hydrochloric, sulfuric, phosphoric and sulfamic acids. Pharmaceutically-acceptable salts of a compound with a hydroxy group include, but are not limited to, the anion of the compound in combination with a suitable cation such as Na^+.

In a further embodiment of the present invention comprises a method for treating the infertility associated with improper nutrition by administration of antibodies to endogenous leptin receptor agonists to a mammal in need of such treatment. Such antibodies may be monoclonal or polyclonal antibodies to leptin receptor agonists, or to antigenic parts thereof.

Both polyclonal and monoclonal antibodies to leptin receptor agonists are obtainable by immunization of an animal with purified leptin receptor agonists, purified recombinant leptin receptor agonists, fragments of these proteins, or purified fusion proteins of leptin receptor agonists, with another protein. In the case of monoclonal antibodies, partially purified proteins or fragments may serve as immunogens. The methods of obtaining both types of antibodies are well known in the art with excellent protocols for antibody production being found in Harlow et al. (1988) *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 726 pp.

Polyclonal sera are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of purified leptin receptor agonists, or parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoadsorbent techniques. Monoclonal antibodies are particularly useful because they can be produced in large quantities and with a high degree of homogeneity. Hybridoma cell lines which produce monoclonal antibodies are prepared

by fusing an immortal cell line with lymphocytes sensitized against the immunogenic preparation and is done by techniques which are well known to those who are skilled in the art. (See, for example, Douillard, I.Y. and Hoffman, T., "Basic Facts About Hybridomas", in *Compendium of Immunology,* Vol. II, L. Schwartz (Ed.) (1981); Kohler, G. and Milstein, C., *Nature* **256**: 495-497 (1975) and *European Journal of Immunology* **6**: 511-519 (1976); Harlow et al.; Koprowski, et al., U.S. Patent 4,172,124; Koprowski et al., U.S. Patent 4,196,265 and Wands, U.S. Patent 4,271,145, the teachings of which are herein incorporated by reference.

A still further part of this invention is a pharmaceutical composition of matter for treating the infertility associated with improper nutrition that comprises at least one of the leptin receptor agonists or antagonists described above, mixtures thereof, and/or pharmaceutical salts thereof, and a pharmaceutically-acceptable carrier therefor. Such compositions are prepared in accordance with accepted pharmaceutical procedures, for example, as described in *Remington's Pharmaceutical Sciences,* seventeenth edition, ed. Alfonso R. Gennaro, Mack Publishing Company, Easton, PA (1985).

For therapeutic use in a method of treating the infertility associated with improper nutrition, a leptin receptor agonist or antagonist, or its salt, can be conveniently administered in the form of a pharmaceutical composition containing one or more leptin receptor agonists or antagonists, or salts thereof, and a pharmaceutically acceptable carrier therefor. Suitable carriers are well known in the art and vary with the desired form and mode of administration of the pharmaceutical composition. For example, they may include diluents or excipients such as fillers, binders, wetting agents, disintegrators, surface-active agents, lubricants, and the like. Typically, the carrier may be a solid, liquid, or vaporizable carrier, or combinations thereof. In one preferred

embodiment, the composition is a therapeutic composition and the carrier is a pharmaceutically acceptable carrier.

The leptin receptor ligands for use in the present invention, or salts thereof, may be formulated together with the carrier into any desired unit dosage form. Typical unit dosage forms include tablets, pills, powders, solutions, suspensions, emulsions, granules, capsules, suppositories; injectable solutions and suspensions are particularly preferred.

Each carrier must be "acceptable" in the sense of being compatible with the other ingredients in the formulation and not injurious to the patient. The carrier must be biologically acceptable and inert, i.e., it must permit the cell to conduct its metabolic reactions so that the leptin receptor ligands suitable for use in the method of the present invention may effect its inhibitory activity.

Formulations include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, and transdermal) administration, with topical ointment formulations, and formulations appropriate for oral administration, being preferred.

For example, to prepare formulations suitable for injection, solutions and suspensions are sterilized and are preferably isotonic to blood. In making injectable preparations, carriers which are commonly used in this field can also be used, for example, water, ethyl alcohol, propylene glycol, ethoxylated isostearyl alcohol, polyoxylated isostearyl alcohol, polyoxyethylene sorbitol and sorbitate esters. In these instances, adequate amounts of isotonicity adjusters such as sodium chloride, glucose or glycerin can be added to make the preparations isotonic. The aqueous sterile injection solutions may further contain anti-oxidants, buffers, bacteriostats, and like additions acceptable for parenteral formulations.

The formulations may conveniently be presented in unit dosage form and may be prepared by any method known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which may encompass one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product. Various unit dose and multidose containers, e.g., sealed ampules and vials, may be used, as is well known in the art.

In addition to the ingredients particularly mentioned above, the formulations of this invention may also include other agents conventional in the art for this type of pharmaceutical formulation.

The leptin receptor ligands suitable for use in the present invention may be present in the composition in an broad proportion to the carrier. For instance, the leptin receptor ligands may be present in the amount of 0.01 to 99.9 wt%, and more preferably in about 0.1 to 99 wt%. Still more preferably, the leptin receptor ligand may be present in an amount of about 1 to 70 wt% of the composition.

The dosage of the leptin receptor agonists or antagonists, pharmaceutically acceptable salts thereof, or mixtures thereof administered to a patient according to the present invention will vary depending on several factors, including, but not limited to, the age, weight, and species of the patient, the general health of the patient, the severity of the symptoms, whether the composition is being administered alone or in combination with other therapeutic agents, the incidence of side effects and the like.

In general, a dose suitable for application in treating the infertility associated with improper nutrition is about 0.001 to 100 mg/kg body weight/dose, preferably about 0.01 to 60 mg/kg body weight/dose, and still more preferably about 0.1 to 40 mg/kg body weight/dose per day. The

desired dose may be administered as 1 to 6 or more subdoses administered at appropriate intervals throughout the day. The leptin receptor ligands may be administered repeatedly over a period of months or years, or it may be slowly and constantly infused to the patient. Higher and lower doses may also be administered.

The daily dose may be adjusted taking into account, for example, the above-identified variety of parameters. Typically, the present compositions may be administered in an amount of about 0.001 to 100 mg/kg body weight/day. However, other amounts may also be administered.

To achieve good plasma concentrations, leptin receptor ligands suitable for use in the present invention may be administered, for instance, by intravenous injection of an approximate 0.1 to 1% solution of the active ingredient, optionally in saline, or orally administered as a bolus.

The active ingredient may be administered for therapy by any suitable routes, including topical, oral, rectal, nasal, vaginal and parenteral (including intraperitoneal, subcutaneous, intramuscular, intravenous, intradermal, and transdermal) routes. It will be appreciated that the preferred route will vary with the condition and age of the patient, the nature of the disorder and the chosen active ingredient including other therapeutic agents. Preferred is the oral route. Also preferred is the topical route. However, other routes may also be utilized depending on the conditions of the patient and how long-lasting the treatment is.

While it is possible for the active ingredient to be administered alone, it is preferably present as a pharmaceutical formulation. The formulations of the present invention comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof and optionally other therapeutic agents.

The above method may be practiced by administration of leptin receptor ligands by themselves or in a combination with other active

ingredients, including therapeutic agents in a pharmaceutical composition. Other therapeutic agents suitable for use herein are any compatible drugs that are effective by the same or other mechanisms for the intended purpose, or drugs that are complementary to those of the present agents. These include agents that are effective for the treatment of infertility and/or associated conditions in humans.

The compounds utilized in combination therapy may be administered simultaneously, in either separate or combined formulations, or at different times than the present compounds, e.g., sequentially, such that a combined effect is achieved. The amounts and regime of administration will be adjusted by the practitioner, by preferably initially lowering their standard doses and then titrating the results obtained. The therapeutic method of the invention may be used in conjunction with other therapies as determined by the practitioner.

While the invention has been described and illustrated herein by references to various specific material, procedures and examples, it is understood that the invention is not restricted to the particular material, combinations of material, and procedures selected for that purpose. Numerous variations of such details can be implied and will be appreciated by those skilled in the art.

WHAT IS CLAIMED IS:

1. A method of treating the infertility associated with improper nutrition comprising administering to a mammal in need of such treatment a leptin receptor ligand in an amount effective to induce fertility.

2. The method of claim 1, wherein said leptin receptor ligand is a leptin receptor agonist.

3. The method of claim 2, wherein said leptin receptor agonist is human leptin.

4. The method of claim 2, wherein said leptin receptor agonist has the amino acid sequence of SEQ ID NO: 1.

4. The method of claim 1, wherein said leptin receptor ligand is a leptin receptor antagonist.

5. The method of claim 1, wherein the leptin receptor ligand is administered in an amount of about 0.001 to 100 mg/kg body weight/dose.

6. The method of claim 1, wherein the leptin receptor ligand is administered orally, intravenously, subcutaneously, topically, transdermally, intramuscularly, or intraperitoneally.

7. The method of claim 1, wherein the leptin receptor ligand is administered orally.

8. The method of claim 1, wherein the composition is administered intravenously.

9. The method of claim 1, wherein the leptin receptor ligand is administered in the form of a pharmaceutical composition of matter which further comprises a pharmaceutically-acceptable carrier.

10. A method of treating precocious puberty comprising administering to a mammal in need of such treatment an amount of a specific antibody to a leptin receptor agonist effective to reverse precocious puberty.

11. A method of treating precocious puberty comprising administering to a mammal in need of such treatment an amount of a leptin receptor antagonist effective to reverse precocious puberty.

12. A method of treating delayed puberty comprising administering to a mammal in need of such treatment an amount of a leptin receptor agonist effective to induce puberty.

13. A pharmaceutical composition of matter for treating the infertility associated with improper nutrition, comprising a leptin receptor ligand in an amount effective to induce fertility, and a pharmaceutically acceptable carrier therefor.

14. The composition of claim 13, wherein said leptin receptor ligand is a leptin receptor agonist.

15. The composition of claim 13, wherein said leptin receptor ligand is a leptin receptor antagonist.

16. A pharmaceutical composition of matter for treating precocious puberty, comprising specific antibodies to a leptin receptor agonist

effective to reverse precocious puberty, and a pharmaceutically acceptable carrier therefor.

17. A pharmaceutical composition of matter for treating precocious puberty, comprising a leptin receptor antagonist in an amount effective to reverse precocious puberty, and a pharmaceutically acceptable carrier therefor.

18. A pharmaceutical composition of matter for treating delayed puberty, comprising a leptin receptor agonist in an amount effective to induce puberty, and a pharmaceutically acceptable carrier therefor.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02909

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/00, 38/19
US CL : 424/85.1; 514/ 2, 8, 12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.1; 514/ 2, 8, 12

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE, BIOSIS, CA, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	HASSINK et al. Serum leptin in children with obesity: Relationship to gender and development. Pediatrics, August 1996, Vol. 98, No.2, pages 201-203, see entire document.	13-15, 19 ---------- 13-15,19
X ---- Y	AHIMA et al. Role of leptin in the neuroendocrine response to fasting. Nature, 18 July 1996, Vol. 382, Number 6588, pages 250-252, see entire document.	13-15, 19 ---------- 13-15,19
X --- Y	AHIMA et al. Leptin accelerates the onset of puberty in normal female mice. J. Clin. Invest., February 1997, Vol. 99, No. 3, pages 391-395, see entire document.	13-15, 19 ---------- 13-15,19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

17 APRIL 1998

Date of mailing of the international search report

05 MAY 1998

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02909

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	CHEHAB et al. Early onset of reproductive function in normal female mice treated with leptin. Science, 03 January 1997, Vol. 275, pages 88-90, see entire document.	13-15, 19 ---------- 13-15,19
X --- Y	VOGEL et al. Leptin: A trigger for puberty. Science, 29 November 1996, Vol. 274, pages 1644-467, see entire document.	13-15, 19 ---------- 13-15,19
X ----- Y	CHEUNG et al. Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinology, February 1997, Vol. 138, No. 2, pages 855-858, see entire document.	13-15, 19 ---------- 13-15,19
X ---- Y	BUTTE et al. Leptin in human reproduction: Serum leptin levels in pregnant and lactating women. J. Clin. Endocrin. and Metab. February 1997, Vol. 89, No. 2, pages 585-589, see entire document.	13-15, 19 ---------- 13-15,19
X --- Y	YU et al. Role of leptin in hypothalamic-pituitary function. Proc. Natl. Acad. Sci., 04 February 1997, Vol. 94, pages 1023-1028, see entire document.	13-15, 19 ---------- 13-15,19
X --- Y	BARASH et al. Leptin in a metabolic signal to the reproductive system. Endocrinology, July 1996, Vol 137, No. 7, pages 3144-3147, see entire document.	1-10, 19 13-15, ---------- 1-10, 13-15, 19
X ---- Y	CHEHAB et al. Correction of the sterility defect in homozygous obese female mice by treatment with the human recobminant leptin. Nature Genetics, March 1996, Volume 12, number 3, pages 318-320, see entire document.	1-10, 14-15, 19 ---------- 1-10, 14-15, 19
X ---- Y	CHEHAB et al. A broader role for leptin. Nature Medicine, July 1996, Vol. 2 No. 7, pages 723--724, see entire document.	1-10, 14-15, 19 ---------- 1-10, 14-15,19
X ----- Y	HAMILTON, B. A new role for a fat actor. Nature Nedicine, March 1996, Vol 2, No. 3, pages 372-273, entire document.	1-10, 14-15, 19 ---------- 1-10, 14-15, 19
X ---- Y	BRZECHFFA et al. Serum immunoreactive leptin concentrations in women with polycystic ovary syndrome. J. Clin. Endocrin. and Metab. November 1996, Vol. 81, No.11, pages 4166-4169, see entire document.	1-10, 14-15, 19 ---------- 1-10, 14-15, 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02909

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	WO 96/23815 A1 (ELI LILLY AND COMPANY) 08 August 1996, see the abstract and claims.	14-19 --------- 14-19
X ----- Y	WO 97/00319 A3 (SMITHKLINE BEECHAM PLC) 03 January 1997, see the abstract and claims.	14-19 -------- 14-19
X ---- Y	WO 96/35787 A1 (CHIRON CORPORATION) 14 November 1996, see the abstract and claims.	14-19 -------- 14-19
X --- Y	WO 96/34885 A2 (SMITHKLINE BEECHAM PLC) 07 November 1996, see the abstract and claims.	14-16, 18-19 -------- 14-16, 18-19
X --- Y	WO 96/40912 A1 (AMGEN INC.) 19 December 1996, (19-12-96), see the abstract and claims.	14-16, 18-19 -------- 14-16, 18-19