COMMONWEALTH OF AUSTRALIA

Patents Act 1952

Form 1 Regulation 9

Case: 37-21(5697)A AS

APPLICATION FOR A STANDARD PATENT OR A STANDARD PATENT OF ADDITION

I/We, MONSANTO COMPANY, a Corporation organised and existing under the laws of the State of Delaware, United States of America, having its principal place of business at 800 North Lindbergh Boulevard, St. Louis, State of Missouri, United States of America,

hereby apply for the grant of a Standard Patent for an invention entitled:

"METHOD AND COMPOSITIONS FOR INCREASING MAMMARY PARENCHYMA"

which is described in the accompanying complete specification.

~~I/We request that the Patent may be granted as a Patent of Addition to the Patent applied for on Application No. filed~~

~~I/We request that the term of the Patent of Addition be the same as that of the patent for the main invention or so much of the term of the patent for the main invention as is unexpired.~~

This application is a Convention application and is based on an application/s numbered 837,477 for a patent or similar protection made in United States of America on 7th March 1986.

APPLICATION ACCEPTED AND AMENDMENTS ALLOWED 19.1.90.

LODGED AT SUB-OFFICE - 6 MAR 1987 Melbourne

LODGED AT SUB-OFFICE - 6 MAR 1987 Melbourne

My/Our address for service is care of EDWIN F. WELLINGTON, Patent Attorney/s, 457 St. Kilda Road, Melbourne, in the State of Victoria, Commonwealth of Australia.

FEE STAMP TO VALUE OF $ 355 ATTACHED
MAIL OFFICER.....

DATED this 6th day of March A.D. 19 87

For and on behalf of
MONSANTO COMPANY,

PATENT OFFICE ONE HUNDRED DOLLARS

LODGED AT SUB-OFFICE - 6 MAR 1987 Melbourne

PATENT OFFICE FIVE DOLLARS

37-21(5697)A AS

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

FORM 8 REGULATION 12(2)

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION ~~UNDER PART XVI~~ FOR A PATENT OR PATENT OF ADDITION.

In support of the Convention Application made ~~under Part XVI of the Patents Act 1952~~ by MONSANTO COMPANY for a patent for an invention entitled:

METHOD AND COMPOSITIONS FOR INCREASING MAMMARY PARENCHYMA

I, Rudolph John Anderson, Jr., General Patent Counsel, Monsanto Company, of 800 North Lindbergh Boulevard, St. Louis, 63167, in the State of Missouri, United States of America, do solemnly and sincerely declare as follows:

1. I am authorized by MONSANTO COMPANY, the applicant for the Patent to make this declaration on its behalf.

2. The basic application as defined by Section 141 of the Act was made at the Patent Office, Washington, District of Columbia, in the United States of America on the 7th of March 19 86, by Robert Joseph Collier and Michael Francis McGrath.

3. ROBERT JOSEPH COLLIER, 6926 Delmar, University City, Missouri 63130 U.S.A.
MICHAEL FRANCIS McGRATH, 933 Orville Wood Road, Chesterfield, Missouri 63017 U.S.A.

~~is~~/are the actual inventor(s) of the invention, and the facts upon which the MONSANTO COMPANY is entitled to make the application are as follows:
The Company is the assignee of the actual inventor(s).

4. The basic application referred to in paragraph 2 of this declaration was the first application made in a Convention country in respect of the invention, the subject of the application.

DECLARED at St. Louis, Missouri, aforesaid this 17th day of February, 19 87.

RUDOLPH JOHN ANDERSON, JR.

To Commissioner of Patents
COMMONWEALTH OF AUSTRALIA

(12) PATENT ABRIDGMENT (11) Document No. AU-B-69788/87
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 595295

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METHOD AND COMPOSITIONS FOR INCREASING MAMMARY PARENCHYMA

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(56) Prior Art Documents
US 4521409
US 4011312

(57) Claim

1. A method for enhancing the growth of mammary parenchyma in a physiologically normal mammal wherein a mammary parenchyma growth-enhancing amount of a substance having a mitogenic effect on mammary epithelial cells in said mammal is administered to the mammal by intramammary infusion during gestation or between about the onset of puberty and the mammal's first gestation.

14. A composition adapted for administration to a ruminant by intramammary infusion, said composition comprising a physiologically acceptable carrier and an amount, effective to thereby enhance the growth of mammary parenchyma in said ruminant, of a substance having a mitogenic effect on mammary epithelial cells in said ruminant.

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PATENTS ACT 1952-1969

595295

FORM 10

COMPLETE SPECIFICATION

(Original)

Application Number: 69788/87
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Class: Int. Class

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Accepted:
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Priority:

Related Art:

This document contains the amendments allowed under Section 83(2) by the Supervising Examiner on

.....

and is correct for printing

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Name of Applicant: MONSANTO COMPANY

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Actual Inventor/s: ROBERT JOSEPH COLLIER; and
MICHAEL FRANCIS McGRATH.

Address for Service: EDWIN F. WELLINGTON
457 St. Kilda Road,
Melbourne, Vic. 3004

This document contains the amendments made under Section 49.

and is correct for printing.

Complete Specification for the invention entitled:

"METHOD AND COMPOSITIONS FOR INCREASING MAMMARY PARENCHYMA"

The following statement is a full description of this invention including the best method of performing it known to me/us:

Background of the Invention

Milk production by a mammal is ultimately limited by the amount of mammary parenchyma (secretory tissue) in the mammary gland. Comparisons of milk yield with total parenchyma wet weight, dry weight, dry fat-free weight and total gland DNA have produced high correlation values. In 43 Proc. N.Z. Soc. Anim. Prod. 71 (1983), Davis et al attributed increased milk production in genetically superior animals entirely to increased udder volume which is also related to the quantity of parenchyma cells.

Classical evidence indicates that mammary parenchyma growth is directed by pituitary, ovarian, adrenal and placental factors. See Cowie, 15 Monographs on Endocrinology (1980) and U.S. Patent 4,521,409 issued June 4, 1985 to Bauman and Sejrsen, in which it is disclosed that growth of mammary parenchyma in a ruminant can be increased by administering a growth hormone (somatotropin) into the ruminant's blood stream between about the onset of puberty and about the first parturition (preferably about the first conception).

If administered into an animal's blood stream, most mitogens for mammary parenchyma cells bind additionally to receptors in many of the animal's other tissues. Due to such dilution of the desired effect on mammary growth-stimulating receptors, increasing mammary parenchyma via administration into the blood stream would be expected to require relatively large quantities of such mitogens. Hence there is great incentive to develop a practical technique for increasing the milk production capacity and/or efficiency of a mammal that is not dependent on administration into the mammal's blood stream.

Using rabbits, mice and rats that were physiologically abnormal, i.e., ovariectomized and/or pretreated (e.g. with a reproductive hormone) to induce pseudopregnancy, studies have been made of effects on mammary gland development by subcutaneous injection of a somatotropin and/or other hormones, implants that released cholera toxin, or intraductal injection of "lactogenic hormone". Lyons et al, 50 Proc. Soc. Exper. Biol. Med. 308-11 (1942) and 14 Recent Prog. Hormone Res. 219-54 (1958); Fiddler et al, 49 J. Endocrin. 459-69 (1971); Daniel et al, 224 Sci. 1245-47 (1984); and Silberstein et al, 81 Proc. Natl. Acad. Sci. USA 4950-54 (1984). Others have studied in vitro effects of mitogens such as an epidermal growth factor, with or without insulin, on the growth of cultured mammary cells of various animal species. Imagawa et al, 79 Proc. Natl. Acad. Sci. USA 4074-77 (1982); Taketani et al, 113(3) Endocrin. 871 (1983) and 80 Proc. Natl. Acad. Sci. USA 2647-50 (1983); Tonelli et al, 285 Nature 250-52 (1980); and Turkington, 57 Exper. Cell Res. 79-85 (1969). The disclosures cited in this paragraph are incorporated here by reference.

Objectives of the Invention

It is an object of this invention to provide a method for enhancing the growth of mammary parenchyma in a mammal. Another object is such a method which provides good results in mammals that are physiologically normal. Another object is such a method having a high degree of specificity in the animal for the desired increase in parenchyma. Another object is a method requiring use of a minimal or near-minimal quantity of a substance effecting such an enhancement in parenchyma growth. Another object is such a method which can be carried out conveniently. Another object is a method that provides predictably uniform effects on treated mammary glands in the animal. Another

object is a method having minimal potential for biological side effects in the animal. Other objects are various compositions suitable for convenient and commercially practical use in such methods, and a process for preparing such compositions. These and other objects of the invention will be more fully apparent from the following detailed disclosure.

Summary of the Invention

We have discovered that these objectives can be achieved in physiologically normal mammals by intramammary infusion of a substance having a mitogenic effect on mammary epithelial cells in that mammal. Surprisingly, substantial enhancement of normal parenchyma growth can be achieved by this method which does not require administration into the animal's blood stream, subcutaneous injection or implantation, but instead utilizes a convenient administration technique of a kind which, in the case of most dairy cows and some other mammals, is routinely carried out for infusion of antibiotics. Substantial enhancement of parenchyma growth over that normally observed can be achieved by this invention utilizing only very small quantities of such substances, and in relatively short intervals of time.

Description of the Invention

This invention is useful for any mammal in which an increase in mammary parenchyma growth is desired. It is especially useful for ruminants, including cattle such as dairy cows, cows in beef herds, and heifers of dairy and beef cows. It is useful in all known breeds of dairy cows, e.g. Holstein, Friesien, Brown Swiss, Jersey, Guernsey, Ayrshire, Milking Shorthorn, and crossbreeds thereof, and in all known breeds of beef cows, e.g. Hereford, Angus, Zebu, Charolais, Brangus, Simmental, Chianina, Dutch Belted, Limousin, and crossbreeds thereof. It is also useful in other ruminants, e.g. sheep and goats of all

breeds, as well as various non-ruminants, e.g. swine of all breeds. Most notably for practical application, the invention is used for treatment of mammals that are physiologically normal which, for present purposes, should be understood to mean not ovariectomized and not pretreated (e.g. with a reproductive hormone such as estrogen, gonadotrophin or the like) to induce pseudopregnancy or puberty.

As used herein, the term "mammary parenchyma" refers to tissue in a mammary gland (including that commonly called "mammary epithelium") which is instrumental in production of milk by that gland. Typically in the female, mammary gland growth occurs mainly after the onset of puberty. Usually (e.g. in the heifer) most mammary gland growth occurs during the first gestation. Further growth and/or regrowth of the parenchyma occurs during subsequent gestations.

To enhance such growth, this invention can be carried out by administering the substance having the desired mitogenic effect to the mammal between about the onset of puberty (including the peripubertal period) and the mammal's first gestation, or during the first or any subsequent gestation. Thus, typically such administration is carried out in a "dry" period during which the mammal is not lactating. In dairy cows and similar mammals, administration during the last trimester before parturition is usually advantageous.

The convenient and surprisingly effective administration technique of this invention is most commonly called intramammary infusion. In mammals such as cows, this technique is routinely used to administer antibiotic-containing ointments or other formulations for prevention or control of mastitis. In practice of the present invention, the substance having a mitogenic effect usually can be administered by a similar technique or, even more conveniently, by

including it (with or without another parenchyma growth stimulant) in a formulation to be infused for mastitis prevention or control, or for any other purpose.

In this technique, a blunt-tip syringe is inserted through the teat orifice and streak canal such that the substance having a mitogenic effect is deposited in the teat cistern. From there, it normally spreads through the teat cistern, gland cistern and ductal system of the mammary gland. This well-known technique is described in "Drugs in Veterinary Practice", pp. 80-83, by Spinelli et al, C. V. Mosby Co., St. Louis, MO (1978), and U.S. Patent 4,011,312 issued March 8, 1977 to Reuter et al, incorporated here by reference. For purposes of this invention, other procedures should be considered equivalents of intramammary infusion to the extent they deposit such a substance in the teat cistern and thereby result in spreading of same through the ductal system and passage therefrom into mammary tissue where it has an enhancing effect on parenchyma growth.

For purposes of this application, "direct" and "paracrine" mitogenic effects are to be understood as effects that do not require, and in most instances do not involve, the infused substance entering the treated animal's blood stream in an amount that raises its concentration therein to a level sufficient to have, through endocrine regulation, a mitogenic effect of the desired magnitude on parenchyma cells in the animal. For a review of paracrine regulation of mammary gland growth, see Oka et al, 15(1) Clinics in Endocrin. and Metab. 79-97 (1986).

This invention can be carried out using any substance having a significant direct or paracrine mitogenic effect on mammary parenchyma in the mammal to be treated. Many important embodiments are carried out using a substance which acts directly on mammary

parenchyma cells in the infused gland to increase their rate of normal cell proliferation. In other embodiments, the substance acts directly on non-parenchyma cells within the gland resulting in such cells providing, through a paracrine effect, a locally increased amount of a substance having a direct mitogenic effect on mammary parenchyma cells in the infused gland.

In some instances, as can be seen from specific examples hereinafter, intramammary infusion of such a substance results in substantial growth of mammary glands adjacent to those infused, and without a significant increase in the level of the infused substance in the animal's bloodstream. However, sufficient receptors for direct or paracrine effects of some substances within a mammary gland may exist only during certain phases of the animal's physiology. For example, there appear to be a greater number of somatotropin-binding receptors in the bovine mammary gland during gestation than when the animals are not pregnant. Compare L'HORMONE PLACENTAIRE SOMATOMAMMOTROPE BOVINE (Chapter V) by Jean-Francois Beckers, Universite de Liege, Faculte de Medecine Veterinaire (1983) with Hormonal Control of Casein Synthesis and Organ Culture of the Bovine Lactating Mammary Gland, Gertler et al, 49 J. Dairy Res. 387 (1982) and Lactogenic Hormones: Binding Sites, Mammary Growth, Secretory Cell Differentiation, and Milk Biosynthesis in Ruminants, Akers, 68 J. Dairy Sci. 501 (1985).

In many embodiments of the invention, the substance having a mitogenic effect is a polypeptide growth factor. Certain classes of such growth factors preferred for use in this invention have molecular weights generally lower than about 45,000, even more typically lower than about 22,500 and, in the case of growth factors having a direct mitogenic effect as described hereinbefore, typically lower than

about 10,000. Some non-polypeptide substances, e.g. ovarian and placental steroids and agonists thereof, can be suitably employed in some instances. In either case, the substance may be naturally occurring, synthesized to simulate a naturally occurring substance, or synthesized to provide a substance that is unlike any naturally occurring compound but nevertheless has such a mitogenic effect.

A preferred class of polypeptide growth factors that can be used are the epidermal growth factors (EGF's). Typically, these interact with receptors on mammary epithelial cells to stimulate proliferation of such cells. EGF's of various mammalian species have been sequenced and identified by their amino acid sequences and/or configurations dictated by internal cross-linking of cysteine residues through disulfide bonds. One such EGF is a well-characterized single-chain polypeptide of 53 amino acids which can be isolated from submaxillary (salivary) glands of adult male mice. Another form of EGF, human urogastrone, is synthesized in the duodenum and salivary glands and can be isolated from human urine. Urogastrone is 70% homologous (37 of 53 common amino acids) with mouse EGF and has three disulfide bonds in the same positions. Available data indicate that human urogastrone and human EGF are identical. For more information on EGF's, see "Epidermal Growth Factor" by Carpenter et al, 48 Ann. Rev. Biochem. 193-216 (1979) and Gregory, 257 Nature 325-27 (1975), both incorporated here by reference.

It is known that the entire amino acid sequence of human or mouse EGF is not necessary for mitogenic activity. E.g., U.S. Patent 3,917,824 issued Nov. 4, 1975 to Gamble et al discloses molecular variations shortened (e.g. by 2, 5 or 6 amino acids) at the carboxy terminus; U.S. Patent 4,035,485 issued July 12, 1977 to Gregory et al discloses a molecular

variation shortened by 7 amino acids; U.S. Patent 3,948,875 issued April 6, 1976 to Cohen discloses EGF derivatives lacking the C-terminal Leu-Arg residues; PCT Appln. WO 83/04030 by Banks et al discloses urogastrone analogs that differ in identity or location of one or more amino acids; and PCT Appln. WO 85/01284 by Komoriya et al discloses polypeptides having EGF-like activity based on the presence of a ten-amino acid sequence corresponding to that between Cys_{20} and Cys_{31} of natural EFG; each incorporated herein by reference. To the extent these or similar molecular variations of EGF, analogs thereof, or other compounds that interact with EGF (or functionally similar) receptors in the mammary gland following intramammary infusion and achieve objectives of this invention in substantial measure, they are equivalents of EGF's for purposes of this invention.

Various procedures for preparing an EGF are known. E.g., Japanese Patent Appln. 59/027858 (Nippon Shinyaku) discloses condensing protected 1-5 amino acid peptides to form a protected tripentacontapeptide which is oxidized, after being deprotected, to make an EGF; European Patent Appln. 128,733 by Lee et al published Dec. 19, 1984 and Patent Appln. WO 85/00369 by Bell published Jan. 31, 1985 disclose EGF preparations by expression of recombined DNA; British Patent 2,092,155 to Sugimoto discloses production of an EGF by multiplying hybridoma cells; and U.S. Patent 4,528,186 issued July 9, 1985 to Nishimura et al discloses recovery of an EGF from human urine. These disclosures are incorporated by reference.

In other preferred embodiments, the growth factor can be selected from fetal EGF's such as the alpha-type transforming growth factors. See European Patent Applns. 105,014 by Sporn et al and 190,018 by Todaro et al (disclosures incorporated here by reference). Typically, these interact with receptors in

the mammary gland to provide the desired mitogenic activity. Another class of the various polypeptides useful in this invention is illustrated by the viral protein analogs disclosed in European Patent Appln. 199,769 based on WO 86/02650 by Brown et al.

In other embodiments, the growth factor can be a polypeptide from the insulin family, an analog of such a polypeptide, or another substance that interacts with insulin-like growth factor (IGF) or functionally similar receptors in the mammary gland to provide a mitogenic effect on the parenchyma. Preferably, the IGF employed is an IGF-I ("somatomedin") or IGF-II. Similar results can generally be obtained using insulin or a precursor thereof (e.g. pro-insulin) although the amounts required are much greater than those of an IGF.

There is extensive literature on identities of these insulin family growth factors and methods for their production. For example, amino acid sequences for human IGF-I and -II and their production by expression of recombinant DNA in microbial hosts are described in European Patent Applns. 128,733 (supra) and 123,228 (Chiron Corp.) published Oct. 31, 1984. IGF-I analogs having valine in amino acid position 59 are described in European Patent Appln. 158,892 by Niwa et al published Oct. 23, 1985. More information on IGF's can be found in Rinderknecht et al, 73 Proc. Natl. Acad. Sci. USA 2365 (1976); Zapf et al, 19 Curr. Top. Cell. Reg. 257 (1981); and Wilson et al, 95 J. Endocrin. 59-64 (1982). Preparations of insulin and pro-insulin are described in Johnson, 219 Sci. 632-37 (1983) and European Patent Appln. 55,945 (Genentech) published July 14, 1982. The disclosures cited in this paragraph are incorporated here by reference.

Substances that can be infused to achieve a paracrine mitogenic effect include placental lactogen,

certain anterior pituitary hormones, and other polypeptides having sufficiently similar chemical structure to provide essentially the same mitogenic effect(s) for purposes of this invention. Generally, anterior pituitary hormones other than prolactin (e.g. somatotropins) are preferred for reasons of efficiency.

The amounts of the mitogenic activity-providing substance required by the present invention are very small, indicating an unexpectedly high efficiency in their use. Unit doses for infusion of a gland in some cases need be no more than about 2 micrograms, and smaller doses may be effective in some instances. Unit doses up to about 100 milligrams or more can usually be administered without interfering with the desired effects, but much smaller doses (e.g. less than about 100 micrograms) are generally preferable for economic reasons. Concentrations of the mitogenic activity-providing substance in the infused composition are commonly most advantageously between about 5 and about 500 ng/ml, although higher or lower concentrations may be useful in some instances.

The most desirable doses, concentrations, frequencies and numbers of infusions can be determined by routine experimentation. In many cases, from 1 to about 5 successive infusions provide good results. These can be suitably spaced by, e.g., from 1 to about 10 (preferably from 2 to about 5) days. The fact that substantial increases in parenchyma growth can be achieved in such short periods of time was quite unexpected and has considerable commercial significance. Satisfactory total doses, i.e., the sum of the successive infusions of an individual mammal during a gestation or puberty prior to gestation, are generally at least about 10 micrograms, typically less than about 500 milligrams, and often preferably less than about 1 milligram of the mitogenic activity-providing substance.

While the invention can be practiced using a single mitogenic activity-providing substance, combinations of such substances may be advantageous in some instances. In many embodiments, it is desirable to infuse a mitogenic activity-providing substance corresponding closely to a substance naturally occurring in the species to be treated, e.g. a bovine EGF, IGF and/or somatotropin for treatment of cattle. However, substances corresponding to those naturally occurring in other species are commonly also suitable.

Normally, the method of this invention is carried out using a composition adapted for administration to a mammal by intramammary infusion, said composition comprising a physiologically acceptable carrier and an amount, effective to thereby enhance the growth of mammary parenchyma in said mammal, of a substance having a mitogenic effect on mammary epithelial cells in that mammal. Such compositions should be physiologically tolerable, preferably substantially non-irritating and essentially free of endotoxins. If desired, the mitogenic activity-providing substance can be infused as a solid which may be finely divided or compressed in an advantageous shape and size, e.g. for prolonged release in the teat cistern. In other embodiments, it is desirable to use a carrier/vehicle that is substantially liquid at the mammal's body temperature, and preferably also at the ambient temperatures of infusion. Whether the infused composition is solid or fluid, it is normally advantageous for it to include a carrier/vehicle that enhances diffusion of the mitogenic activity-providing substance through the mammary gland. In some, but not all cases, it is advantageous for the carrier/vehicle to be substantially water-soluble.

The most desirable type and quantity of carrier/vehicle for infusion of the mitogenic activity-providing substance can be determined by routine experimentation. To illustrate, 1-10 ml of a liquid carrier/vehicle

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having a pH in the physiological range, e.g. a physiological saline solution, Freund's Incomplete Adjuvant or a vegetable (e.g. peanut or sesame) or mineral oil, is typically satisfactory. Emulsions of two or more of such vehicles are commonly advantageous. Appropriate stabilizers, e.g. mono- and/or disaccharides, sugar and alcohols or neutral amino acids, may be included if desired.

This invention also provides a process for preparing compositions useful for enhancing the growth of mammary parenchyma in a mammal. In a generic embodiment, such a process comprises combining (e.g. dispersing) a substance having a mitogenic effect on mammary epithelial cells in said mammal in a vehicle that enhances diffusion of the substance through a mammary gland of said mammal, said composition being adapted by said combining for administration to the mammal by intramammary infusion.

The following descriptions of specific embodiments of the invention are illustrative only and do not imply any limitation on the scope of the invention. All animals in these examples were physiologically normal (as defined hereinbefore). All temperatures are in degrees Celsius. Parts and percentages are by weight except where otherwise indicated.

A. Epidermal Growth Factors

Examples 1-2

12 pregnant, non-lactating, cross-bred beef cows within their last 40-80 days of gestation were housed in a free-stall facility, fed a ration of alfalfa hay and pelleted concentrate, and administered 10 ml of an excipient (Freund's Incomplete Adjuvant emulsified with an equal volume of 0.9% sterile saline) into each quarter-udder by intramammary

infusion through the streak canal on days 1, 3, 5, 7 and 9 of this study. Infusions were carried out using a 3.2 centimeter long, approximately 12 gauge, blunt-tipped plastic teat infusion cannula obtained from Jorgensen Laboratories, Loveland, CO. Because front and rear udder halves are not of equal size, treatment vs. control comparisons were made between laterally opposite quarters, i.e., front right vs. front left and rear right vs. rear left. The 2 quarters in each udder half were randomly assigned to treatment and its control. In the treatment infusions, the excipient contained 25 micrograms of human EGF (hEGF) obtained from G. D. Searle & Co., Ltd., U.K. (6 animals) or mouse EGF (mEGF) obtained from Collaborative Research Corp., Lexington, MA (the other 6 animals). The hEGF had been prepared by Searle by expression of recombinant DNA and subsequently further purified by filtration on a 10,000 MW filter to a purity above 90% as determined by HPLC. The mEGF was culture-grade, Catalog No. 40001, from mouse submaxillary glands, prepared by the method of Savage et al, 247 J. Biol. Chem. 7609 (1972) with a purity of 98% determined on SDS polyacrylamide gel.

Prior to treatment, each animal was teat-dipped for 3 consecutive days and each teat was pre-cleaned with 70% ethanol in water on the day of treatment. On day 14 the cows were sacrificed and their mammary glands excised. Each udder (milked out if necessary) was skinned, divided along the median suspensory ligament and then into front right, front left, rear right and rear left udder quarters. Each quarter-udder was ground, a representative 200 gm sample was homogenized with 4 times its volume of water, and the total homogenate volume of each was measured.

To determine dry weight of each quarter-udder, three 10 ml aliquots of a 1:5 diluted homogenate

were placed in pre-weighed pans, dried overnight at 60° and then dried and weighed to a consistent value.

To determine dry fat-free tissue weight of each quarter-udder, three 5 ml aliquots of the undiluted homogenate were extracted by the method of Anderson, 41 J. Anim. Sci. 118 (1975) except that samples were placed in pre-weighed 25 x 150 mm glass centrifuge tubes and extracted overnight with 10 ml ethanol:ether (3:1), then centrifuged (5 min at 500 rpm) and the supernatant aspirated; an additional 10 ml aliquot of ethanol:ether was added for a second extraction; and the sample was dried under nitrogen until a consistent value was obtained.

DNA in each quarter-udder was determined by the method of Burton, 12B Methods in Enzymol. 163-66 (1968) using an assay solution of 15% trichloroacetic acid (TCA) in 2 N HCl. In these determinations, aliquots of gland homogenate were diluted 1:5 with water and rehomogenized. Using triplicate 0.25 ml or 0.5 ml aliquots of these homogenates, each aliquot was mixed with 2 ml of the assay solution. After 30 min, the samples were centrifuged for 10 min at 3000 rpm and the resulting pellets were washed with 2 ml of a 10% solution of TCA in distilled water. After recentrifugation, the resulting pellets were disrupted in 2 ml of 0.5 N perchloric acid and heated for 30 min at 70° to extract DNA. Samples were centrifuged and 1 ml of each resulting supernatant was placed in a 12 x 75 mm tube. After addition of 2 ml of a solution of 1.5 g diphenylamine and 1.5 ml H_2SO_4 in 100 ml glacial acetic acid (and 0.1 ml of 1.6% aqueous acetaldehyde per 20 ml reagent just prior to use), the samples were vortexed and incubated overnight at 27°. Calf thymus DNA was the standard.

Results are in Tables 1 and 2.

Table 1

Effects of Intramammary Infusion of hEGF on Quarter-Udders in Pregnant Beef Cows

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, μg
A. Treatment Mean	389.9	120.0	63.2	794.5
B. Control Mean (Excipient only)	357.3	111.4	58.3	710
C. Mean Increase (A-B)	32.6	8.6	4.9	84.5
D. % Increase, (A-B)/B	9.1	7.7	8.4	11.9
E. Avg % Increase/Animal	12.9	13.1	16.1	14.8
F. Significance Level of % Increase, p<	0.01	0.09	0.16	0.06

Table 2

Effects of Intramammary Infusion of mEGF on Quarter-Udders in Pregnant Beef Cows

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, μg
A. Treatment Mean	419.6	150.2	69.6	905.1
B. Control Mean (Excipient only)	402.0	147.9	64.6	794.9
C. Mean Increase (A-B)	17.6	2.3	5.0	110.2
D. % Increase, (A-B)/B	4.4	1.6	7.9	13.8
E. Avg % Increase/Animal	1.8	2.1	5.2	12.0
F. Sig. Level of % Increase, p<	0.22	0.40	0.19	0.05

As shown in Tables 1 and 2, quarter-udders infused with hEGF or mEGF were consistently heavier and contained more DNA than their laterally-opposite control quarters that received excipient only, and at generally high levels of statistical significance.

Examples 3-6

24 pregnant, non-lactating, cross-bred beef cows were divided into 4 treatment groups with 6 animals in each group. The effects of intramammary infusion of human EGF on quarter-udders in these animals were determined as described in Example 1 except that (A) as the excipient, the Freund's emulsion was used for Group 1 and an emulsion containing 60 parts sesame oil and 40 parts injectable grade 0.9% sterile saline containing 0.5% Tween-20 was used for Groups 2, 3 and 4 and (B) the amounts of hEGF included in each treatment infusion were 25 micrograms for Groups 1 and 2, 2.5 micrograms for Group 3, and 250 micrograms for Group 4. DNA determinations were made as in Examples 1-2 except that the initial triplicate 0.5 ml aliquots of homogenate were extracted with 2 ml of ethanol:ether (3:1) for 1 hour, centrifuged for 15 min at 3000 rpm, washed with 2 ml of 70% ethanol for 10 min, centrifuged at 3000 rpm for 15 min and aspirated of ethanol prior to mixing of each aliquot with the TCA solution. Results for Groups 1-4 are in Tables 3-6, respectively.

Table 3

Effects of Intramammary Infusion of a Total of 125 Micrograms of hEGF in Freund's Emulsion on Quarter-Udders in Pregnant Beef Cows (Group 1)

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, µg
A. Treatment Mean	364.9	107.2	60.3	723.0
B. Control Mean (Excipient only)	349.5	101.0	56.8	629.0
C. Mean Increase (A-B)	15.4	6.2	3.5	94.0
D. % Increase, (A-B)/B	4.4	6.1	6.2	14.9
E. Avg % Increase/Animal	8.1	10.3	8.3	23.0
F. Sig. Level of % Increase, p<	0.15	0.11	0.13	0.18

Table 4

Effects of Intramammary Infusion of a Total of 125 Micrograms of hEGF in Sesame Emulsion on Quarter-Udders in Pregnant Beef Cows (Group 2)

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, µg
A. Treatment Mean	370.2	122.1	71.0	995.0
B. Control Mean (Excipient only)	356.2	111.1	68.8	703.0
C. Mean Increase (A-B)	14.0	11.0	2.2	292.0
D. % Increase, (A-B)/B	3.9	9.9	3.2	41.5
E. Avg % Increase/Animal	10.4	20.2	15.9	70.0
F. Sig. Level of % Increase, p<	0.2	0.02	0.3	0.09

Table 5

Effects of Intramammary Infusion of a Total of 12.5 Micrograms of hEGF in Sesame Emulsion on Quarter-Udders in Pregnant Beef Cows (Group 3)

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, µg
A. Treatment Mean	397.8	132.4	79.4	867.6
B. Control Mean (Excipient only)	400.0	127.0	72.7	896.6
C. Mean Increase (A-B)	(2.2)	5.4	6.7	(29.0)
D. % Increase, (A-B)/B	(0.5)	4.2	9.2	(3.3)
E. Avg % Increase/Animal	0.6	7.8	16.3	(2.2)
F. Sig. Level of % Increase, p<	—	0.17	0.1	—

Table 6

Effects of Intramammary Infusion of a Total of 1.25 Milligrams of hEGF in Sesame Emulsion on Quarter-Udders in Pregnant Beef Cows (Group 4)

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, µg
A. Treatment Mean	391.9	131.5	81.7	733.2
B. Control Mean (Excipient only)	360.2	117.9	75.1	729.2
C. Mean Increase (A-B)	31.7	13.6	6.6	4.0
D. % Increase, (A-B)/B	8.8	11.6	8.8	0.5
E. Avg % Increase/Animal	9.9	12.2	11.9	1.6
F. Sig. Level of % Increase, p<	0.001	0.004	0.03	—

As shown in Tables 3-6, quarter-udders infused with hEGF were generally substantially heavier and contained more DNA than their laterally-opposite quarters that received excipient only.

Examples 7-8

24 pregnant, non-lactating, cross-bred beef cows were randomly divided into 3 treatment groups with 8 animals in each group. The effects of intramammary infusion of human EGF on quarter-udders in these animals were determined as described in Examples 1-2 except that the amounts of hEGF included in each treatment infusion were 25 micrograms for Group 1, 250 micrograms for Group 2, and 2.5 micrograms for Group 3. Gland weights and DNA were determined as in Examples 1-2. Results with the low dose used for Group 3 were obscured by swellings of some glands. Results for Groups 1 and 2 are in Tables 7 and 8, respectively.

Table 7

Effects of Intramammary Infusion of a Total of 125 Micrograms of hEGF on Quarter-Udders in Pregnant Beef Cows (Group 1)

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, µg
A. Treatment Mean	363.0	113.0	52.8	396.9
B. Control Mean (Excipient only)	358.0	109.7	50.7	358.9
C. Mean Increase (A-B)	5.0	3.3	2.1	38.0
D. % Increase, (A-B)/B	1.5	3.0	4.1	10.6
E. Sig. Level of % Increase, p<	0.34	0.21	0.16	0.12

Table 8

Effects of Intramammary Infusion of a Total of 1.25 Milligrams of hEGF on Quarter-Udders in Pregnant Beef Cows (Group 2)

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, µg
A. Treatment Mean	392.5	122.0	63.8	344.0
B. Control Mean (Excipient only)	388.2	145.7	61.4	384.0
C. Mean Increase (A-B)	4.3	6 3	2.4	(40.0)
D. % Increase, (A-B)/B	1.1	5.3	3.9	(10.4)
E. Sig. Level of % Increase, p<	0.4	0.12	0.26	-

As shown in Tables 7-8, quarter-udders infused with hEGF were heavier than their laterally-opposite quarter-udders that received excipient only.

Example 8A

48 pregnant Holstein heifers within their last 40 days (approximately) of gestation were housed in a free-stall facility, fed a ration of alfalfa hay and pelleted concentrate, and administered 10 ml of an excipient into each quarter-udder by intramammary infusion through the streak canal every third day until parturition with the exception of one animal which was treated with human EGF as described in this Example only until 15 days before parturition. Infusions were carried out using a 3.2 centimeter long, approximately 12 gauge, blunt-tipped plastic teat infusion cannula obtained from Jorgensen Laboratories, Loveland, CO. Animals were randomly assigned to each of the following 4 treatments (12 animals each): (A) 250 micrograms of human EGF obtained from G. D. Searle & Co., Ltd. (U.K.), solubilized (62.5 micrograms/ml) in sterile, injectable grade 0.9% saline containing 0.5% Tween-20 and then emulsified just prior to use in sterile sesame oil containing 5% Arlacel A mannide monooleate

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(3:2 oil: saline) with a final concentration of 25 micrograms/ml of hEGF in the excipient; (B) 250 micrograms of bovine IGF-I prepared as in Examples 14-16, purified and then solubilized and emulsified as just described for hEGF; (C) 50 milligrams of N-methionyl bovine somatotropin (BST) prepared as in Examples 16-17 and then diluted to 10mg/ml in sterile sesame oil; and (D) a control using excipient (sterile sesame oil) alone. In each animal, all 4 quarter-udders received the same treatment. Each udder was sprayed with 70% ethanol and wiped dry prior to treatment and dipped with an iodine solution following treatment. At parturition, 4 animals from each treatment group were sacrificed and their mammary glands were removed for analysis. The other 8 animals from each treatment group were milked normally each a.m. and p.m. for the first 105 days of lactation.

During those 105 days, the animal treated with hEGF only until 15 days before parturition produced an average of 9.6 kg milk/day (34.8%) more than the average produced by the 8 control cows. On average, milk production by the cows treated until parturition did not exceed that of the control cows.

B. Insulin-like Growth Factors

Examples 9-11

24 pregnant, non-lactating, cross-bred beef cows within their last 40-80 days of gestation were housed in a free-stall facility, fed a ration of alfalfa hay and pelleted concentrate, and administered 10 ml of an excipient (a sesame oil emulsion of the kind used in Examples 4-6) into each quarter-udder by intramammary infusion through the streak canal on days 1, 3, 5, 7 and 9 of this study. The infusion technique random assignment of quarter-udders to treatment for control and treatment vs. control comparisons were as in Examples 1-2. In the treatment infusions, the excipient contained bovine insulin-like growth factor-I (bIGF-I) prepared by expression in E. coli (Prla

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Strain 079) of recombinant DNA coding for bIGF-I fused to the "lam" B cell wall protein signal sequence, secretion into the E. coli periplasmic space, and recovery/purification by conventional techniques including gel filtration, cation exchange and reverse-phase HPLC. This bIGF-I had the same amino acid sequence and secondary structure as the AMgen human IGF-I used in Example 13. In standard L6 myoblast assays, its activity was essentially the same (90-123%) as that of the Example 13 hIGF-I. The amounts of bIGF-I in each infusion were: Group 1 - 2.5 micrograms; Group 2 - 25 micrograms; Group 3 - 250 micrograms. Animals were prepared and infused, and their quarter-udder weights and DNA were determined as in Examples 3-6. Results for Group 1 are in Table 9.

Table 9

Effects of Intramammary Infusion of a Total of 12.5 Micrograms of bIGF-I on Quarter-Udders in Pregnant Beef Cows (Group 1)

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, µg
A. Treatment Mean	408.4	111.7	54.9	1307
B. Control Mean (Excipient only)	369.0	102.7	48.0	1219
C. Mean Increase (A-B)	39.4	9.0	6.9	88
D. % Increase, (A-B)/B	10.7	8.7	14.2	7.2
E. Avg. % Increase/Animal	11.0	8.8	16.0	12.5
F. Sig. Level of % Increase, p<	0.01	0.003	0.03	0.2

In this study, the two higher doses of bIGF-I (Groups 2-3) did not produce consistently greater growth of treatment quarters vs. control

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quarters. However, substantial growth of the non-infused quarters was observed. When the procedures described for Groups 1-3 were repeated, results were essentially the same. Pooling results from the original study and its replication, it was determined that both treatment and control quarter-udders had grown as follows:

Table 10

Doses	Controls	Treatment	Combined
Medium vs. Low	+27%, $p<0.08$	+23.1%, $p<0.12$	+25%
High vs. Medium	+34.4%, $p<0.08$	+31.5%, $p<0.09$	+33%
High vs. Low	+71.3%, $p<0.007$	+62%, $p<0.01$	+67%

The above increases in growth of the control quarter-udders occurred even though plasma levels of bIGF-I did not increase significantly during the study. Results were essentially the same when these tests were repeated using other pregnant, non-lactating beef cows grouped on the basis of essentially equal beginning udder volumes to remove that variable as much as possible from the test results.

Example 12

When dairy or beef heifers or pregnant cows are infused according to the procedure in Examples 9-11 with a total of from 125 micrograms to 12.5 milligrams of bovine insulin-like growth factor-II purified essentially free from all other bovine peptides as in Example 1 of U.S. Patent Appln. 888,996 filed July 31, 1986 corresponding to European Patent Appln. 86870128.5, both incorporated herein by reference, the results are essentially the same as in Examples 9-11, i.e., substantial increases in the weights and DNA of treated quarter-udders.

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C. Combinations of IGF's and EGF's

Example 13

30 mixed-breed sheep within their last 35-45 days of gestation were fed a ration of alfalfa hay and sheep chow and administered 2.5 ml of an excipient (a Freund's emulsion of the kind used in Examples 1-2 for 15 sheep; physiological (0.9%) saline solution for the other 15) into each half-udder by intramammary infusion through the streak canal on days 1, 3, 5, 7 and 9 of this study. Infusions were carried out using a 3.2 centimeter long, 16 gauge, blunt-tipped Teflon sheath from a C. R. Bard, Inc. A-cath cannula for placement of indwelling catheters. Udder halves in each animal were randomly assigned to treatment in which the excipient contained additionally 2.5 micrograms of mouse EGF of the kind used in Examples 1-2 and 2.5 micrograms of human IGF-I prepared by expression of recombinant DNA and sold by AMgen Biological Corp., Thousand Oaks, CA (Catalog No. 04111, Lot 407; over 90% pure as determined by HPLC). The other udder half in each animal was assigned to control in which the excipient only was infused. Prior to treatment, each animal was teat-dipped for 7 consecutive days and each teat was pre-cleaned with 70% ethanol in water on the day of treatment.

On day 13 the sheep were sacrificed and their mammary glands excised. Each udder (milked out if necessary) was skinned and divided along the median suspensory ligament into right and left udder halves. Each half-udder was homogenized with 4 times its weight of water, and the total homogenate volume of each was measured.

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To determine dry weight of each half-udder, three 10 ml aliquots of a 1:5 diluted homogenate were placed in pre-weighed pans, dried overnight at 60° and then dried and weighed to a consistent value.

To determine dry fat-free tissue weight of each half-udder, three 5 ml aliquots of the undiluted homogenate were extracted by the method of Anderson, 41 J. Anim. Sci. 118 (1975) except that samples were placed in preweighed 25 x 150 mm glass centrifuge tubes and extracted overnight with 10 ml of ethanol:ether (3:1), then centrifuged (5 min at 500 rpm) and the supernatant aspirated; an additional 10 ml aliquot of ethanol:ether was added for a second extraction; and the sample was dried under nitrogen and weighed until a constant value was obtained.

DNA in each half-udder was determined by the method of Burton, Biochemistry 62315 (1956) using an assay solution of 15% trichloroacetic acid (TCA) in 2N HCl. In these determinations, aliquots of gland homogenate were thawed, diluted 1:5 with water and rehomogenized. Using triplicate 0.5 ml aliquots of these homogenates, each aliquot was mixed with 2 ml of the assay solution. After 30 min, the samples were centrifuged for 10 min at 3000 rpm and the resulting pellets were washed with 2 ml of a 10% solution of TCA in distilled water. After recentrifugation, the resulting pellets were disrupted in 2 ml of 0.5 N perchloric acid and heated for 30 min at 70° to extract DNA. Samples were centrifuged and 1 ml of each resulting supernatant was placed in a 12 x 75 mm tube. After addition of 2 ml of a solution of 1.5 g diphenylamine and 1.5 ml H_2SO_4 in 100 ml glacial acetic acid (and 0.1 ml of 1.6% aqueous acetaldehyde per 20 ml reagent just prior to use), the samples were vortexed and incubated overnight at 20°. Calf thymus DNA was the standard.

Results are in Table 11.

Table 11

Effects of Intramammary Infusion of a Combination of mEGF and hIGF-I on Half-Udders in Pregnant Sheep

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, µg
A. Treatment Mean	277.3	73.6	75.2	1021.2
B. Control Mean (Excipient only)	264.7	66.2	56.4	849.5
C. Mean Increase (A-B)	12.5	7.4	18.8	171.7
D. % Increase, (A-B/B	4.7	11.0	33.0	20.2
E. Sig. Level of Increase, p<	0.016	0.03	0.001	0.002

The data in Table 11 show that DNA and wet, dry and dry fat-free weights of the half-udders treated in accordance with this invention were substantially increased relative to the untreated half-udders in the same animals, and at exceptionally high levels of statistical significance.

Example 14

8 non-pregnant, Holstein heifers about 18 months of age were tested for the effects of intramammary infusion of a combination of bovine IGF-I and human EGF according to the procedures used in Examples 9-11 with the exceptions that (A) the volume of excipient in each infusion was 2.5 ml and (B) each treatment infusion contained 6.25 micrograms of bIGF-I and 625 micrograms of hEGF of the kinds used in Examples 9-11 and 1-2, respectively. Results are in Table 12.

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Table 12

Effects of Intramammary Infusion of a Combination of hEGF and bIGF-I on Quarter-Udders in Holstein Heifers

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g
A. Treatment Mean	205.1	93.2	10.7
B. Control Mean (Excipient only)	188.2	89.0	9.5
C. Mean Increase (A-B)	16.9	4.2	1.2
D. % Increase (A-B/B)	8.9	4.7	12.1
E. Avg. % Increase/Animal	11.3	7.7	18.8
F. Sig. Level of Increase, p<	0.06	0.2	0.09

The data in Table 12 show that the wet, dry and dry fat-free weights of Holstein heifer quarter-udders treated in accordance with this invention were substantially greater than their laterally-opposite quarter-udders that received excipient only, and at high levels of statistical significance.

Example 15

The effects of intramammary infusion of a combination of bovine IGF-I and human EGF on 8 pregnant, non-lactating, cross-bred beef cows within their last 40-80 days of gestation were determined by the procedures described in Examples 9-11. Each treatment infusion contained 25 micrograms of hEGF of the kind used in Examples 1-2 and 250 micrograms of bIGF-I of the kind used in Examples 9-11. Results are in Table 13.

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Table 13

Effects of Intramammary Infusion of a Combination of bIGF-I (a Total of 1.25 Milligrams) and hEGF (a Total of 125 Micrograms) on Quarter-Udders in Pregnant Beef Cattle

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, µg
A. Treatment Mean	496.1	156.4	72.0	1392
B. Control Mean (Excipient only)	474.0	144.0	60.7	1243
C. Mean Increase (A-B)	22.1	12.4	11.3	149
D. % Increase, (A-B)/B	4.7	8.6	18.7	12.0
E. Avg. % Increase/Animal	8.2	13.0	11.0	12.0
F. Sig. Level of % Increase, p<	0.1	0.03	0.08	0.15

As shown in Table 13, quarter-udders receiving the combination of bIGF-I and hEGF were consistently heavier and contained more DNA than their laterally-opposite control quarters, and at high levels of statistical significance.

Examples 15A-15B

18 pregnant Holstein heifers within their last 40-60 days of expected calving were housed in a free-stall facility, fed a ration of alfalfa hay and pelleted concentrate and administered 10 ml of an excipient (sterile sesame oil) into each quarter-udder by intramammary infusion through the streak canal every third day for the number of treatments shown below.

The heifers in Groups 1 and 3 were treated no later than their fifth day before parturition; treatments of those in Groups 2 and 4 were continued into their last five days. Treatments containing a mitogenic substance were administered to both quarter-udders on one side of each animal. Excipient only was administered to both quarter-udders on the opposite side of that animal. All infusions were carried out with a Jorgensen Laboratories infusion canula of the kind used in Examples 1-2. Each teat was sprayed with 70% ethanol and wiped dry prior to treatment, and dipped with an iodine solution following treatment.

Group	No. of Heifers	Mitogenic Substance In The Excipient	No. of Treatments Range	Avg.
1	4	hEGF & IGF-I* (0.25 mg each)	10	10
2	5	"	7-20	14
3	5	BST** (50 mg)	10-20	12
4	4	"	4-16	9

*Obtained as in Examples 1-2 and 9-11, respectively, solubilized (62.5 micrograms/ml of each) in sterile, injectable-grade 0.9% saline containing 0.5% Tween-20 and emulsified into the sterile sesame oil excipient (3:2 oil: saline) and 5% Arlacel A (Sigma Chemical Co., St. Louis, MO).

**Prepared as in Examples 16-17 and diluted to 10 mg/ml in the sesame oil excipient. See results in Examples 17A-17B.

These animals were milked normally each a.m. and p.m. for the first 30 days after parturition, with milk production recorded separately for each quarter-udder. On average, milk production by the half-udders that had been treated in Group 2 did not exceed that of their control half-udders.

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Table 14
Effects of Intramammary Infusion
of a Combination of hEGF and IGF-I on Quarter-Udders
in Pregnant Heifers (Group 1)

Average Milk Production, kg/day			
Treated Side (A)	Control Side (B)	% Increase, (A-B)/B	Sig. Level of Increase, p<
10.0	9.0	11	0.14

As shown in Table 14, quarter-udders infused with a combination of hEGF and IGF-I no later than the fifth day before parturition produced, on average, substantially more milk than their laterally-opposite quarters that received excipient only.

D. Somatotropins

Examples 16-17

24 pregnant, non-lactating, cross-bred beef cows in their last 40-80 days of gestation were divided into 2 groups and tested for the effects of intramammary infusion using the procedures of Examples 3-6 except that (A) the volume of excipient in each infusion was 5 ml and (B) each treatment infusion contained a 1% zinc salt of an N-methionyl bovine somatotropin (BST) (Group 1 - 100mg; Group 2-40 mg) prepared by expression of recombinant DNA and purified essentially as described in Example 1A of U.S. Patent Appln. 787,873 filed Oct. 16, 1985 based on European Patent Appln. 177,478 published April 9, 1986, both incorporated herein by reference. Results are in Tables 15-16.

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Table 15

Effects of Intramammary Infusion of a Total of 500 Milligrams of BST on Quarter-Udders in Pregnant Beef Cows (Group 1)

		Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, µg
A.	Treatment Mean	790.1	207.6	146.7	2185
B.	Control Mean (Excipient only)	736.6	190.6	141.5	2054
C.	Mean Increase (A-B)	53.5	17.0	5.2	131
D.	% Increase, (A-B)/B	7.3	8.9	3.6	6.4
E.	Avg. % Increase/Animal	9.8	10.7	7.9	10.5
F.	Sig. Level of % Increase, p<	0.004	0.03	0.18	0.3

Table 16

Effects of Intramammary Infusion of a Total of 200 Milligrams of BST on Quarter-Udders in Pregnant Beef Cows (Group 2)

	Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, µg
A. Treatment Mean	396.9	96.4	49.5	1371
B. Control Mean (Excipient only)	345.4	85.1	44.8	1299
C. Mean Increase (A-B)	51.5	11.3	4.7	72
D. % Increase, (A-B)/B	14.9	13.3	10.5	5.5
E. Avg. % Increase/Animal	13.4	10.7	7.8	15.8
F. Sig. Level of % Increase, p<	0.007	0.06	0.06	0.1

The data in Tables 15-16 show that the increases in DNA and wet, dry and dry fat-free weights of pregnant beef cow quarter-udders treated in accordance with this invention were substantially greater than those of their laterally-opposite quarter-udders that received excipient only, and that the differences between treatment and control increases are at high levels of statistical significance.

Examples 17A-17B

Shown in Table 17 are the milk production results obtained with the heifers of Group 3 in Examples 15A-15B. On average, milk production by the half-udders that had been treated in Group 4 of Examples 15A-15B slightly exceeded (2%, $p<0.4$) that of their control half-udders.

Table 17

Effects of Intramammary Infusion of BST on Quarter-Udders in Pregnant Holstein Heifers (Group 4)

Average Milk Production, kg/day			
Treated Side (A)	Control Side (B)	% Increase, (A-B)/B	Sig. Level of Increase, p<
10.9	9.8	11	0.15

As shown in Table 17, quarter-udders infused with BST no later than the fifth day before parturition produced, on average, substantially more milk than their laterally-opposite quarters that received excipient only.

E. Transforming Growth Factors

Examples 18-21

When dairy or beef heifers or pregnant cows are infused using the procedures and dosages of Examples 3-6 with human alpha-type transforming growth factor from Bachem Inc. Fine Chemicals, Torrance, CA (Catalog #PGRO-30), results are essentially the

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same, i.e., substantial and statistically significant increases in the weights and DNA of the treated quarter-udders.

F. Insulin

Examples 22-24

When dairy or beef heifers or pregnant beef or dairy cows are infused according to the procedures of Examples 3-6 with 25, 100 or 250 mg per infusion of bovine pancreas insulin from Sigma Chemical Co., St. Louis, MO (Catalog #I-5500; 24 IU/mg), results are similar, i.e., substantial and statistically significant increases in the weights and DNA of the treated quarter-udders.

G. Placental Lactogens

Examples 25-27

When dairy or beef heifers or pregnant beef or dairy cows are infused according to the procedures of Examples 3-6 with a total of 0.25, 10 or 250 milligrams of natural bovine placental lactogen purified by the method of Byatt, 119 Endocrin. 1343-50 (1986), results are very similar, i.e., substantial and statistically significant increases in the weights and DNA of the treated quarter-udders.

D. Prostaglandins

Examples 28-31

24 pregnant, non-lactating, cross-bred beef cows in their last 45-105 days of gestation were

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housed in a free-stall facility, fed a ration of alfalfa hay and pelleted concentrate, and administered 5 ml of an excepient (MCT-Oil, a Mead Johnson Corp. medium chain triglyceride which is a lipid fraction of coconut oil consisting primarily of triglycerides of C_8 and C_{10} fatty acids as follows: C_8-67%; C_{10}-23%; $<C_8$-6%; $>C_{10}$-4%) into each quarter-udder by intramammary infusion through the streak cannal on days 1, 3, 5, 7 and 9 of this study. The 48 front and rear udder halves were randomly divided into 4 groups, with one quarter of each udder half treated and its laterally opposite quarter-udder used as a control. In the treatment infusions, the excipient contained Prostaglandin (PG) E_1 or E_2 from Sigma Chemical Co., St. Louis, MO. (Catalog Nos. 86F-0406 and -0404). The amounts of PG in each infusion were: Group 1 - 87.5 micrograms of E_2; Group 2 - 875 micrograms of E_2; Group 3 - 87.5 micrograms of E_1; Group 4 - 875 micrograms of E_1. Animals were prepared and infused as in Examples 23-24; the effects were determined as in Examples 1-2. Results for Groups 1-4 are in Tables 18-21, respectively.

Table 18

Effects of Intramammary Infusion of Prostaglandin E_2 (a Total of 0.4375 Milligrams) on Quarter-Udders in Pregnant Beef Cattle (Group 1)

		Wet Weight, g	Dry Weight, g	DryFat-Free Weight, g	DNA, mg
A.	Treatment Mean	1966.9	525.5	351.8	5203
B.	Control Mean (Excipient Only)	1735.5	484.9	297.8	4724
C.	Mean Increase (A-B)	231.4	40.6	54.0	479
D.	% Increase (A-B)/B	13.3	8.3	18.1	10.1
E.	Sig. Level of % Increase, p<	0.01	0.04	0.18	0.02

Table 19

Effects of Intramammary Infusion of Prostaglandin E_2 (a Total of 4.375 Milligrams) on Quarter-Udders in Pregnant Beef Cattle (Group 2)

		Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, mg
A.	Treatment Mean	1030.8	312.7	194.5	3397
B.	Control Mean (Excipient Only)	742.0	219.0	144.3	2348
C.	Mean Increase (A-B)	288.8	93.7	50.2	1049
D.	% Increase, (A-B)/B	38.9	42.8	34.8	44.6
E.	Sig. Level of % Increase, p<	0.0003	0.0001	0.004	0.001

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Table 20

Effects of Intramammary Infusion of Prostaglandin E_1 (a Total of 0.4375 Milligrams) on Quarter-Udders in Pregnant Beef Cattle (Group 3)

		Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, mg
A.	Treatment Mean	972.5	259.3	180.9	2881
B.	Control Mean (Excipient Only)	778.0	210.6	200.3	2284
C.	Mean Increase, (A-B)	194.5	48.7	(19.4)	597
D.	% Increase, (A-B)/B	25.0	23.0	(9.7)	26
E.	Sig. Level of % Increase, p<	0.002	0.002	0.58	0.07

Table 21

Effects of Intramammary Infusion of Prostaglandin E_1 (a Total of 4.375 Milligrams) on Quarter-Udders in Pregnant Beef Cattle (Group 4)

		Wet Weight, g	Dry Weight, g	Dry Fat-Free Weight, g	DNA, mg
A.	Treatment Mean	1435.5	379.6	247.7	4218
B.	Control Mean (Excipient Only)	949.3	268.2	189.0	2792
C.	Mean Increase (A-B)	486.2	111.4	58.7	1426
D.	% Increase (A-B)/B	51.2	41.5	31.0	51.1
E.	Sig. Level of % Increase, p<	0.03	0.02	0.07	0.06

As shown in Tables 18-21, quarter-udders receiving the prostaglandins were consistently heavier and contained more DNA than their laterally-opposite control quarters that received excipient only, and at generally very high levels of statistical significance.

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While the biological mechanisms of this invention are not fully understood, it is apparent that it can be used to provide, in accordance with conventionally recognized criteria, increases in mammary parenchyma and corresponding milk production capacity of treated animals that will persist through the animal's next lactation period, and in many cases through multiple subsequent lactations.

The matter contained in each of the following claims is to be read as part of the general description of the present invention.

The claims defining the invention are as follows:

1. A method for enhancing the growth of mammary parenchyma in a physiologically normal mammal wherein a mammary parenchyma growth-enhancing amount of a substance having a mitogenic effect on mammary epithelial cells in said mammal is administered to the mammal by intramammary infusion during gestation or between about the onset of puberty and the mammal's first gestation.

2. A method of Claim 1 wherein the substance has a direct mitogenic effect on said cells.

3. A method of Claim 1 or 2 wherein the substance comprises a polypeptide growth factor.

4. A method of Claim 3 wherein the growth factor is selected from epidermal growth factors, insulin family growth factors, alpha-type transforming growth factors, and combinations thereof.

5. A method of Claim 3 wherein the growth factor comprises an epidermal growth factor.

6. A method of Claim 3 wherein the growth factor comprises an IGF-I.

7. A method of Claim 3 wherein the growth factor comprises an alpha-type transforming growth factor.

8. A method of Claim 1 wherein the substance has a paracrine mitogenic effect on said cells.

9. A method of Claim 1 wherein the substance comprises a somatotropin or placental lactogen.

10. A method of Claim 1 wherein the substance is administered during the mammal's last trimester of gestation.

11. A method of any one of Claims 1 to 10 wherein the mammal is a cow.

12. A method of any one of Claims 1 to 10 wherein the mammal is a sheep.

13. A method of any one of Claims 1 to 10 wherein the mammal is a goat.

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14. A composition adapted for administration to a ruminant by intramammary infusion, said composition comprising a physiologically acceptable carrier and an amount, effective to thereby enhance the growth of mammary parenchyma in said ruminant, of a substance having a mitogenic effect on mammary epithelial cells in said ruminant.

15. A composition of Claim 14 wherein the substance has a direct mitogenic effect on said cells.

16. A composition of Claim 14 or 15 wherein the substance comprises a polypeptide growth factor.

17. A composition of Claim 16 wherein the growth factor is selected from epidermal growth factors, insulin family growth factors, alpha-type transforming growth factors, and combinations thereof.

18. A composition of Claim 16 wherein the growth factor comprises an epidermal growth factor.

19. A composition of Claim 16 wherein the growth factor comprises an IGF-I.

20. A composition of Claim 16 wherein the growth factor comprises an alpha-type transforming growth factor.

21. A composition of Claim 14 wherein the substance has a paracrine mitogenic effect on said cell.

22. A composition of Claim 14 wherein the substance comprises a somatotropin or placental lactogen.

23. A composition of any one of Claims 14 to 22 wherein the carrier for the substance enhances diffusion of the substance through the infused mammary gland.

24. A composition of any one of Claims 14 to 22 wherein the carrier for the substance is liquid at the ruminant's body temperature.

25. A composition of any one of Claims 14 to 22 wherein the carrier for the substance is substantially water-soluble.

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26. A composition of any one of Claims 14 to 25 which is in a form suitable for administration to a cow in gestation or between about the onset of puberty and the cow's first gestation.

27. A composition of any one of Claims 14 to 25 which is in a form suitable for administration to a sheep in gestation or between about the onset of puberty and the sheep's first gestation.

28. A composition of any one of Claims 14 to 25 which is in a form suitable for administration to a goat in gestation or between about the onset of puberty and the goat's first gestation.

29. A unit dose of a composition of any one of Claims 14 to 28 containing between about 2 micrograms and about 100 milligrams of said substance.

30. A process for preparing a composition adapted for intramammary infusion to a ruminant to enhance the growth of mammary parenchyma in said ruminant, which comprises combining a substance having a mitogenic effect on mammary epithelial cells in said ruminant with a vehicle that enhances diffusion of said substance through the infused mammary gland of said ruminant.

31. A process of Claim 30, wherein said vehicle is substantially water-soluble and substantially liquid at the ruminant's body temperature.

32. A composition obtained by the process of Claim 30 or 31.

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