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(54) Title: NEW GDF-9 AND GDF-9B (BMP-15) SEQUENCES FOR ALTERING MAMMALIAN OVARIAN FUNCTION AND OVULATION RATE

(57) Abstract: The present invention provides compositions and methods for modulating the ovulation rate and therefore fertility in female mammals including humans. The invention also relates to novel mutations in the GDF-9 and GDF-9B genes which are associated with changes in fertility.



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NEW GDF-9 AND GDF-9B (BMP-15) SEQUENCES FOR ALTERING MAMMALIAN
OVARIAN FUNCTION AND OVULATION RATE

TECHNICAL FIELD

The present invention relates to new sequences for altering mammalian ovarian function
5 and ovulation rate.

In particular, the invention broadly concerns a novel mutation in the GDF-9 gene and two novel mutations in the GDF-9B gene. These mutations have been found to be involved in increasing the ovulation rate in heterozygous female mammals; or causing sterility in homozygous female mammals.

10 **BACKGROUND ART**

The genes GDF-9 and GDF-9B (also known as BMP15) code for proteins which are expressed exclusively in the oocyte of the developing follicle, and which play an essential role in mammalian fertility. GDF-9 is a member of the transforming growth factor beta (TGFB) superfamily (McPherron and Lee, 1993) which is expressed in
15 oocytes from the primary stage of follicular development until ovulation (McGrath *et al.*, 1995; Laitinen *et al.*, 1998). GDF-9B is closely related to GDF-9 (Dube *et al.*, 1998; Laitinen *et al.*, 1998) and is expressed in mouse oocytes at the same time as GDF-9, but in human primary follicles slightly later than GDF-9. In the ovary GDF-9 and GDF-9B have now been shown to be expressed exclusively in the developing oocyte in humans
20 (Aaltonen *et al.*, 1999), rodents (Laitinen *et al.*, 1998; Dube *et al.*, 1998; Jaatinen *et al.*, 1999), ruminants (Bodensteiner *et al.*, 1999; Bodensteiner *et al.*, 2000; Galloway *et al.*, 2000) and marsupials (Eckery *et al.*, 2002). In sheep expression of GDF-9 can be seen in primordial follicles whereas GDF-9B is expressed in primary follicles (Bodensteiner *et al.*, 1999; Galloway *et al.*, 2000).

GDF-9 is an essential growth factor for folliculogenesis in mice. Female GDF-9 knockout mice (GDF-9 $-/-$) are infertile due to a block in follicular development at the primary stage (Dong *et al.*, 1996). GDF-9B does not appear to be crucial for mouse folliculogenesis as knockout female mice (BMP15 $-/-$) are fertile (Yan *et al.*, 2001),
5 even though fecundity is somewhat reduced. However, GDF-9B is essential for folliculogenesis in sheep as those carrying two copies of naturally-occurring inactivating GDF-9B mutations are infertile due to a block in follicular development at the primary stage (Galloway *et al.*, 2000).

In sheep it is also clear that heterozygotes carrying inactivating mutations in one copy of
10 GDF-9B (whereby only one copy of the gene produces active protein) have an increased ovulation rate (Galloway *et al.*, 2000). A similar increase in ovulation rate in heterozygote mice with knockouts in either GDF-9 or GDF-9B has not been observed (Yan *et al.*, 2001). Double knockouts of both GDF-9 and GDF-9B in mice are infertile with a similar phenotype to GDF-9 $-/-$ mice alone, but GDF-9B knockout mice (BMP15
15 $-/-$) with one active copy of GDF-9, have a lower fecundity than BMP15 $-/-$ females (Yan *et al.*, 2001), suggesting that the relative dose of these gene products may also play a role in mice. Collectively these findings suggest that important differences exist in the actions of GDF-9 and GDF-9B between species with a high ovulation rate phenotype (e.g. mice, rats) and those with a low ovulation rate phenotype (e.g. sheep, humans).

20 GDF-9 maps to a region of sheep chromosome 5 (Sadighi *et al.*, 2002) which is syntenic to the map locations for GDF-9 on human chromosome 5 and mouse chromosome 11. GDF-9B maps to the sheep X chromosome (Galloway *et al.*, 2000) in a region of the chromosome syntenic to the map locations for GDF-9B on the human and mouse X chromosomes (Dube *et al.*, 1998; Aaltonen *et al.*, 1999).

GDF-9 and GDF-9B, like other members of the TGF β family, are coded as prepropeptides containing a signal peptide, a proregion and a C-terminal mature region which is the biologically active peptide. Cleavage of the mature region from the proregion is carried out by an intracellular furin-like protease, and occurs at a conserved
5 furin protease cleavage site. Members of the TGF β superfamily are biologically active as dimers, and although GDF-9 and GDF-9B do not contain the cysteine molecule responsible for covalent interchain disulphide bonding seen in other members of the family, these molecules are thought to be biologically active as dimers (Galloway *et al.*, 2000; Yan *et al.*, 2001). However it is unclear whether the physiologically active dimers
10 are homodimers (GDF-9-GDF-9 and GDF-9B-GDF-9B), or heterodimers (GDF-9-GDF-9B) or whether all three dimer forms play a role. It has been postulated based on the above models that GDF-9 homodimers play a more important role in the mouse but in sheep the GDF-9B homodimers are the most bioactive (Yan *et al.*, 2001). It is unclear whether any such difference is related to the fact that sheep are mono-ovulatory animals
15 (maturing usually only one egg per cycle) whereas mice are poly-ovulatory. Clearly both GDF-9 and GDF-9B play crucial roles in controlling and maintaining fertility in mammals, and understanding the nature of their actions is essential for the development of therapies.

GDF-9 and GDF-9B in sheep

20 The sheep GDF-9 gene spans about 2.5 kb and contains 2 exons separated by a single 1126 bp intron (Bodensteiner *et al.*, 1999). The full length coding sequence is 1359 nucleotides long and encodes a pre-propeptide of 453 amino acid residues (Genbank accession number AF078545). A pre-pro region of 318 residues includes a predicted signal sequence, and ends with the RHRR furin protease cleavage site at residues 315 –
25 318. Residues 319 to 453 beyond the cleavage site code for the 135 amino acid mature

active peptide. The amino acid sequence of the sheep GDF-9 mature peptide is 92.8 % similar to the human mature peptide and 87.1 % similar to the mouse mature peptide.

Sheep GDF-9B has previously been sequenced by us (Galloway *et al.* 2000; Genbank accession nos. AF236078, AF236079) and has a very similar gene structure to GDF-9.

- 5 The full length coding sequence of 1179 nucleotides is contained in two exons, separated by an intron of about 5.4 kb, and encodes a pre-propeptide of 393 amino acid residues. A pre-pro region of 268 residues includes a predicted signal sequence, and ends with the RRAR furin protease cleavage site at residues 265 – 268. Residues 269 to 393 beyond the cleavage site code for a 125 amino acid mature active peptide. The
- 10 amino acid sequence of the sheep GDF-9B mature peptide is 78.3 % similar to the human mature peptide and 78.6 % similar to the mouse mature peptide.

- We have previously shown that the effects on prolificacy in Inverdale and Hanna sheep is due to naturally-occurring mutations in GDF-9B (Galloway *et al.*, 2000). Both Inverdale and Hanna sheep have increased ovulation rates in heterozygous carriers of
- 15 mutated GDF-9B, but female homozygous carriers are infertile with ‘streak’ ovaries (Davis *et al.*, 2001). Infertility in these sheep is due to primary ovarian failure caused by the inability of the follicle to develop beyond the primary stage. Hanna sheep have a single C to T mutation at nucleotide 871 of the GDF-9B coding sequence (nucleotide 67 of the mature GDF-9B peptide coding region) which produces a premature stop codon
- 20 in the place of a glutamic acid (Q) at amino acid residue 291 (residue 23 of the mature protein). Inverdale sheep have a distinct T to A mutation at nucleotide 896 (nucleotide 92 of the mature GDF-9B peptide coding region) which substitutes valine (V) for aspartic acid (D) at residue 299 (residue 31 of the mature peptide). This substitution of a hydrophobic valine with a negatively charged aspartate changes the electrostatic surface
- 25 potentials of an area involved in dimer formation and appears to disrupt dimerisation and hence abolish biological activity (Galloway *et al.*, 2000).

In addition to the Inverdale and Hanna lines of sheep discussed above, the Cambridge and F700 Belclare strains of sheep have also been shown to carry genes affecting prolificacy as evidenced in high ovulation rate (Hanrahan, 1991) and the presence of sterile ewes with 'streak-like' ovaries (Hanrahan, 1991).

- 5 The Cambridge breed was established at the Cambridge University farm in 1964 by screening 54 ewes selected for their high prolificacy from nine British sheep breeds. Ewes within the screened flock were subsequently selected on high litter size. Ewes with the highest ovulation rates were selected from this flock in 1984 to provide the foundation animals for the flock now maintained at Teagasc Sheep Research Centre in
10 Ireland (Hanrahan, 1991). A progeny test of 10 Cambridge rams, descended from the flock in Ireland, gave progeny mean ovulation rates ranging from 2.1 - 4.2.

- The Belclare breed was established in 1978 at the Belclare Research Centre of Teagasc in Ireland by crossing three populations of prolific sheep assembled by Teagasc in Ireland. These were Fingalway, High Fertility, and Lleyn sheep (Hanrahan, 1991). The
15 Fingalway was an interbred cross (from F1) of the Finnish Landrace and Galway breeds; the Lleyn is a breed native to north west Wales and selected animals were imported into Ireland in 1975 by Teagasc for the purposed of developing the Belclare breed; the High Fertility was developed in Ireland during the 1960s from ewes with exceptional litter size performance collected from farms in Ireland between 1963 and 1965. A subline of
20 the Belclare (called F700 line) was derived from Belclare sheep that had exceptionally high ovulation rates (Hanrahan 1991). Progeny of 10 Belclare rams had mean ovulation rates ranging from 1.9 – 4.2.

- All references, including any patents or patent applications cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes
25 prior art. The discussion of the references states what their authors assert, and the

applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents form part of the common general knowledge in the art, in New Zealand or in any other country.

It is acknowledged that the term 'comprise' may, under varying jurisdictions, be attributed with either an exclusive or an inclusive meaning. For the purpose of this specification, and unless otherwise noted, the term 'comprise' shall have an inclusive meaning - i.e. that it will be taken to mean an inclusion of not only the listed components it directly references, but also other non-specified components or elements. This rationale will also be used when the term 'comprised' or 'comprising' is used in relation to one or more steps in a method or process.

It is desirable to provide new sequence mutations in the GDF-9B and GDF-9 genes linked to altered fertility rates.

It is an object of the present invention to go some way towards fulfilling this desideratum and/or provide the public with a useful choice.

Further aspects and advantages of the present invention will become apparent from the ensuing description which is given by way of example only.

SUMMARY OF INVENTION

The present invention is concerned with novel mutated GDF-9 and GDF-9B gene sequences which alter mammalian ovarian function and ovulation rate. The invention broadly has application in increasing or decreasing the ovulation rate, or causing sterility in a female mammal, and additionally encompasses regulation of the function of the corpus luteum.

In particular, the present invention concerns a novel mutation in GDF-9 which increases ovulation rate in heterozygotes and causes sterility in homozygotes for this gene.

The present invention also concerns two mutations in GDF-9B. Heterozygotes for either one of these mutations have an increased ovulation rate. Mammals which are
5 heterozygotes for both mutations in GDF-9B (where each mutation is on a separate X chromosome) are sterile.

Surprisingly, the inventors have also discerned that in female mammals that are heterozygous for the mutated GDF-9 gene and heterozygous for one of the GDF-9B gene mutations, an even higher ovulation rate exists than in mammals heterozygous for
10 one mutation in either GDF-9 or GDF-9B alone.

Knowledge of the mutated gene sequences can be applied to a test for identifying heterozygous or homozygous female and male mammals carrying the mutated GDF-9B and/or GDF-9 genes. This knowledge of the biological function of the genes and their mutations can also be utilised to increase or decrease the ovulation rate of female
15 mammals, or to induce sterility or reduced fertility in female mammals. In particular, an increase in ovulation rate in mammals may be induced by mimicking the heterozygous state, e.g. by reducing the biological activity of GDF-9 and/or GDF-9B by around 50%. This can be achieved by a partial or short-term active immunisation regime whereby wild-type GDF-9 and/or GDF-9B or a functional variant or fragment thereof is
20 administered to raise sufficient antibodies to partially neutralise endogenous GDF-9 and/or GDF-9B. Alternatively, said antibodies may be administered directly in a partial or short term passive immunisation regime.

A decrease in ovulation rate sufficient to reduce fertility or induce sterility may be induced by mimicking the homozygous state, e.g. by reducing the biological activity of
25 GDF-9 and/or GDF-9B to around zero. This can be achieved by a full or long term

active immunisation regime whereby wild-type GDF-9 and/or GDF-9B or a functional variant or fragment thereof is administered to raise sufficient antibodies to affectively neutralise all of the endogenous GDF-9 and/or GDF-9B. Alternatively, said antibodies may be administered directly in a full or long term passive immunisation regime. Where
5 the effect is permanent, sterility in the mammal is induced. Where the effect is reversible or temporary, a contraceptive effect is induced.

While the invention is broadly defined above, it will be appreciated by those persons skilled in the art that it is not limited thereto and includes embodiments of which the following descriptions give examples.

10 **BRIEF DESCRIPTION OF DRAWINGS**

Further aspects of the present invention will become apparent from the following description which is given by way of example only and with reference to the accompanying drawings in which:

15 **Figure 1a** shows the predicted amino acid sequence of sheep GDF9 protein. Numbers at the start of each line indicate amino acid positions of full-length unprocessed protein. Numbers in brackets indicate amino acid positions of the mature peptide. The RRHR furin protease cleavage site and predicted start of the mature processed peptide is shaded in grey.
20 The filled triangle indicates the position of the single intron within the gene. The open triangles indicate positions of mutations that confer amino acid substitutions but are not associated with the sterility phenotype. The position of the [787] mutation associated with sterility is shaded black;

Figure 1b shows nucleotide substitution of four GDF-9 sequence mutations which result in an amino acid change compared to the wild-type sequence in Irish Cambridge and F700 Belclare sheep;

Figure 2a shows the predicted amino acid sequence of sheep GDF9B protein. Numbers at the start of each line indicate amino acid positions of full-length unprocessed protein. Numbers in brackets indicate amino acid positions of the mature peptide. The RRAR furin protease cleavage site and predicted start of the mature processed peptide is shaded in grey. The filled triangle indicates the position of the single intron within the gene. The open triangle indicates the position of a single Leu deletion polymorphism. The position of the [S1] and [S2] mutations associated with sterility are shaded black;

Figure 2b shows nucleotide substitutions of two GDF-9B sequence mutations which result in an amino acid change compared to the wild-type sequence in Irish Cambridge and F700 Belclare sheep;

Figure 3a shows a table representing the genotype and phenotype of sire R830 mated to three ewes 9704, 8783 and 7810, and their six female offspring. The phenotype of each animal is indicated as sterile S, fertile F or n/a (not applicable) for the male. The genotype of each animal is shown as ++ (wild type female for X-linked [S1] and [S2] mutations, wild type male and female [787] autosomal mutation), +Y (wild type male for X-linked [S1] or [S2]). Double copy carriers of the mutation are T/T, S1/S1 or S2/S2. Single copy carriers are T/+, S1/+ or S2/+. Sire R830 is hemizygous for X-linked [S2] as he can only carry one copy;

Figure 3b shows a table representing the genotypes and phenotypes within a F700 Cambridge pedigree. The pedigree represents sire 962101 mated to two ewes 962158 and 976234, and their four female offspring, and sire 930142 mated to ewe 8874 and their one female offspring. The phenotype of each animal is indicated as sterile S, fertile F or n/a (not applicable) for the male. The genotype of each animal is shown as ++ (wild type female for X-linked [S1] and [S2] mutations, wild type male and female for [787] autosomal mutations), +Y (wild type male for X-linked [S1] or [S2]. Double copy carriers of the mutations are T/T or S1/S1. Single copy carriers are T/+ or S1/+. Sires are hemizygous for X-linked [S1] as they can only carry one copy.

Figure 4 shows the nucleotide and amino acid sequences of wildtype sheep GDF-9 showing the positions of mutations in Irish Cambridge and F700 Belclare sheep. Numbers on the right indicate nucleotide position from the atg start codon. Numbers under each line indicate amino acid residue numbering from the start of the mature processed active peptide. Negative numbers indicate amino acids in the pro-region of the protein. The position of the single intron is marked by white boxes inserted into the sequence. Positions of the eight nucleotide polymorphisms are marked in bold within boxes, and named according to Table 1 using square brackets []. The amino acids residues which are changed by the nucleotide polymorphisms are underlined. The taa stop codon indicates the end of the protein;

Figure 5 shows the nucleotide and amino acid sequences of sheep GDF-9B showing the positions of mutations in Irish Cambridge and F700 Belclare sheep. Numbers on the right indicate nucleotide position from the atg start codon. Numbers under each line indicate amino acid residue numbering from the start of the mature processed active peptide.

Negative numbers indicate amino acids in the pro-region of the protein. The position of the single intron is marked by a dashed inserted into the sequence. Positions of the four nucleotide polymorphisms are marked in bold within boxes, and named according to Table 1 using square brackets []. The amino acids residues which are changed by the nucleotide polymorphisms are underlined. Asterisks (***) indicate the positions of the previously reported Hanna (Gln to Stop codon) and Inverdale (Val to Asp codon) mutations. The tga stop codon indicates the end of the protein;

Figure 6

shows the alignment of GDF-9 and GDF-9B protein sequence with other members of the TGF β superfamily members for which structures have been determined. The furin processing site is indicated as a solid gray block at the start of the sequences. The mature processed protein begins at amino acid residue position 4. Conserved cysteine molecules involved in disulphide bonds are shown in grey shading. Numbers along the bottom provide a relative reference to amino acid position, but do not represent the real amino acid residue number of each protein because gaps have been introduced to allow alignment of conserved protein regions. The asterisk * indicates the conserved cysteine that is present in most other TGF β family members except GDF9 and GDF9B, and which is responsible for the interchain disulphide bond present in most dimers. Boxed letters indicate the [787] serine (S) in GDF9 which is changed to phenylalanine in the mutants (position 86 on this diagram), and the [S2] serine (S) in GDF9B which is changed to isoleucine (position 118 in this diagram);

Figure 7

shows examples of the pattern of progesterone concentrations in plasma of actively immunized ewes. Antigen used for immunization and the ewe identification numbers are shown at the top of each graph. Markings by

vasectomized rams are indicated with arrows. Day 0 = corresponds to the beginning of thrice weekly sampling period; and

Figure 8 shows the average concentrations of progesterone in plasma following synchronization of luteal regression. Ewes were administered 100 ml of KLH, GDF9 peptide or BMP15 peptide antiplasma i.v. 4 days before synchronization with Estrumate (i.e. PGF_{2α}, arrowed).

DETAILED DESCRIPTION OF INVENTION

The present invention is directed to new naturally-occurring mutations in the sheep GDF-9 and GDF-9B genes. It is shown for the first time that mutation of the GDF-9 gene causes increased ovulation rate as well as infertility in a manner similar to inactivating mutations in GDF-9B, and that GDF-9 is also essential for maintaining normal ovarian folliculogenesis in sheep. Furthermore, it is shown for the first time in any species, that sheep which are heterozygous for both GDF-9 and GDF-9B mutations have higher ovulation rates than sheep that are heterozygous for GDF-9 or GDF-9B mutations alone. These observations are supported by genotype, phenotype and immunisation data discussed below.

According to a first aspect of the present invention there is provided an isolated mutated GDF-9 nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- a) SEQ ID NOs. 1, 3 or 5;
- b) a sequence complementary to the molecule defined in a);
- c) a functional fragment or variant of the sequences in a) or b);
- d) an anti-sense sequence to any of the molecules defined in a), b) or c).

The present invention also provides an isolated mutated GDF-9 polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4 or 6, or a functional fragment or variant thereof.

5 Whilst eight polymorphisms were located in the GDF-9 nucleotide sequence of the Belclare and Cambridge genomic DNA, only four resulted in amino acid substitutions in the corresponding polypeptide. However, of these, only one amino acid substitution was considered likely to change the function of the encoded polypeptide and therefore be involved in the observed changes in fertility rate seen in these sheep. This [787] serine to phenylalanine substitution at residue 395 replaces an uncharged polar group
10 with a non-polar group at residue 77 of the mature coding region. Figures 1a, 1b, and 4, and Table 1 illustrate these nucleotide and amino acid changes.

Cambridge and Belclare ewes which were genotyped and found to be heterozygous for the GDF-9 [787] mutation were associated with a significant increase in ovulation rate over control ewes having none of the observed mutations (Table 3).

15 Cambridge and Belclare ewes which were genotyped and found to be homozygous for the GDF-9 [787] mutation were sterile.

The discovery of an inactivating mutation in GDF-9 associated with infertility and increased ovulation rate in sheep is the first evidence that GDF-9 is important for
20 increasing ovulation rate. Although a knockout mutation of GDF-9 in mice has been shown to cause infertility, no effects for GDF-9 on increasing ovulation rate have been described. The present invention shows that small perturbations of protein structure within the GDF-9 mature peptide have severe consequences on protein activity.

25 The present invention also provides an isolated mutated GDF-9B nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- a) SEQ ID NOs. 7, 9, 11, 13, 15 or 17;
- b) a sequence complementary to the molecule defined in a)
- c) an anti-sense sequence to any of the molecules defined in a) or b).

The present invention further provides an isolated mutated GDF-9B polypeptide
5 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.
8, 10, 12, 14, 16 or 18.

Four polymorphisms were located in the GDF-9B nucleotide sequence of the Belclare
and Cambridge genomic DNA. Two of these polymorphisms resulted in amino acid
substitutions in the corresponding polypeptide sequence which were considered to
10 change the polypeptide function and be involved in the observed changes in fertility
rates seen in these sheep. The [S1] polymorphism resulted in C to T change at
nucleotide 718 and introduced a premature stop codon (TAG) in the place of glutamic
acid (Q, CAG) at amino acid residue 239 which presumably resulted in a complete loss
of GDF-9B function. The [S2] polymorphism resulted in a serine to isoleucine
15 substitution at residue 367 of the unprocessed protein replacing an uncharged polar
group with a non polar group. Figures 2 and 5 and Table 1 illustrate these nucleotide
and amino acid changes.

Cambridge and Belclare ewes which were genotyped and found to be heterozygous for
the GDF-9B [S1] or [S2] mutation were associated with a significant increase in
20 ovulation rate over control ewes having none of the observed mutations (Table 3).

Cambridge and Belclare ewes which were genotyped and found to be homozygous for
the GDF-9B [S1] or [S2] mutations were sterile.

These two new functional mutations in GDF-9B support the findings of previous
studies, that mutations in this gene in sheep are associated with fertility control

(Galloway *et al.*, 2000). Inactivating mutations in GDF-9B cause increased ovulation rate and infertility in a dosage dependent manner. The serine to isoleucine change in the [S2] mutation and premature stop codon in the [S1] mutation, support the notion that perturbations of protein structure within the GDF-9B mature peptide have serious consequences in protein activity.

Surprisingly, ewes which are heterozygous for both of the GDF-9 [787] mutation and one of the GDF-9B [S1] or [S2] mutations have an even higher ovulation rate than animals that are heterozygous for a GDF-9 or GDF-9B mutation alone. The effects of the combination of GDF-9 [787] and GDF-9B [S1] mutations in Cambridge sheep and the combination of GDF-9 [787] and GDF-9B [S2] mutation in Belclare sheep appears to be additive (Table 3).

Thus, it is contemplated that an immunisation regime which could mimic these genotypes would be useful in modulating ovulation in female mammals. For example, a regime which would reduce the activity of endogenous GDF-9B and/or GDF-9 to about one half (as in heterozygous animals whereby only 50% of active molecules are expressed) could be used to increase ovulation and enhance fertility in female mammals. Conversely, an immunisation regime which would reduce the activity of endogenous GDF-9 and/or GDF-9B to approximately zero (as in homozygous animals where no active molecules are expressed) could be used to induce sterility.

The present invention further provides an isolated GDF-9 nucleic acid molecule comprising a mutation in at least one codon associated with receptor binding and/or dimerisation. The mutation preferably results in an amino acid substitution in the polypeptide encoded by the nucleic acid molecule, and said amino acid substitution is preferably present in the receptor binding domain and causes a disruption in receptor

binding. Alternatively, the amino acid substitution may be present in the dimersation domain to cause a disruption in dimerisation.

The invention further provides an isolated GDF-9B nucleic acid molecule comprising a mutation in at least one codon associated with receptor binding and/or dimerisation. The
5 mutation preferably results in an amino acid substitution in the polypeptide encoded by the nucleic acid molecule, and said amino acid substitution is preferably present in the receptor binding domain and causes a disruption in receptor binding. Alternatively, the amino acid substitution may be present in the dimerisation domain to cause disruption in dimerisation.

10 Suitable programs for ascertaining the structure of polypeptides from the amino acid sequence which can be used to determine the regions of the nucleotide sequence associated with dimerisation and/or receptor binding will be known to persons skilled in the art. Examples of suitable computer programs include The Modeller by Rockerfeller University and The SWISS Model developed by Swiss Protein database.

15 The mutations seen in the GDF-9 and GDF-9B genes which are associated with changes in fertility in the Cambridge and Belclare breeds have been shown to be associated with alterations in the function of the encoded polypeptides due to amino acid substitutions. Comparison between the location of these amino acid substitutions, with mutations, in other closely related TGF- β molecules support the hypothesis that the biological activity
20 of GDF-9 [787] is abolished due to a disruption in dimerisation whilst the GDF-9B [S2] mutation may abolish biological activity by disrupting receptor binding.

It is anticipated that other amino acid changes in the receptor-binding and dimerisation domains, or regions of the protein that disrupt protein folding of the mature peptide will have similar effects as would be appreciated by a skilled person and are included within
25 the scope of the present invention.

The present invention also provides a method of identifying a mammal which carries a mutated nucleic acid molecule encoding GDF-9B, said method comprising the steps of:

- i) obtaining a tissue or blood sample from the mammal;
- ii) isolating DNA from the sample; and optionally
- 5 iii) isolating GDF-9B DNA from the DNA obtained at step i) or ii);
- iv) probing said DNA with a probe complementary to either strand of the mutated GDF-9B DNA of SEQ ID NOs 11 or 17;
- v) amplifying the amount of mutated GDF-9B DNA;
- vi) determining whether the GDF-9B sequence DNA obtained in step v) carries a
10 mutation associated with sterility or increased ovulation.

The present invention further provides a method of identifying a mammal which carries a mutated nucleic acid molecule encoding GDF-9, said method comprising the steps of:

- i) obtaining a tissue or blood sample from the mammal;
- ii) isolating DNA from the sample; and optionally
- 15 iii) isolating GDF-9 DNA from the DNA obtained at step i) or ii);
- iv) probing said DNA with a probe complementary to either strand of the mutated GDF-9 DNA of SEQ ID NO 5;
- v) amplifying the amount of mutated GDF-9 DNA;
- vi) determining whether the GDF-9 sequence DNA obtained in step v) carries a
20 mutation associated with sterility or increased ovulation.

The probe and primers that can be used in this method also forms a part of this invention. Said probes and primers may comprise a fragment of the nucleic acid molecule of the invention capable of hybridising under stringent conditions to a mutated GDF-9 or GDF-9B gene sequence. Such probes and primers are also useful, in studying
5 the structure and function of the mutated genes, and for obtaining homologues of the genes from mammals other than sheep expressing the Cambridge and Belclare phenotypes.

Nucleic acid probes and primers can be prepared based on nucleic acids according to the present invention or sequences complementary thereto. A "probe" comprises a single
10 stranded nucleic acid molecule having a known sequence which is attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. Such probes are used to locate and mark target DNA or RNA sequence by hybridizing to it.

A "primer" is a short nucleic acid, preferably DNA, 15 or more nucleotides in length,
15 which are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, preferably a DNA polymerase. Primer pairs can be used for amplification of a nucleic acid sequence, eg by the polymerase chain reaction (PCR) or other nucleic acid amplification methods well known in the art. PCR-primer
20 pairs can be derived from the sequence of a nucleic acid according to the present invention, for example, by using computer programs intended for that purpose such as Primer (Version 0.5[©] 1991, Whitehead Institute for Biomedical Research, Cambridge, MA).

Methods for preparing and using probes and primers are described, for example, in
25 Sambrook et al. Molecular Cloning: A Laboratory Manual, 2nd ed, vol. 1-3, ed Sambrook et al. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY, 1989.

Probes or primers can be free in solution or covalently or noncovalently attached to a solid support by standard means.

For the amplification of a target nucleic acid sequence (eg by PCR) using a particular amplification primer pair, stringent conditions are conditions that permit the primer pair to hybridise only to the target nucleic acid sequence to which a primer having the corresponding wild type sequence (or its complement) would bind.

- 5 Nucleic acid hybridization is affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridising nucleic acids, as will be readily appreciated by those skilled in the art.

- 10 When referring to a probe or primer, the term “specific for (a target sequence)” indicates that the probe or primer hybridises under stringent conditions only to the target sequence in a given sample comprising the target sequence. Commonly, stringent hybridization conditions are 6 x SSC at 55°C.

- In another embodiment, the present invention provides the use of a nucleic acid molecule which is complementary to either strand of the mutated DNA of SEQ ID NOs.
15 11 or 17 as a marker to identify a mammal carrying a mutated nucleic acid molecule encoding GDF-9B.

The term ‘either strand’ refers to both the strand of DNA shown in the Sequence ID Number that is being referred to or its complementary strand which is not shown in the sequence listing but which can be determined therefrom.

- 20 In a further embodiment, the present invention provides the use of a marker as described above in a method of DNA marker assisted selection of mammals carrying mutated GDF-9B associated with either enhanced ovulation or sterility.

In another embodiment, the present invention provides the use of a nucleic acid molecule which is complementary to either strand of the mutated DNA of SEQ ID NO 5

as a marker to identify a mammal carrying a mutated nucleic acid molecule encoding GDF-9.

The present invention further provides the use of a marker as described above in a method of DNA assisted selection of mammals carrying mutated GDF-9 either
5 enhanced ovulation or sterility.

The above markers and methods of marker assisted selection are useful to identify sequence variants in individual animals that are associated with increased ovulation of that animal. Although these variants may not necessarily give rise to the increased ovulation or sterility trait directly, they will be sufficiently closely associated with it to
10 predict the trait. The methods by which these sequence variants are identified are known in the art, and include, but are not limited to, restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism AFLP, direct sequencing of DNA within or associated with the GDF-9 gene, or identification and characterisation of variable number of tandem repeats (VNTR), also known as
15 microsatellite polymorphisms. Thus, the genetic marker may have utility in DNA selection of animals having increased ovulation.

In a further embodiment, the present invention provides a construct or vector comprising a nucleic acid molecule substantially as described above.

The term "construct" as used herein refers to an artificially assembled or isolated
20 nucleic acid molecule which includes the gene of interest. In general a construct may include the gene or genes of interest and appropriate regulatory sequences. It should be appreciated that the inclusion of regulatory sequences in a construct is optional for example, such sequences may not be required in situations where the regulatory sequences of a host cell are to be used. The term construct includes vectors but should
25 not be seen as being limited thereto.

The term “vector” as used herein encompasses both cloning and expression vectors. Vectors are often recombinant molecules containing nucleic acid molecules from several sources.

- 5 A “cloning vector” refers to a nucleic acid molecule originating or derived from a virus, a plasmid or a cell of a higher organism into which another exogenous (foreign) nucleic acid molecule of interest, of appropriate size can be integrated without loss of the vector’s capacity for self-replication. Thus vectors can be used to introduce at least one foreign nucleic acid molecule of interest (e.g. gene of interest) into host cells, where the gene can be reproduced in large quantities.
- 10 An “expression vector” refers to a cloning vector which also contains the necessary regulatory sequences to allow for transcription and translation of the integrated gene of interest, so that the gene product of the gene can be expressed.

The cloning vector may be selected according to the host or host cell to be used. Useful vectors will generally have the following characteristics:

- 15 (a) the ability to self-replicate;
- (b) the possession of a single target for any particular restriction endonuclease; and
- (c) desirably, carry genes for a readily selectable marker such as antibiotic resistance.

- 20 Two major types of vector possessing these characteristics are plasmids and bacterial viruses (bacteriophages or phages). Presently preferred vectors are bacterial, insect or mammalian vectors and may include the following: the pUC, pBlueScript, pGEM, PGEX, pBK-CMV, lambda ZAP, lambda GEM, pEFIREP, pUB6/V5/His, pBC1, pADTrack-CMV, pAdenovator, pAdEasy-1, pSFV-PD, pCA3, pBABE, pPIC9,

pA0815, pET and pSP series. However, this list should not be seen as limiting the scope of the present invention.

Examples of preferred expression systems are as follows:

1. For an *in vitro* cell expression system, the 293T cell system with a pEFIRE5-P
5 vector (Hobbs S *et al.*, 1998, Biochem Biophys Res Commun. 252: 368-72)
which confers puromycin resistance may be used. For coexpression of two
genes, the aforementioned vector may be modified to change the antibiotic
resistance gene to bleomycin resistance. Alternatively, the co-expression of two
genes and the selection gene can be achieved by constructing a tricistronic
10 expression vector. A corresponding stably transfected insect cell system can
also be used, e.g. the S2 cell system using "DES" vector expression system;
www.invitrogen.com.
2. With respect to expressing GDF's in all tissues of transgenic animals, one
approach is to use the pUB6/V5-His A vector (www.invitrogen.com) to make
15 the constructs. For tissue-specific expression the rat PEPCK 0.6 kb promoter
for liver and kidney expression can be included in the construct by replacing the
Ubi-C promoter in the pUB6/V5-His A vector with the PEPCK promoter. For
GDF expression in mammary tissue another promoter system would be
preferred. For this tissue one approach would be to use the bovine β -
20 lactoglobulin gene promoter and/or the bovine α S1 casein promoter (e.g. pBC1
vector, www.invitrogen.com) to drive the expression of the GDFs into milk.
For global over-expression in transgenic animals, the CMV enhanced β -actin

promoter (Okabe M, et al.; FEBS Letters 407: 313-319, 1997) or a modified EF1 α -promoter can be used also (Taboit-Dameron F, et al., Transgenic Research 8: 223-235, 1998).

Adenoviruses, retroviruses and alphaviruses are other suitable mammalian expression systems. A typical approach to those skilled in the art is that described by (TC He *et al.*, 1998, Proc Natl Acad Sci USA. 95: 2509-14). With respect to GDF expression the pAd Track-CMV vector or pAdenovator vectors (www.qbiogene.com) can be used to make the construct which is then co-transformed with pAd Easy-1 adenoviral plasmid into *E. coli* to generate a recombinant adenoviral genome which contains a CMV-promoter driven GDF expression cassette. This recombinant adenoviral genome is then transfected into 293T cells to make the virus stock. Alternative methods for generating adenoviruses can also be used for the same purpose (e.g. PCA3 plasmid based gene transfer (www.microbix.com); or COS-TPC method (Miyake S *et al.*, 1996, Proc Natl Acad Sci USA. 93: 1320-4).

3. Non-cytopathogenic Semliki Forest viruses expressing GDF's can be generated using, for example, pSFV-PD vectors as described by Lundstrom et al., Histochem Cell Biol 115: 83-91, 2001. Furthermore, retroviral expression systems based on, for example, pBABE vectors, can be used for expressing GDF's in mammalian cells (Morgenstern, JP and Land, H, 1990; Nucleic Acids Res 18: 3587-3596).

4. Yeast cells (e.g. *Pichia pastoris*, *Saccharomyces cerevisiae*) are another well established expression system to those skilled in the art (MA Romanos *et al.*,

1992, Yeast. 8: 423-88). For example, the pPIC9 vector (www.invitrogen.com) can be used in *Pichia pastoris* for the expression of GDF's. For coexpression of two genes, the vector pA0815 (www.invitrogen.com) is a preferred candidate.

5. *Echerichia coli* (*E. coli*) is a standard laboratory expression system in
5 widespread use. For example, the pET expression system (www.novagen.com) can be used to express recombinant mammalian GDF-9 and GDF-9B

The DNA molecules of the invention may be expressed by placing them in operable linkage with suitable control sequences in a replicable expression vector. Control sequences may include origins of replication, a promoter, enhancer and transcriptional
10 terminator sequences amongst others. The selection of the control sequence to be included in the expression vector is dependent on the type of host or host cell intended to be used for expressing the DNA as would be understood by a person skilled in the art.

The term "operably linked" or grammatical variant thereof as used herein means that the regulatory sequences necessary for expression of the gene of interest are placed in the
15 nucleic acid molecule in the appropriate positions relative to the gene to enable expression of the gene.

As used herein the term "regulatory sequences" refers to certain nucleic acid sequences such as origins of replication, promoters, enhancers, polyadenylation signals, terminators and the like, that enable expression of the nucleic acid molecule of interest.

20 The term "expression" as used herein broadly refers to the process by which a nucleic acid molecule is converted by transcription and then translation into a protein.

The term "gene" as used herein refers to a nucleic acid molecule comprising an ordered series of nucleotides that encodes a gene product (i.e. specific protein). The expression vectors useful in the present invention may contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed.

- 5 The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the glycolytic promoters of yeast acid phosphatase, e.g. Pho5, the promoters of the yeast alpha-mating factors, and promoters
- 10 derived from polyoma, adenovirus, retrovirus, and simian virus, and cytomegalovirus e.g. the early and late promoters of SV40, and other sequences known to control the expression of genes of prokaryotic and eukaryotic cells and their viruses or combinations thereof.
- 15 In the construction of a vector it is also an advantage to be able to identify the bacterial clone carrying the vector incorporating the foreign DNA. Such assays include measurable colour changes, antibiotic resistance and the like. In one preferred vector, the β -galactosidase gene is used, in which clones are detectable as blue or white phenotypes on X-gal plates. This facilitates selection. Once selected, the vectors may
- 20 be isolated from the culture using standard procedures.

Depending on the host used, transformation and transfection is performed according to standard techniques appropriate to such cells. For prokaryotes or other cells that contain substantial cell walls, the calcium treatment process (Cohen, S N Proceedings, National Academy of Science, USA 69 2110 (1972)) may be employed. For

25 mammalian cells without such cell walls, the calcium phosphate precipitation method of Graeme and Van Der Eb, Virology 52:546 (1978) or liposomal reagents are preferred.

Upon transformation of the selected host with an appropriate vector the polypeptide encoded can be produced, often in the form of a fusion protein, by culturing the host cells. The polypeptide of the invention may be detected by rapid assays as indicated above. The polypeptide is then recovered and purified as necessary. Recovery and
5 purification can be achieved using any procedures known in the art, for example by absorption onto and elution from an anion exchange resin. This method of producing a polypeptide of the invention constitutes a further aspect of the present invention.

Host cells transformed with the vectors or constructs of the invention also form a further aspect of the present invention.

- 10 The term "host cell" refers to a cell which is capable of containing a vector or construct and supports the replication and/or expression of the vector or construct. Suitable hosts cells may include *E.coli*, yeast or mammalian cells but should not be limited thereto.

The present invention also provides a cell line comprising a host cell substantially as described above.

- 15 Knowledge of the mutated gene sequences can be applied to a test for identifying heterozygous or homozygous female and male mammals carrying the mutated GDF-9B and/or GDF-9 genes. This knowledge of the biological function of the genes and their mutations can also be utilised to increase or decrease the ovulation rate of female mammals, or to induce sterility or reduced fertility in female mammals. In particular, an
20 increase in ovulation rate in mammals may be induced by mimicking the heterozygous state whereby only half of the 'normal' amount of GDF-9 and/or GDF-9B is expressed. This can be achieved by a partial or short-term active regime whereby wild-type GDF-9 and/or GDF-9B or a functional variant or fragment thereof is administered to raise sufficient antibodies to partially neutralise endogenous GDF-9 and/or GDF-9B.

Alternatively, said antibodies may be administered directly in a partial or short term passive immunisation regime.

A decrease in ovulation rate sufficient to reduce fertility or induce sterility may be induced by mimicking the homozygous state, whereby no active GDF-9 and/or GDF-9B is expressed. This can be achieved by a full or long term active immunisation regime whereby wild-type GDF-9 and/or GDF-9B or a functional variant or fragment thereof is administered to raise sufficient antibodies to affectively neutralise all of the endogenous GDF-9 and/or GDF-9B. Alternatively, said antibodies may be administered directly in a full or long term passive immunisation regime. Where the effect is permanent, sterility in the mammal is induced. Where the effect is reversible or temporary, a contraceptive effect is induced.

Thus in a further aspect, the present invention provides a method altering GDF-9 and/or GDF-9B bioactivity in a female mammal so as to modulate ovulation comprising the steps of either:

- 15 (a) inducing a partial immunisation response to endogenous GDF-9 and/or GDF-9B to partially reduce bioactivity thereof and enhance ovulation; or
- (b) inducing a full immunisation response to endogenous GDF-9 and/or GDF-9B to substantially reduce bioactivity thereof and induce sterility.

The immunisation response may be induced by administration of an antigenic composition comprising:

- i) a GDF-9 polypeptide or a functional fragment or variant of GDF9; and/or
- ii) a GDF-9B polypeptide or a functional fragment or variant of GDF-9B;

together with a pharmaceutically or veterinarily acceptable carrier and/or diluent to a mammal in need thereof.

The antigenic composition may include an adjuvant to induce a partial immunisation response and enhance ovulation.

- 5 An example of such an adjuvant includes DEAE-Dextran adjuvant.

Adjuvants such as DEAE-Dextran are selected so as to provoke a partial immune response upon administration to a mammal.

The antigenic composition may comprise an alternative adjuvant to induce a strong immunisation response and reduce fertility or induce sterility.

- 10 Examples of such an adjuvant include Freund's adjuvant.

Adjuvants such as Freund's are selected so as to provoke a strong immune response upon administration to a mammal.

- Partial or strong immunisation may be induced either actively by the administration of the aforementioned antigenic compositions, or passively, by administration of antibodies raised against said antigenic composition or anti-sera comprising said antibodies.
- 15

Partial immunisation may also be induced by a short term administration regime, whereby the antigenic composition or antibodies thereto are administered over a short time period such as one to two months.

- 20 Full immunisation may be induced by a long term administration regime, whereby the antigenic composition or antibodies therein are administered over a long time period such as six months or more.

The type of adjuvant, antigenic composition, and administration regime are selected to induce the desired response as would be understood by a skilled worker. A particularly desired response is an enhanced ovulation rate and associated increased fertility. Such a method is useful to enhance the reproductive efficiencies and/or enhance multiple
5 ovulations from high value mammals.

A further desired response is a decrease in ovulation rate and associated reduced fertility. Preferably such a response is permanent and results in sterility of the female mammal. This method provides an alternative to surgical methods that are presently used to induce sterility and is much less invasive and carries no risk of infection etc as
10 is the case with surgery.

When the response induced is temporary or reversible, the method may be used as a method of contraception. Again such a method provides advantages over the currently used methods of contraception (i.e. administration of progesterone and/or oestrogen) which are associated with health risks. In addition, the method of the present invention
15 may be used to prevent ovulation in a female mammal, such as a race horse, greyhound etc, when the natural oestrous cycle would interfere with the performance of such a mammal. As such an effect is temporary or reversible, the mammal will not suffer any deleterious effects.

Such a temporary/reversible effect has been observed in sheep following administration
20 of GDF-9B peptide over a period of time sufficient to inhibit ovulation, which upon cessation of administration, subsequently became pregnant.

The present invention further contemplates inducing a desired response by administration of the mutated GDF-9B polypeptide molecules of the invention. Although such polypeptide molecules are likely to be inactive as they comprise a
25 mutation in the receptor binding and/or dimerisation domains, such polypeptides, when

administered in sufficient amounts, may compete with the endogenous GDF-9 and/or GDF-9B binding and/or dimerisation to reduce the biological activity thereof. Such a response, if it results in a partial reduction of endogenous GDF-9 and/or GDF-9B activity will result in enhanced ovulation and fertility. If such administration results in a full reduction of endogenous GDF-9 and/or GDF-9B activity, the response induced will be sterility.

Also encompassed by the present invention is a transgenic non-human mammal wherein one copy of the endogenous GDF-9 and/or GDF-9B gene has been knocked out. Such a mammal would have increased ovulation and enhanced fertility.

Such a transgenic mammal may be produced by known methods (Wells et al, 1998. *Reprod Fertil Dev*: 10:615-26; Clark 2002, *Methods Mol Biol*, 180: 273-87; Cousens et al 1994, *Mol Reprod Dev*. 39:384-91; Chen et al 2002, *Biol Reprod*. 67: 1488-92; Arat et al 2001, *Mol Reprod Dev*. 60: 20-6) and may comprise the steps of introducing to the genetic material of the mammal at least one nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- SEQ ID NOs 1 or a functional fragment or variant thereof; and
- SEQ ID NOs 7 or 13 but not both; or
- SEQ ID NOs 3 or a functional fragment or variant thereof; and
- SEQ ID NOs 9 or 15 but not both, using a vector or construct according to the invention.

In a further aspect the present invention provides a method of modulating the ovulation rate of a female mammal comprising the steps of:

a) identifying the nucleotide sequences of GDF-9 or GDF-9B carried by the female mammal;

b) administering as appropriate having regard to the GDF-9 and/or GDF-9B genes present in the mammal, an effective amount of an agent selected from the group consisting of:

5

1) an immunising effective amount of a GDF-9 polypeptide and/or an immunising effective amount of a GDF-9B polypeptide substantially as described above;

2) antisense nucleic acid molecule(s) directed towards nucleic acid(s) encoding:

10

i) a GDF-9 polypeptide substantially as described above; and/or

ii) a GDF-9B polypeptide substantially as described above.

In a further embodiment, the present invention provides a method for breeding a mammal having increased ovulation comprising the steps of:

15 a) identifying the nucleotide sequences of GDF-9 or GDF-9B carried by the female mammal it is proposed to breed from;

b) identifying the nucleotide sequences of GDF-9 or GDF-9B carried by the male mammal it is proposed to breed from;

c) selecting the female and male animals that will result in progeny having the following characteristics:

20

i) a single copy of a mutated GDF-9 nucleotide sequence comprising:

- A) SEQ ID NO 5; or
 - B) a functional variant or fragment of the molecule in A); or
 - C) a sequence complementary to the molecule in A) or B); and/or
- ii) a single copy of mutated GDF-9B nucleotide sequence comprising:
- 5 A) SEQ ID NOs 11 or 17; or
- B) a sequence complementary to the molecule(s) in A).

The mammals selected for breeding according to the method described above may result in progeny having the following characteristics:

- i) a single copy of a mutated GDF-9 nucleotide sequence comprising:
- 10 A) SEQ ID NO 5; or
- B) a functional variant or fragment of the molecule in A); or
- C) a sequence complementary to the molecule in A) or B);
- ii) a single copy of a mutated GDF-9B nucleotide sequence comprising:
- A) SEQ ID NOs 11 or 17; or
- 15 B) a sequence complementary to the molecule(s) in A).

In a still further embodiment, the present invention provides a method for selecting a female mammal for breeding on the basis of possessing an increased rate of ovulation comprising the steps of identifying a female mammal possessing only a single mutated copy of:

- 1) a mutated GDF-9 nucleotide sequence comprising:
- a) SEQ ID NO 5; or
 - b) a functional variant of the molecule of a); or
 - c) a sequence complementary to the molecules in a) or b);
- 5 and/or
- 2) a mutated GDF-9B nucleotide sequence comprising:
- a) SEQ ID NOs 11 or 17; or
 - b) a sequence complementary to the molecules in a).

Preferably the mammal selected has both a single mutated copy of GDF-9 and GDF-9B.

- 10 In a further embodiment, the present invention provides a composition comprising:
- i) a mutated GDF-9 polypeptide comprising an amino acid sequence selected from the group consisting of:
 - A) SEQ ID NOs. 2, 4 or 6; or
 - B) a functional fragment or variant of the sequences in A); and/or
 - 15 ii) a mutated GDF-9B polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 8, 10,12,14,16 or 18

together with a pharmaceutically or veterinarily acceptable carrier and/or diluent.

The preparation of pharmaceutical compositions including pharmaceutical carriers are well known in the art, and are set out in textbooks such as Remington's Pharmaceutical

- 20 Sciences, 19th Edition, Mack Publishing Company, Easton, Pennsylvania, USA.

The compounds and compositions of the invention may be administered by any suitable route, and the person skilled in the art will readily be able to determine the most suitable route and dose for the condition to be treated. Dosage will be at the discretion of the attendant physician or veterinarian, and will depend on the nature and state of the
5 condition to be treated, the age and general state of health of the subject to be treated, the route of administration, and any previous treatment which may have been administered.

The carrier or diluent, and other excipients, will depend on the route of administration, and again the person skilled in the art will readily be able to determine the most suitable
10 formulation for each particular case.

As mentioned above, the polypeptide of the present invention may be administered directly to a female mammal.

The term "protein, or polypeptide" as used herein refers to a protein encoded by the nucleic acid molecule of the invention, including fragments, mutations and homologues
15 having the same biological activity i.e. ovulation modulating activity. The protein or polypeptide of the invention can be isolated from a natural source, produced by the expression of a recombinant nucleic acid molecule, or chemically synthesized.

It is to be clearly understood that the invention also encompasses peptide analogues, which include but are not limited to the following:

- 20 1. Compounds in which one or more amino acids is replaced by its corresponding D-amino acid. The skilled person will be aware that retro-inverso amino acid sequences can be synthesised by standard methods; see for example Choreo and Goodman, 1993;

2. Peptidomimetic compounds, in which the peptide bond is replaced by a structure more resistant to metabolic degradation. See for example Olson et al, 1993; and
3. Compounds in which individual amino acids are replaced by analogous structures for example, gem-diaminoalkyl groups or alkylmalonyl groups, with or without
5 modified termini or alkyl, acyl or amine substitutions to modify their charge.

The use of such alternative structures can provide significantly longer half-life in the body, since they are more resistant to breakdown under physiological conditions.

Methods for combinatorial synthesis of peptide analogues and for screening of peptides and peptide analogues are well known in the art (see for example Gallop et al, 1997,
10 Nature Biotechnology, 15: 328-330).

For the purposes of this specification, the term "peptide and peptide analogue" includes compounds made up of units which have an amino and carboxy terminus separated in a 1, 2, 1, 3, 1, 4 or larger substitution pattern. This includes the 20 naturally-occurring or "common" α -amino acids, in either the L or D configuration, the biosynthetically-
15 available or "uncommon" amino acids not usually found in proteins, such as 4-hydroxyproline, 5-hydroxylysine, citrulline and ornithine; synthetically-derived α -amino acids, such as α -methylalanine, norleucine, norvaline, C α - and N-alkylated amino acids, homocysteine, and homoserine; and many others as known in the art.

It also includes compounds that have an amine and carboxyl functional group separated
20 in a 1,3 or larger substitution pattern, such as β -alanine, γ -amino butyric acid, Freidinger lactam the bicyclic dipeptide (BTD) (Freidinger *et al*, 1982, J. Org. Chem 59: 104-109; Nagai and Sato, 1985, Tetrahedron Lett. 26: 647-650), amino-methyl benzoic acid, and others well known in the art. Statine-like isosteres, hydroxyethylene isosteres, reduced amide bond isosteres, thioamide isosteres, urea isosteres, carbamate

isosteres, thioether isosteres, vinyl isosteres and other amide bond isosteres known to the art are also useful for the purposes of the invention.

A “common” amino acid is a L-amino acid selected from the group consisting of glycine, leucine, isoleucine, valine, alanine, phenylalanine, tyrosine, tryptophan, 5 aspartate, asparagine, glutamate, glutamine, cysteine, methionine, arginine, lysine, proline, serine, threonine and histidine. These are referred to herein by their conventional three-letter or one-letter abbreviations.

An “uncommon” amino acid includes, but is not restricted to, one selected from the group consisting of D-amino acids, homo-amino acids, N-alkyl amino acids, 10 dehydroamino acids, aromatic amino acids (other than phenylalanine, tyrosine and tryptophan), ortho-, meta- or para-aminobenzoic acid, ornithine, citrulline, norleucine, α -glutamic acid, aminobutyric acid (Abu), and α - α disubstituted amino acids.

In a further embodiment, the present invention provides a method of modifying the function of the corpus luteum by administering supplementary GDF-9 or GDF-9 B, or 15 analogues thereof, or GDF-9 or GDF-9B antagonists to female mammals.

The present invention also encompasses ligands directed to the polypeptides of the invention.

The term “ligand” refers to any molecule which can bind to another molecule such as a polypeptides or peptide, and should be taken to include, but not be limited to, 20 antibodies, cell surface receptors or phage display molecules.

It should be appreciated that the term “antibody” encompasses fragments or analogues of antibodies which retain the ability to bind to a polypeptide of the invention, including but not limited to Fr, F (ab)₂ fragments, ScFv molecules and the like. The antibody may

be polyclonal but is preferably monoclonal. In some embodiments the ligand may be a phage display molecule.

The invention also includes adenovirus-based gene therapy techniques for expressing GDF-9B and GDF-9/GDF-9B in cell cultures, organ cultures and whole experimental
5 animals for manipulating ovarian follicular protein synthesis or production.

Definitions

Throughout this specification it should be understood that the nucleic acid molecule may be a RNA, cRNA, genomic DNA or cDNA molecule, and may be single or double-stranded. The nucleic acid molecule may also optionally comprise one or more
10 synthetic non-natural or altered nucleotide bases, or combinations thereof.

The term “analogues” above refers to a compound which has a biological function with improved characteristics over the native compounds (e.g. such an analogue may have a longer half-life than the native compound.)

The term “antagonist” refers to a compound which inhibits the effect of another
15 compound. In this context, the antagonist could refer to a purified antibody, a sera or serum containing an antibody or a plasma or pool of plasma containing an antibody that would neutralise GDF-9 or GDF-9B.

The term “partial immunisation” refers to immunisation of an animal either active or passive of sufficient antigen/antibody to allow for instigation of an immune response to
20 be mounted against the antigen; but the degree of antigen/antibody administered and/or the means of administration are such that insufficient antibodies are produced by the immunised animal to effectively neutralise all the antigen of interest.

The term “full immune response” refers to the immune response of animal which has been fully immunised i.e. the response mounted by the immunised animal results in production of sufficient antibodies to effectively neutralise all the antigen of interest.

5 The term “introducing” (or grammatical variations thereof) when used in the context of inserting a nucleic acid molecule into a cell, means “transfection” or “transformation” or “transduction” and includes reference to any method for incorporation or transfer of a nucleic acid molecule into a eukaryotic or prokaryotic cell for expression or replication thereof (for example this may include but should not be limited to insertion of a nucleic acid into: a chromosome, mitochondrial DNA, an autonomous replicon (eg. a plasmid).

10 The term “transduction” as used herein, refers to the process of transferring genetic information from a nucleic acid molecule from one cell to another by way of a viral vector. The term “transfection” as used herein, refers to the uptake, incorporation, and expression of recombinant DNA by eukaryotic cells.

15 The term “transformation” as used herein refers to a process by which the genetic material carried by an individual cell is altered by incorporation of exogenous DNA into its genome.

The term “variant” as used herein refers to nucleotide and polypeptide sequences wherein the nucleotide or amino acid sequence exhibits substantially 60% or greater homology with the nucleotide or amino acid sequence of the Figures, preferably 75%
20 homology and most preferably 90-95% homology to the sequences of the present invention. — as assessed by GAP or BESTFIT (nucleotides and peptides), or BLASTP (peptides) or BLAST X (nucleotides). The variant may result from modification of the native nucleotide or amino acid sequence by such modifications as insertion, substitution or deletion of one or more nucleotides or amino acids or it may be a
25 naturally-occurring variant. The term “variant” also includes homologous sequences

which hybridise to the sequences of the invention under standard hybridisation conditions defined as 2 x SSC at 65°C, or preferably under stringent hybridisation conditions defined as 6 x SSC at 55°C, provided that the variant is capable modulating the ovulation rate of a female mammal or altering ovarian function. Where such a variant is desired, the nucleotide sequence of the native DNA is altered appropriately. This alteration can be effected by synthesis of the DNA or by modification of the native DNA, for example, by site-specific or cassette mutagenesis. Preferably, where portions of cDNA or genomic DNA require sequence modifications, site-specific primer directed mutagenesis is employed, using techniques standard in the art.

- 10 A “fragment” of a nucleic acid is a portion of the nucleic acid that is less than full length, and comprises at least a minimum sequence capable of hybridizing specifically with a nucleic acid molecule according to the invention, or a sequence complementary thereto, under stringent conditions as defined below. A “fragment” of a polypeptide is a portion of the polypeptide which is less than full length, but which still retains the biological function of either; increasing or decreasing the ovulation rate of a mammal, causing sterility in a mammal; or altering the regulation of the corpus luteum. Hence, a fragment according to the invention has at least one of the biological activities of the nucleic acid or polypeptide of the invention. However, it will be appreciated that the biological activity of a fragment of the GDF-9 sequence of the present invention encompass only those mutations which will increase the ovulation rate in female mammals heterozygous for the mutation.

The term “isolated” means substantially separated or purified away from contaminating sequences in the cell or organism in which the nucleic acid naturally occurs and includes nucleic acids purified by standard purification techniques as well as nucleic acids prepared by recombinant technology, including PCR technology, and those chemically synthesised.

The term “modulation of ovulation” means increasing or decreasing the rate of ovulation compared to the endogenous rate observed in an untreated animal.

The term “hybridization” or grammatical variants thereof means the process of joining two complementary strands of DNA or one each of DNA and RNA to form a double
5 stranded molecule.

When referring to a probe or primer, the term “specific for (a target sequence)” indicates that the probe or primer hybridizes under stringent conditions only to the target sequence in a given sample comprising the target sequence.

EXAMPLES

10 Non-limiting examples illustrating the invention will now be provided. It will be appreciated that the above description is provided by way of example only and variations in both the materials and techniques used which are known to those persons skilled in the art are contemplated.

Methodology

15 Animals

The flocks of Cambridge and Belclare sheep at The Sheep Research Centre of Teagasc, Athenry, were routinely examined for ovulation rate at the beginning of each mating season using laparoscopy. The examination was done once before joining and once after the first mating of the joining period. This data has been collected each year since these
20 flocks were established. The flocks were self-contained with at least 5 males used for mating each year. In addition rams from these flocks were progeny tested for ovulation rate by crossing with Galway and Scottish Blackface ewes – both low prolificacy

breeds. Ovulation rate measurements were done by laparoscopy under licence from The Minister of Health under the Cruelty to Animals Act (1876) EU Directive 86/609/EC.

When sterile ewes were first detected they were checked for the possibility that they were freemartins but this could not be confirmed (Hanrahan, 1991). Blood samples
5 were retained for DNA extraction from the sterile Cambridge ewes born in 1990 and later years and from essentially all of the F700 Belclare sterile females born since 1993. This material has been supplemented by blood samples for DNA extraction collected from fertile ewes in these flock from 1992 onwards.

Ovulation rate data were analysed by least squares procedures with the individual
10 animal as the experimental unit using the GLM procedure of SAS. The factors in the models were ewe, age, year of record, and the number of copies (0 or 1) of each of the mutations described below.

Samples

Genomic DNA was isolated from Irish Cambridge and F700 Belclare sheep either from
15 frozen stored buffy coat or directly from white blood cells in whole blood. Parentage of key pedigrees was verified with autosomal sheep microsatellite markers OarHH64 (sheep chromosome 4), OarCP34 (sheep chromosome 3) and OarFCB304 (sheep chromosome 19).

Sequencing and Mutation Detection

20 The sheep GDF-9 and GDF-9B genes were amplified using the polymerase chain reaction (PCR) with primers designed from published sheep sequences (sheep genomic GDF-9B exon 1, AF236078; sheep genomic GDF-9B exon 2, AF236079; sheep genomic GDF-9 exon 1 and 2, AF078545).

The PCR primers used were as follows:

GDF-9B exon 1 B13: 5'-ACTGCTGCCTTGTCCCAC-3'

B28: 5'-AGGCAATGTGAAGCCTGACA-3'

GDF-9B exon 2 B25: 5'-CAGTTTGTACTGAGCAGGTC-3'

5 O4: 5'-TTCTTGGGAAACCTGAGCTAGC-3'

GDF-9 exon 1 G1: 5'-GAATTGAACCTAGCCCACCCAC-3'

G4: 5'-AGCCTACATCAACCCATGAGGC-3'

GDF-9 exon 2 G5: 5'-ATCCCACCCTGACGTTTAAGGC-3'

G7: 5'-TCCTCCCAAAGGCATAGACAGG-3'

10 The resulting PCR products were sequenced on an ABI 373 sequencer.

Single Stranded Conformational Polymorphism Detection

SSCP (single stranded conformational polymorphism) was carried out on 9 Belclare rams involved in the progeny testing programme and on the half sib progeny of three of these rams (n = 58 (29, 17, and 12 progeny respectively)) and also on 2 Cambridge rams one of whom was progeny tested. In addition, seven purebred daughters of two of the Belclare rams were tested along with four of the five dams involved.

GDF-9B genotypes were determined by analysis of three nucleotide fragments which spanned most of exon 2. Fragments analysed by SSCP were:

353 bp, exon 2 primer 9B-359 5'-CGC TTT GCT CTT GTT CCC TCT-3'

20 primer 9B-691 5'-CCT CAC TAC CTC TTG GCT GCT-3'

273 bp, exon 2 primer 9B-664 5'-GGG TTC TAC GAC TCC GCT TC-3'
 primer 9B-916 5'-GGT TAC TTT CAG GCC CAT CAT-3'

312 bp, exon 2 primer 9B-915 5'-CAT GAT GGG CCT GAA AGT AAC-3'
 primer 9B-1205 5'-GGC AAT CAT ACC CTC ATA CTC C-3'

- 5 Primers were designed from nucleotide sequence Genbank Accession number AF236079 and primer names correspond to nucleotide position within that sequence.

GDF-9 genotypes were determined by analysis of five fragments which spanned exon 1, part of the intron and most of exon 2. Fragments analysed by SSCP were:

462 bp, exon 1 primer G9-1734 5'-GAA GAC TGG TAT GGG GAA ATG-3'

10 primer G9-2175 5'-CCA ATC TGC TCC TAC ACA CCT-3'

294 bp, intron primer G9-2676 5'-GTG TGA GAG AGA TGG GAG CA-3'
 primer G9-2947 5'-AAG AGG AAA ACT ATC AAA AGA CA-3'

296 bp, exon 2 primer G9-3270: 5'-TGG CAT TAC TGT TGG ATT GTT TT-3'

15 primer G9-3546: 5'-CAA GAG GAG CCG TCA CAT CA-3'

206 bp, exon 2 primer G9-3543: 5'-GAT TGA TGT GAC GGC TCC TCT-3'
 primer G9-3728: 5'-GGG AAT GCC ACC TGT GAA AAG-3'

221 bp, exon 2 primer G9-3939: 5'-TCT TTT TCC CCA GAA TGA ATG T-3'
 primer G9-4140: 5'-CAC AGG ATG GTC TTG GCA CT-3'

Primers were designed from nucleotide sequence Genbank Accession number AF078545 and primer names correspond to nucleotide position within that sequence.

Amplification was carried out for 30 cycles in a 40 µL reaction mixture, using 150 ng of genomic DNA, with 1.5 mM or 3 mM magnesium and an annealing temperature of 55 to 58° C. PCR fragments were analysed by SSCP in polyacrylamide gels with overnight migration at 9-15 V/cm, 15°C.

Single Nucleotide Polymorphism Detection Assays

The [E1] polymorphism identified in GDF-9 exon 1 produced a G to A nucleotide change which disrupts a *Hha* I restriction enzyme cleavage site (GCGC to GCAC) at nucleotide 260 of the 462 bp PCR fragment produced by primers G9-1734 and G9-2175 above. Digestion was carried out using 9 µl of PCR product and 3 U *Hha* I in 15 µl final volume, for 6 h at 37° C. Restriction digestion of the PCR product from wildtype animals with *Hha* I resulted in cleavage of the 462 bp product (at two internal *Hha* I sites) into fragments of 52 bp, 156 bp and 254 bp. However, DNA fragments containing the A nucleotide are not cleaved at this site and fragment sizes of 52 bp and 410 bp are seen. Animals heterozygous for the mutation have fragments of all four sizes (52 bp, 156 bp, 254 bp and 410 bp).

The remaining single nucleotide polymorphisms (SNPs) in GDF-9 and GDF-9B identified by sequencing did not affect common restriction endonuclease cleavage sites. In order to screen these polymorphisms through the F700 Belclare and Cambridge flocks of sheep, PCR was carried out using primers with single mismatches in order to deliberately generate products that contained restriction enzyme sites. Assays were designed so that digestion with the appropriate restriction enzyme cleaved either PCR products from wild-type animals or PCR products from animals containing the SNP, as specified below. The resulting band shift was resolved on a high percentage agarose gel.

The primer sequences and PCR conditions for each assay were as follows. The mismatch created in the appropriate primer to generate the restriction enzyme cleavage site is underlined.

In all five assays below, amplification was carried out at: 94°C for 5 min; 35 cycles of
5 94°C for 30 sec, an annealing step for 40 sec (at the specific temperature stated below for each assay) and 72°C for 30 sec; followed by a final extension of 72°C for 4 min. Magnesium concentration was 1.5 mM.

1. The primers used for the GDF-9 [324] nucleotide change amplify a 161 bp PCR product were:

10 [324]-Sfu1F 5'-GGAATATTCACATGTCTGTAAATTTACATGTTCG-3'

[324]-Sfu3R 5'-GAGGGAATGCCACCTGTGAAAAGCC-3'

The annealing temperature was 63°C.

The non-wildtype strand was cleaved by restriction enzyme *Sfu* I.

2. The primers used for the GDF-9 [714] nucleotide change amplify a 158 bp PCR
15 product were:

[714]-Tru1R 5'-CAGTATCGAGGGTTGTATTTGTGTGGGGCCT-3'

[714]-Tru3F 5'-GCCTCTGGTTCCAGCTTCAGTC-3'

The annealing temperature was 63°C.

The non-wildtype strand was cleaved by restriction enzyme *Mse* I.

3. The primers used for the GDF-9 [787] nucleotide change amplify a 139 bp PCR
20 product were:

[787]-Dde1R: 5'-CATGGATGATGTTCTGCACCATGGTGTGAACCTGA-3'

[787]-Dde3F: 5'-CTTTAGTCAGCTGAAGTGGGACAAC-3'

The annealing temperature was 62°C.

The wildtype strand was cleaved by restriction enzyme *Dde* I.

- 5 4. The primers used for the GDF-9B [S1] nucleotide change amplify a 141 bp PCR product were:

[S1]-Hinf1F: 5'-CACTGTCTTCTTGTTACTGTATTTCAATGAGAC-3'

B26: 5'-GATGCAATACTGCCTGCTTG-3'

The annealing temperature was 63°C.

- 10 The wildtype strand was cleaved by restriction enzyme *Hinf* I.

5. The primers used for the GDF-9B [S2] nucleotide change amplify a 153 bp PCR product were:

[S2]-Dde1F: 5'-GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA-3'

O4: 5'-TTCTTGGGAAACCTGAGCTAGC-3'

- 15 The annealing temperature was 64°C.

The wildtype strand was cleaved by restriction enzyme *Dde* I.

Restriction digestion of PCR with *Hinf* I [S1] or *Dde* I [[787] and [S2]] resulted in a cleavage of the longer primer from the fragment amplified from wild-type alleles (thus producing a 30-35 bp smaller product than the uncleaved fragment from animals containing alleles with the mutation). Restriction digestion of PCR with *Sfu* I [324] or

20

Mse I [714] resulted in a cleavage of the longer primer from the fragment amplified from mutant alleles (thus producing a 30-35 bp smaller product than the uncleaved fragment from animals containing wild-type alleles). Animals heterozygous for any of the mutations had fragments of both sizes. The digested fragments were separated on a 4% agarose gel and visualised with ethidium bromide staining. The gels were scored for the presence or absence of the mutations. Homozygous, heterozygous and negative controls were included with each assay.

Immunisation experiments

All experiments were performed with the approval of the Animals Ethics Committee at Wallaceville Animal Research Centre in accordance with the 1987 Animal Protection (Codes of Ethical Conduct) Regulations of New Zealand. The animals used in the immunization studies (n=54) were 5 to 6 year old parous Romney ewes.

Generation of antigens for immunization of sheep

Peptides KKPLVPASVNLSEYFC (GDF-9) and SEVPGPSREHDGPESC (GDF-9B) were synthesized and conjugated to KLH through the C terminal cysteine residue by Macromolecular Resources (Colorado State University, Fort Collins, CO).

Long-term active immunization of ewes against GDF-9 and GDF-9B peptides

Ewes were injected (i.m) with 0.4 mg KLH (control, n=10), 0.4 mg KLH-GDF-9 peptide conjugate (GDF-9 peptide; n=10) or 0.4 mg KLH-GDF-9B peptide conjugate (GDF-9B peptide; n=10) in 1 ml of Freund's complete adjuvant for the initial immunization. Thereafter, ewes were immunized once monthly with 0.2 mg KLH, GDF-9 peptide or GDF-9B peptide in 1 ml of saline mixed with 1.25 ml STM (Span-Tween-Marcol) for 6 months. After the 5th injection, vasectomised rams with marking harnesses were run with the ewes to monitor estrous cycles. The length of the estrous

cycle was calculated as the days between first observed markings by the vasectomised ram of successive cycles. In addition, blood samples were collected via the jugular vein 3 times a week for determination of plasma progesterone concentrations. Ovulation rates of the ewes that displayed estrous behaviour were determined by laparoscopy once
5 all of the control ewes had been observed in estrus and for each successive estrous cycle. In addition, ovulation rate of all ewes was determined by laparoscopy 3-4 weeks prior to ovarian collection. Approximately 2 weeks following the final injection, ewes were killed using a captive bolt and exsanguinated. The blood collected from all ewes was to be used in subsequent passive immunisation studies. Both ovaries were
10 recovered and the number of corpora lutea present was recorded and one ovary from each ewe was fixed in Bouins fluid for morphological examination and analysis of follicular populations.

Passive immunisation of ewes against KLH, KLH-GDF-9 peptide and KLH-GDF-9B peptide

15 Pools of antiplasma from KLH (n=9), GDF-9 peptide (n=7, all anovulatory ewes) and GDF-9B peptide (n=9, all anovulatory ewes) treated ewes were generated by combining the plasmas obtained from some of the actively immunized ewes within each treatment group. The estrous cycles of ewes were synchronized by using a prostaglandin $F_{2\alpha}$ derivative (Estrumate; 125 μ g). Estrus was detected with the aid of a vasectomised ram
20 wearing a marking harness. On day 4 or 5 of the estrus cycle (estrus = day 0) ewes were laparoscoped to determine ovulation rate and fitted with an indwelling jugular cannula. The following day ewes (n=4-5 per group) were administered 100 ml of antiplasma to KLH, GDF-9 peptide or GDF-9B peptide through the indwelling jugular cannula. Ewes were given another injection of Estrumate, at 96h after administration of the antiplasma
25 to induce a follicular phase and ovulation rate was determine by laparoscopy at 10 days after the injection of Estrumate and every 15-18 days thereafter until the end of the

breeding season (as assessed by lack of estrous activity in non-experimental sheep). Blood samples were collected from the ewes at 5 minutes, 1 h and 96 h after injection of the antiplasma and thereafter 3 times a week from the 2nd injection of Estrumate for determination of antibody titers and concentrations of progesterone in plasma.

5 **Determination of progesterone concentrations**

Concentrations of progesterone in plasma were determined by radioimmunoassay (RIA). The inter- and intra-assay co-efficients of variation were <10% and assay sensitivity was 0.1 ng/ml. All samples below the sensitivity of the assay were assigned a value of 0.1 ng/ml for statistical analysis.

10 **Short-term immunisations**

Romney ewes were immunized with KLH (N=50), KLH conjugated to GDF-9 peptide (N=30) or KLH conjugated to GDF-9B peptide (N=30). The antigens were administered in DEAE Dextran (4% w/v) on 2 occasions one month apart. The number of corpora lutea (CL) was determined following the first observed oestrus which occurred after the
15 booster immunization. In addition, in a selected subpopulation of these ewes (N=26 KLH, N=15 GDF-9, N=16 GDF-9B) the number of CL present following the next oestrus was also determined. The average number of CL for each ewe was analysed by Chi-square analysis.

Statistical analysis

20 For the long-term, actively immunized ewes, ovulation rate for individual ewes was calculated as the mean of the number of corpora lutea observed at all observations for that ewe when at least 1 corpus luteum (CL) was present (i.e. observations of no CL were excluded from the calculation). The Kruskal-Wallis test was used to compare ovulation rates between the KLH-GDF-9B mature protein and the KLH treated groups.

No other groups were included in this comparison since none had sufficient numbers of ewes ovulating. The Chi Square test was used to compare the proportion of ewes observed in estrus by the time all the control ewes had been observed in estrus. In addition the Chi Square test was used to compare the proportion of ewes with corpora lutea on their ovaries 3-4 weeks before and at ovarian collection.

When examining the effects of active immunization treatments on ovarian volumes, numbers of follicles or oocyte or follicular diameters, the data were analysed within each follicle type after normalising the data by log transformation. For each parameter a one-way ANOVA was performed, after blocking on animals where appropriate, and differences between treatment groups were determined by least significant difference.

For the passively immunized ewes, differences in the number of ewes with corpora lutea at each laparoscopy were determined using Fisher's exact test. The areas under the curves were calculated using Genstat using the area function for progesterone values from 2 to 19 days following injection of Estrumate that was given 4 days after administration of plasma. Resulting values were analysed with one-way ANOVA and differences between the control and treated ewes determined with Fisher's pairwise comparisons.

For the short term active immunizations, ovulation rate for individual ewes was calculated as the average of the number of corpora lutea observed at both observations. Data was analysed using the general linear models procedures of SAS. Differences between least-squares means were evaluated by least significant differences.

RESULTS and ANALYSIS OF RESULTS

Finding mutations in Cambridge and F700 Belclare animals

In order to determine whether mutations in GDF-9 or GDF-9B were contributing to sterility in these animals sequence information was obtained for the entire coding sequence of both genes in a subset of Irish Cambridge (N = 9) and F700 Belclare sheep (N = 10). Animals were chosen for full-length sequencing based on their sterility phenotype or their pedigree relationship to sterile animals. In addition, mutation detection was also carried out by single-stranded conformational polymorphism (SSCP) analysis independently of the above sequencing in F700 Belclare pedigrees (23 animals and 58 progeny test daughters of three rams) and also on 2 Cambridge rams.

Mutations in GDF-9

Sequence of GDF-9 revealed eight single nucleotide polymorphisms across the entire coding region (Table 1, Figure 4). SSCP analysis identified five fragments across the gene which contained conformational differences. These differences correspond to one single nucleotide polymorphism (SNP) in exon 1, one SNP in the intron and five SNPs in exon 2.

Original naming of the mutations (numbers in square brackets [], Table 1) refers to the nucleotide position from the start of exon 2, except for [E1] which refers to the polymorphism found in exon 1 of GDF-9. Table 1 shows the relationship between (a) the original numbering system, (b) the coding nucleotide position in the full length coding sequence numbered from the first atg, (c) the position of the coding amino acid residue involved, and (d) the position of the coding residue (if any) within the mature coding sequence, numbering from the first amino acid after the furin protease processing site. For example mutation [581] is a G to A nucleotide substitution at

coding nucleotide 978 of GDF-9 which corresponds to an unchanged glutamate (Glu) coding residue 326 of the full length unprocessed protein, or residue 8 of the processed mature peptide.

Three of the eight polymorphisms are nucleotide changes which do not result in an altered amino acid ([74] at nucleotide position 471, [80] at nucleotide 477, and [581] at nucleotide position 978). The five remaining nucleotide changes [324], [597], [714], and [787] gave rise to amino acid changes (Table 1), Figure 1, Figure 4), although three of them were relatively conservative changes. The [E1] arginine to histidine change at amino acid residue 87 in exon 1 substituted one basic charged polar group with another, and occurred at a position prior to the furin processing site, so was unlikely to affect the activity of the mature protein. Both the [597] valine to isoleucine change at amino acid residue 332 of the unprocessed protein (residue 14 of the mature coding region) and the [714] valine to methionine at residue 371 of the unprocessed protein (residue 53 of the mature coding region) substituted non-polar groups with non-polar groups. The remaining two changes resulted in non-conservative substitutions. The [324] glutamic acid to lysine change at amino acid residue 241 of the unprocessed protein replaced an acidic group with a basic group, but this occurred at a position prior to the furin processing site and was unlikely to affect the mature active coding region. However the [787] serine to phenylalanine change at residue 395 replaced an uncharged polar group with a non-polar group at residue 77 of the mature coding region. The nucleotide and amino acid changes are illustrated in Figure 1 and Figure 4.

Mutations in GDF-9B

Both DNA sequencing and independent SSCP analysis of GDF-9B in Cambridge and F700 Belclare sheep revealed four polymorphisms across the entire coding region (Table 1, Figure 5). Original naming of these mutations (in square brackets []) refers

specifically to the leucine deletion [Leu], or for the conservative [422] T to C mutation, the nucleotide position from the start of exon 2. GDF-9B mutations which changed amino acids were named [S1] and [S2]. Table 1 shows the relationship between (a) the original numbering system, (b) the coding nucleotide position in the full length coding sequence numbered from the first atg, (c) the position of the coding amino acid residue involved, and (d) the position of the coding residue (if any) within the mature coding sequence, numbering from the first amino acid after the furin protease processing site. For example mutation [S2] was a G to T nucleotide substitution at coding nucleotide 1100 of GDF-9B which corresponds to an serine (Ser) residue changing to an isoleucine (Ile) residue at coding residue 367 of the full length unprocessed protein, or residue 99 of the processed mature peptide.

The first of these four polymorphisms (Table 1) was a previously-reported leucine deletion polymorphism [leu] in the predicted signal sequence (Galloway *et al.*, 2000) whereby some sheep have two leucine codons (CTT) at this position and some sheep have only one. This polymorphism has been shown to be unrelated to fertility and ovulation rate in Inverdale sheep (Galloway *et al.*, 2000). One other nucleotide change, [422], did not result in an altered amino acid (nucleotide position 747). The remaining two nucleotide changes ([S1] and [S2]) gave rise to more critical changes in the protein (Figure 2, Figure 5). The [S1] C to T change at nucleotide 718 introduced a premature stop codon (TAG) in the place of glutamic acid (Q, CAG) at amino acid residue 239 of the unprocessed protein, which presumably resulted in complete loss of GDF-9B function. The [S2] G to T change at nucleotide 1100 changed the serine residue at amino acid 99 of the mature active protein (residue 367 of the unprocessed protein) to an isoleucine, thereby substituting an uncharged polar group with a nonpolar group. The nucleotide and amino acid changes are illustrated in Figure 2 and Figure 5.

Screening for mutations in more animals

Initial sequencing of a smaller number of animals from each family identified the [74], [80], [324], [714], and [787] nucleotide changes in GDF-9, and the [S1], [S2] and [422] changes in GDF-9B. Forced RFLP (restriction fragment length polymorphism) assays to
5 detect the specific SNPs were developed for [324], [714], [787] (GDF-9) and for [S1] and [S2] (GDF-9B), and these assays were carried out on larger numbers of animals (Table 2). Subsequent sequencing of full length GDF-9 and GDF-9B in more animals revealed the [581] and [597] nucleotide changes in GDF-9 in the Cambridge sheep but not the F700 Belclares. Independent SSCP analysis identified the [E1] polymorphism in
10 exon 1 of GDF-9 in one ram, and this was also screened through further animals. [E1] was found to be associated with the wildtype alleles in this ram and his backcross progeny, and not associated with ovulation rate.

Homozygous mutations relate to sterility

Presence or absence of each of these nucleotide changes was analysed in relation to
15 sterility or fertility in all of the animals tested, revealing that only the [787] change in GDF-9 and the [S1] and [S2] changes in GDF-9B contributed to infertility. Female sheep which were homozygous for [787] were sterile; female sheep which were homozygous for [S1], or homozygous for [S2] were sterile; female sheep which were heterozygous for [S1] and [S2] simultaneously (whereby both copies of the X
20 chromosome carried a different GDF-9B mutation) were sterile. Figures 3a and 3b show data from small pedigrees illustrating what was seen in the larger set of animals.

Figure 3a illustrates a F700 Belclare pedigree. The sire R830 carried the GDF-9B [S2] mutation on his X chromosome and a single copy of the GDF-9 [787] mutation on chromosome 5, but did not have the GDF-9B [S1] mutation. Dam 9704 carried a single
25 copy of the GDF-9B [S1] mutation on her X chromosome and their two female

offspring (930458 and 930459) were sterile since they had inherited mutated copies of GDF-9B from both parents. Dam 8783 carried a single copy of the GDF-9 [787] mutation on chromosome 5 and the female offspring of her mating with sire R830 were infertile and were homozygous for the GDF-9 [787] mutation. Their infertility cannot be explained by GDF-9B mutations. Offspring 930810 and 948302 were not homozygous for any of these mutations and hence were fertile. All three functional mutations ([S1], [S2], and [787]) were seen in the F700 Belclare flock (Table 2).

Figure 3b illustrates two Cambridge pedigrees. The sire 962101 carried the GDF-9B [S1] mutation on his X chromosome and a single copy of the GDF-9 [787] mutation on chromosome 5, but did not have the GDF-9B [S2] mutation. Dam 962152 carried a single copy of the [S1] mutation on her X chromosome and a single copy of the [787] mutation on chromosome 5. Their two female offspring (997634 and 997635) were sterile and had inherited mutated copies of both GDF-9B ([S1]) and GDF-9 ([787]) from both parents. Dam 976234 only carried a single copy of the [S1] mutation and one female offspring (997553) was infertile, having inherited mutated copies of GDF-9B ([S1]) from both parents, whereas 997552 was fertile. Sire 930142 was homozygous for the GDF-9 [787] mutation and carried the GDF-9B [S1] mutation on his X chromosome, whereas dam 8874 was only heterozygous for the GDF-9 [787] mutation and carried no GDF-9B mutation. Their daughter (948093) had inherited two copies of the GDF-9 [787] mutation and was sterile even though she was also heterozygous for the GDF-9B [S1] mutation which she inherited from her sire. The [S2] mutation was not seen in any animals tested from the Cambridge flock (Table 2).

Among the animals tested for these changes, fertile animals homozygous for both GDF-9 [324] and [714] were found and it is therefore concluded that neither of those changes result in disruption of the genes sufficient to cause sterility. Animals were also found

which were heterozygous for GDF-9 and GDF-9B mutations together, and these animals were not sterile.

Structural effects of mutations on activity

Structural data is available for members of the TGF β superfamily which may provide
5 information about the likely effects of each of the three mutations ([S1], [S2] and [787])
on the biological activity of GDF-9 and GDF-9B, and hence explain the association
with sterility. Structures have been reported for TGF- β 1 (Hinck *et al.*, 1996); TGF- β 2
(Daopin *et al.*, 1992), TGF- β 3 (Mittl *et al.*, 1996), BMP7/OP1 (Griffith *et al.*, 1996) and
BMP2 (Scheufler *et al.*, 1999). Receptor binding structures have also been reported for
10 BMP2 with the BRIA receptor binding ectodomain (Kirsch *et al.*, 2000) and for TGF- β 3
with the T β R2 receptor binding ectodomain (Hart *et al.*, 2002).

In the present invention, the [S1] mutation resulted in premature termination of GDF-
9B protein prior to the mature active protein processing site. It is thus expected that this
mutation would result in no mature protein being translated, and appears to be an even
15 more severe effect than the Hanna mutation (Galloway *et al.*, 2000) which results in
infertility in sheep. The GDF-9B [S2] mutation changed an uncharged polar serine
residue (residue 99 of mature GDF-9B) which is conserved across most members of the
TGF β superfamily, to a non-polar isoleucine (Figure 6). This serine (and the nearby
conserved leucine) has been shown to be essential for receptor binding by structural and
20 site-directed mutagenesis studies of BMP2. In F700 Belclare sheep it appears that this
mutation abolishes biological activity of GDF-9B, possibly by affecting receptor
binding. The GDF-9 [787] mutation changed an uncharged polar serine residue (residue
77 of mature GDF-9) to a non-polar phenylalanine in a region of the molecule which
appears to be involved in dimerisation. This change occurred only three residues away
25 from a conserved histidine (H80) of the mature GDF-9 peptide (Figure 6). In BMP7 this

conserved histidine exhibits hydrogen bonding to three residues of the paired molecule in the BMP7 dimer (Griffith *et al.*, 1996) and TGF β 3 (Mittl *et al.*, 1996). GDF-9 lacks the interchain disulphide bond which forms a covalent link between both monomers of the biologically active dimer that is found in most other members of the TGF β superfamily. It is therefore likely that in GDF-9 the hydrogen bonds between monomers would be even more critical for maintaining dimer stability, so that the GDF-9 [787] mutation possibly abolished biological activity by disrupting dimerisation.

Heterozygous animals have increased ovulation rate

Irish Cambridge and F700 Belclare sheep have increased ovulation rates as well as infertility (Hanrahan, 1996). Ovulation rate data of fertile ewes which had been genotyped for the [S1], [S2] and [787] mutations was collected (Table 3). Heterozygous carriers of mutations in GDF-9B (either [S1] or [S2]) showed an increased ovulation rate similar to those seen in Inverdale and Hanna sheep (Davis *et al.*, 2001). Animals which were heterozygous for both a GDF-9B mutation and a GDF-9 mutation had an even higher ovulation rate, and this effect appeared to be additive.

The additive effects of the GDF-9 mutation and GDF-9B mutations together in one animal imply that GDF-9 and GDF-9B work independently so that a combination of both proteins would alter ovarian function more effectively than by altering either GDF-9 or GDF-9B alone.

Effect of immunising sheep against GDF-9

In addition to the genotype effects above, both long-term active immunisation and short-term passive immunisation of sheep with GDF-9 was carried out and shown to cause sterility and/or abnormal corpus luteum function. This finding provides additional evidence that a homozygous GDF-9 mutant phenotype is one of sterility.

Ewes were actively immunised against KLH (control, n = 9) or KLH conjugated to GDF-9 peptide (n = 10). The ewes actively immunized with the GDF-9 peptide showed no cyclic estrous behaviour (Table 4) as evidenced by repeated laparoscopy. High (normal) progesterone concentrations were only seen in one or two samples (Figure 7).

- 5 Generally, when corpora lutea and/or luteal-like structures were observed following long-term immunisation against KLH conjugated to GDF-9 peptide, progesterone concentrations were abnormal. In addition many of the ewes did not have any visible antral follicles at laparoscopy or at ovarian collection. Figure 7 also shows data for ewes immunised against GDF-9B/(BMP15) for comparison.
- 10 Passive immunisation using 100 ml of GDF-9 peptide antiplasma caused abnormal luteal function within 30 days of administration of the antiplasma (Figure 8). There were no differences in ovulation rates among the groups before administration of the antiplasma. Administration of antiplasma against GDF-9 peptide 4 days before induction of the follicular phase did not affect ovulation rate. However, at laparoscopy
- 15 the corpora lutea of two of the animals treated with GDF-9 antiplasma appeared smaller than normal. In addition, the overall mean concentration of progesterone during the subsequent luteal phase was less ($P < 0.05$) than that observed in the control animals (Figure 8). This was the result of the progesterone concentrations being normal in two of the animals but in the other three animals, the post ovulation rise in progesterone was
- 20 delayed even though luteolysis occurred at the normal time. Figure 8 also shows data for passive immunisation with GDF-9B/(BMP15) for comparison.

The finding of abnormal luteal function following GDF-9 immunisation (both passive and active) has not been previously reported. It is postulated, therefore, that the administration of supplementary GDF-9 and GDF-9B, or analogue thereof, or GDF-9 or

25 GDF-9B antagonists, may modify corpus luteum function.

In a further experiment, short-term active immunisation of sheep with GDF-9 or GDF-9B was shown to mimic the heterozygous effects of mutations in these genes. Short-term immunisation using milder adjuvant than in the previous experiments (2 immunisations in DEAE-Dextran adjuvant), with either KLH conjugated to GDF-9 peptide or with KLH conjugated to GDF-9B peptide, acted to increase ovulation rate in the animals which ovulated as measured by the number of corpora lutea (CL) (Table 5).

The observation that an increased ovulation rate effect (ie as seen in heterozygous carriers of these inactivating mutations) can also be induced in sheep by short-term active immunisation against peptides of GDF-9 and GDF-9B provides new methods for altering ovarian function.

Temporary/reversible effects in immunization with GDF-9B

Four of the 30 ewes immunised (0.4 mg GDF-9B peptide-KLH followed by 0.2 mg GDF-9B peptide-KLH 30 days apart) displayed transient infertility. After the second immunization, ewes failed to show oestrus and upon examination of their ovaries, failed to show any evidence of ovulation (no corpora lutea). These ewes returned to estrous over the next 30 days and were placed with a fertile ram. The oocytes release at oestrus were healthy as indicated by the following. Three of the four ewes were flushed to recover embryos for transfer as they had higher ovulation rates upon return to fertility. While no embryos were recovered from one of the ewes, developing embryos were recovered from the other two ewes. In addition, while the embryos from one of the ewes were too advanced for recovery (by approximately one week) the two embryos from the other ewe were transferred to a recipient ewe and resulted in the birth of one lamb. The fourth ewe was allowed to carry the pregnancy to term and gave birth to two lambs, one stillborn and one live. These results provide evidence that the sterility induced by the

method of the present invention can be temporary or reversible and may therefore be used in methods of contraception.

CONCLUSIONS

These findings provide the first evidence that mutations in GDF-9 and GDF-9B are
5 associated with the reproductive effects seen in the Cambridge and Belclare breeds of
sheep. The increased ovulation rate and sterility phenotypes in these animals can be
explained by the presence of heterozygous mutations and homozygous mutations,
respectively, in these genes. Methods which are able to induce changes in the biological
activity of GDF-9 and/or GDF-9B to mimic these genotypes have been developed to
10 modulate fertility in mammals in need thereof.

INDUSTRIAL APPLICATION

The present invention provides compositions and methods for modulating the ovulation
rate and therefore fertility in female mammals including humans.

TABLE 1

Sequence variations in GDF-9 and GDF-9B within the Irish Cambridge and F700 Belclare F700 flocks

Gene	[original nucl. change name]	coding nucl.(bp)	coding residue	mature residue	result
5	GDF-9 [E1]	G-A 260	87		Arg (R) – His (H)
	[74]	C-T 471	157		unchanged Val (V)
	[80]	G-A 477	159		unchanged Leu (L)
	[324]	G-A 721	241		Glu (E) – Lys (K)
	[581]	A-G 978	326	8	unchanged Glu (E)
10	[597]	G-A 994	332	14	Val (V) – Ile (I)
	[714]	G-A 1111	371	53	Val (V) – Met (M)
	[787]	C-T 1184	395	77	Ser (S) – Phe (F)
	GDF-9B [Leu]	CTT del 28-30	10		Leu deletion
15	[S1]	C-T 718	239		Gln (Q) – STOP
	[422]	T-C 747	249		unchanged Pro (P)
	[S2]	G-T 1100	367	99	Ser (S) – Ile (I)

Columns indicate the relationship between (a) the original naming system used for each polymorphism, (b) the nucleotide change, (c) the coding nucleotide position (in base

pairs (bp)) in the full length coding sequence numbered from the first atg start codon, (d) the position of the coding amino acid residue involved (starting from the first Met residue), and (e) the position of the coding residue (if any) within the mature coding sequence, numbering from the first amino acid after the furin protease processing site.

- 5 For example mutation [581] is a G to A nucleotide substitution at coding nucleotide 978 of GDF-9 which corresponds to an unchanged glutamate (Glu) coding residue 326 of the full length unprocessed protein, or residue 8 of the processed mature peptide. Polymorphisms associated with infertility and ovulation rate traits are in bold.

10 **TABLE 2.** Genotype analysis of nucleotide changes in GDF-9B and GDF-9 genes from Irish Cambridge and F700 Belclare sheep.

Numbers shown are the number of individuals carrying at least one copy of the given mutation with the total number of individuals genotyped indicated in brackets underneath. Genotypes were determined by specific SNP assay and/or sequencing.

- 15 * The [E1] polymorphism in exon 1 of GDF-9 (see Table 1) was identified by SSCP analysis and was not tested in the same set of animals used for the above table.

	GDF-9B			GDF-9*						
	[S1]	[S2]	[422]	[74]	[80]	[324]	[581]	[597]	[714]	[787]
F700 Belclares	9 (83)	71 (86)	2 (13)	6 (10)	6 (10)	13 (29)	0 (10)	0 (10)	2 (19)	11 (86)
Cambridge	74 (129)	0 (131)	0 (9)	0 (9)	7 (9)	1 (26)	3 (9)	2 (9)	7 (24)	95 (126)

TABLE 3. Least squares means for ovulation rate of sheep carrying the different genotypes for GDF-9 and GDF-9B mutations

<i>Genotype</i>			<i>Breed</i>	
GDF-9B [S1]	GDF-9B [S2]	GDF-9 [787]	F700 Belclare	Cambridge
0	0	0	1.92±0.277 (n = 11)	2.27±0.488 (n = 10)
0	0	1	2.67±0.895 (n = 1)	4.39±0.308 (n = 28)
0	1	0	3.26±0.184 (n = 32)	-
0	1	1	6.09±0.549 (n = 3)	-
1	0	0	2.69±0.475 (n = 4)	3.11±0.438 (n = 15)
1	0	1	-	5.77±0.270 (n = 38)
Effect of GDF-9B [S1]			0.77±0.537 (P = 0.16)	1.18±0.387 (P<0.01)
Effect of GDF-9B [S2]			2.38±0.548 (P<0.01)	-
Effect of GDF-9 [787]			1.79±0.548 (P<0.01)	2.35±0.392 (P<0.01)

(n) = no. of ewes

- 5 **TABLE 4.** Proportions of ewes immunized against KLH, GDF-9 peptide or GDF-9B peptide in estrus at the time of first laparoscopy (1st), with visible luteal structures at laparoscopy 3-4 weeks before collection (2nd) and at ovarian collection (3rd).

Immunized Group	1 st	2 nd	3 rd
KLH	9/9	9/9	9/9
GDF-9 peptide	2/10*	2/10*	3/10*
GDF-9B peptide	1/10*	1/10*	1/10*

*Signifies a value that is different from the control (KLH) value (P<0.05)

TABLE 5. Short-term immunisation of sheep with GDF-9 or GDF-9B

Number of CL		1 to < 2	2 to < 3	=3
Treatment		Number of CL recorded in treated ewes		
5	KLH (n=50)	21	29	0
	GDF-9 peptide* (n=30)	7	17	6
10	GDF-9B peptide* (n=30)	2	18	10

* Using Chi-square analysis, the GDF-9 and GDF-9B immunized ewes have significantly higher ovulation rates (as measured by corpora lutea (CL) than KLH control ewes ($P < 0.01$).

Aspects of the present invention have been described by way of example only and it should be appreciated that modifications and additions may be made thereto without departing from the appended claims.

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WHAT WE CLAIM IS:

1. An isolated mutated GDF-9 nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
 - a) SEQ ID NOs. 1, 3 or 5;
 - 5 b) a sequence complementary to the molecule defined in a);
 - c) a functional fragment or variant of the sequences in a) or b);
 - d) an anti-sense sequence to any of the molecules defined in a), b) or c).
2. An isolated mutated GDF-9B nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
 - 10 a) SEQ ID NOs. 7, 9, 11, 13, 15 or 17;
 - b) a sequence complementary to the molecule defined in a)
 - c) an anti-sense sequence to any of the molecules defined in a) or b).
3. An isolated GDF-9 nucleic acid molecule comprising a mutation in at least one codon of the sequence associated with receptor binding and/or dimerisation.
- 15 4. An isolated GDF-9 nucleic acid molecule as claimed in claim 3, wherein said mutation results in an amino acid substitution in the polypeptide encoded by said nucleic acid sequence.
5. An isolated GDF-9 nucleic acid molecule as claimed in claim 4, wherein said amino acid substitution is present in a receptor binding domain and disrupts
20 receptor binding.

6. An isolated GDF-9 nucleic acid molecule as claimed in claim 4, wherein said amino acid substitution is present in a dimerisation domain and disrupts dimerisation.
7. An isolated GDF-9B nucleic acid molecule comprising a mutation in at least one codon sequence associated with receptor binding and/or dimerisation.
8. An isolated GDF-9B nucleic acid molecule as claimed in claim 7, wherein said mutation results in an amino acid substitution in the polypeptide encoded by said nucleic acid sequence.
9. An isolated GDF-9B nucleic acid molecule as claimed in claim 8, wherein said amino acid substitution is present in a receptor binding domain and disrupts receptor binding.
10. An isolated GDF-9B nucleic acid molecule as claimed in claim 8, wherein said amino acid substitution is present in a dimerisation domain and disrupts dimerisation.
11. A method of identifying a mammal which carries a mutated nucleic acid molecule encoding GDF-9B, said method comprising the steps of:
 - i) obtaining a tissue or blood sample from the mammal;
 - ii) isolating DNA from the sample; and optionally
 - iii) isolating GDF-9B DNA from the DNA obtained at step i) or ii);
 - iv) probing said DNA with a probe complementary to either strand of the mutated GDF-9B DNA of SEQ ID NOs 11 or 17;
 - v) amplifying the amount of mutated GDF-9B DNA;

vi) determining whether the GDF-9B sequence DNA obtained in step v) carries a mutation associated with sterility or increased ovulation.

12. A method of identifying a mammal which carries a mutated nucleic acid molecule encoding GDF-9, said method comprising the steps of:

5 i) obtaining a tissue or blood sample from the mammal;

ii) isolating DNA from the sample; and optionally

iii) isolating GDF-9 DNA from the DNA obtained at step i) or ii);

iv) probing said DNA with a probe complementary to either strand of the mutated GDF-9 DNA of SEQ ID NO 5;

10 v) amplifying the amount of mutated GDF-9 DNA;

vi) determining whether the GDF-9 sequence DNA obtained in step v) carries a mutation associated with sterility or increased ovulation.

13. A use of a nucleic acid molecule which is complementary to either strand of the mutated DNA of SEQ ID NOs. 11 or 17 as a marker to identify a mammal
15 carrying a mutated nucleic acid molecule encoding GDF-9B.

14. A use of a marker as defined in claim 13 in a method for marker assisted selection of a mammal which possesses a genotype which is associated with either enhanced ovulation or sterility.

15. A use of a nucleic acid molecule which is complementary to either strand of the
20 mutated DNA of SEQ ID NO 5 as a marker to identify a mammal carrying a mutated nucleic acid molecule encoding GDF-9.

16. A use of a marker as defined in claim 15, in a method for marker assisted selection of a mammal which possesses a genotype which is associated with either enhanced ovulation or sterility
17. A probe capable of specifically hybridising to either strand of the mutated GDF-9B DNA of SEQ ID NOs 11 or 17 under stringent hybridisation conditions.
18. A probe capable of hybridising to either strand of the mutated GDF-9 DNA of SEQ ID NO 5 under stringent hybridisation conditions.
19. A construct comprising a nucleic acid molecule as claimed in claim 1 or 2.
20. A vector comprising a nucleic acid molecule as claimed in claim 1 or 2.
21. A host cell which comprises a construct or vector as claimed in claim 19 or 20 which has been introduced therein.
22. A cell line comprising a host cell as claimed in claim 21.
23. A method of altering GDF-9 and/or GDF-9B bioactivity in a female mammal so as to modulate ovulation comprising the steps of either:
 - (a) inducing a partial immunisation response to endogenous GDF-9 and/or GDF-9B to partially reduce bioactivity thereof and enhance ovulation; or
 - (b) inducing a full immunisation response to endogenous GDF-9 and/or GDF-9B to substantially reduce bioactivity thereof and induce sterility.
24. A method as claimed in claim 23, wherein said immunisation response is induced by administration of an antigenic composition comprising:
 - i) a GDF-9 polypeptide or a functional fragment or variant of GDF9; and/or

ii) a GDF-9B polypeptide or a functional fragment or variant of GDF-9B;

together with a pharmaceutically or veterinarily acceptable carrier and/or diluent;

to a mammal in need thereof.

- 5 25. A method as claimed in claim 24, wherein said antigenic composition comprises a mild adjuvant to induce a partial immunisation response and induce enhanced ovulation.
26. A method as claimed in claim 24, wherein said antigenic composition comprises a strong adjuvant to induce a full immunization response and induce sterility.
- 10 27. A method as claimed in any one of claims 23 to 26, wherein said partial immunization response is induced by a short term immunization regime.
28. A method as claimed in any one of claims 23 to 26, wherein said full immunization response is induced by a long term immunization regime.
29. A method as claimed in claim 24, wherein said immunization response is induced
15 passively by administration of antibodies raised against said antigenic composition.
30. A method as claimed in claim 29, wherein said antibodies are administered according to a short term regime to induce a partial immunization response and induce enhanced ovulation.
- 20 31. A method as claimed in claim 29, wherein said antibodies are administered according to a long term regime to induce a full immunization response and induce sterility.

32. A method as claimed in any one of claims 23, 24, 26, 28, 29, and 31, wherein said full immunization response is temporary and/or reversible and wherein said sterility induced comprises contraception.
33. A method as claimed in any one of claims 23, 24, 26, 28, 29, and 31, wherein said full immunization response and said sterility induced is permanent.
34. A method for breeding a mammal having increased ovulation comprising the steps of:
- a) identifying the nucleotide sequences of GDF-9 or GDF-9B carried by the female mammal it is proposed to breed from;
 - b) identifying the nucleotide sequences of GDF-9 or GDF-9B carried by the male mammal it is proposed to breed from;
 - c) selecting the female and male animals that will result in progeny having the following characteristics:
 - i) a single copy of a mutated GDF-9 nucleotide sequence comprising:
 - A) SEQ ID NO 5; or
 - B) a functional variant or fragment of the molecule in A); or
 - C) a sequence complementary to the molecule in A) or B); and/or
 - ii) a single copy of mutated GDF-9B nucleotide sequence comprising:
 - A) SEQ ID NOs 11 or 17; or
 - B) a sequence complementary to the molecule(s) in A).

35. A method as claimed in claim 34, wherein said mammal is selected to have a single mutated copy of GDF-9 and GDF-9B.

36. A method for selecting a female mammal for breeding which possesses a genotype indicative of an increased rate of ovulation, said genotype comprising a single mutated copy of:

1) a mutated GDF-9 nucleotide sequence comprising:

a) SEQ ID NO 5; or

b) a functional variant of the molecule of a); or

c) a sequence complementary to the molecules in a) or b);

and/or

2) a mutated GDF-9B nucleotide sequence comprising:

a) SEQ ID NOs 11 or 17; or

b) a sequence complementary to the molecules in a)

said method comprising identifying said mammal according to the method of claim 11 and/or 12 and selecting said mammal.

37. A method as claimed in claim 36 wherein the mammal selected has both a single mutated copy of GDF-9 and GDF-9B.

38. A method of modifying the function of the corpus luteum by administering supplementary GDF-9 or GDF-9B, or analogues thereof, or GDF-9 or GDF9-B antagonists to female mammals.

39. A transgenic non-human animal wherein one copy of the endogenous GDF-9 and/or GDF-9B gene has been knocked out.
40. A transgenic non-human animal as claimed in claim 39, comprising a transgenic ovine having a genome lacking one copy of a gene encoding a protein having biological activity of GDF-9 and/or GDF-9B.
41. An isolated mutated GDF-9 polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, or 6 or a functional fragment or variant thereof.
42. An isolated mutated GDF-9B polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 10, 12, 14, 16, or 18.
43. A composition comprising an isolated nucleic acid as claimed in any one of claims 1 to 10, or an isolated polypeptide as claimed in claim 41 or 42 and a pharmaceutically acceptable carrier.

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Figure 1a

1 MALPNKFFLW FCCFAWLCFP ISLDSLPSRG EAQIVARTAL ESEAETWSLL

R87H [E1]



51 NHLGGRHRPG LLSPLLEVLY DGHGEPPLQ PDDRALRYMK RLYKAYATKE



101 GTPKSNRRHL YNTVRLFTPC AQHKQAPGDL AAGTFPSVDL LFNLDRTTVV

151 EHLFKSVLLY TFNNSISFPF PVKCICNLVI KEPEFSSKTL PRAPYSFTYN

E241K [324]



201 SQFEFRKKYK WMEIDVTAPL EPLVASHKRN IHMSVNFTCA EDQLQHPSAR

251 DSLFNMTLLV APSLLLYLND TSAQAFHRWH SLHPKRKPSQ GPDQKRGLSA

(1)

V332I [597]



301 YPVGEEAAEG VRSS[REDACTED]DQ ESASSELKKP LVPASVNLSE YFKQFLFPQN

V371M [714]



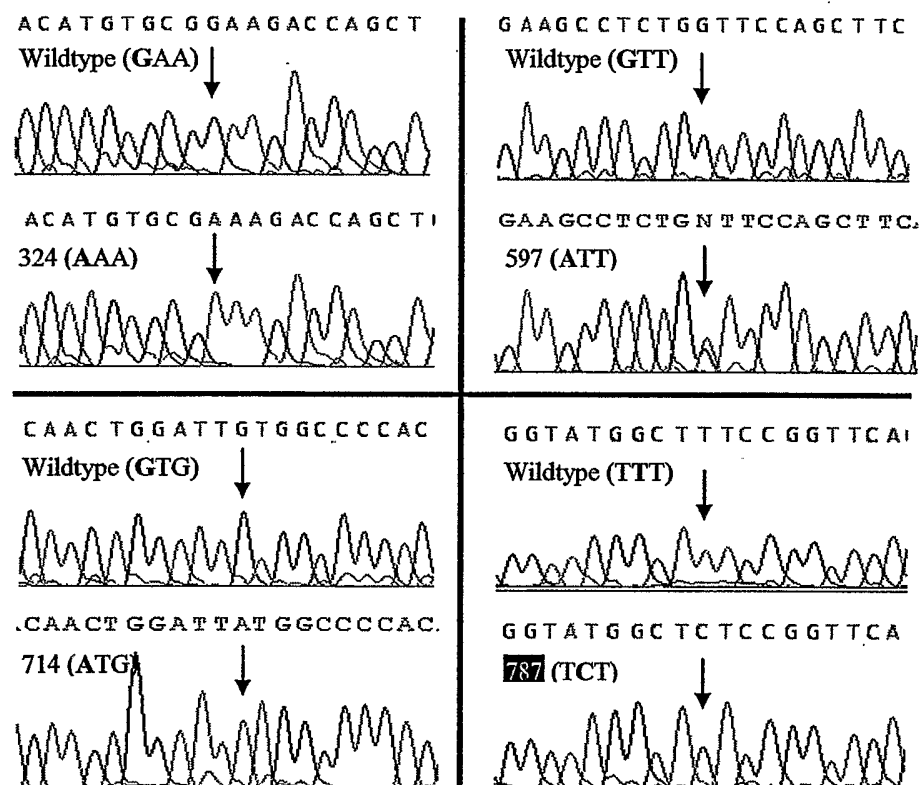
S395F [787]

351 ECELHDFRLS FSQKWDNWI VAPHKYNPRY CKGDCPRAVG HRYGSPVHTM

401 VQNIIEKLD SSVPRPSCVP AKYSPLSVLA IEPDGSIAKY EYEDMIATKC

(135)

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Figure 1b

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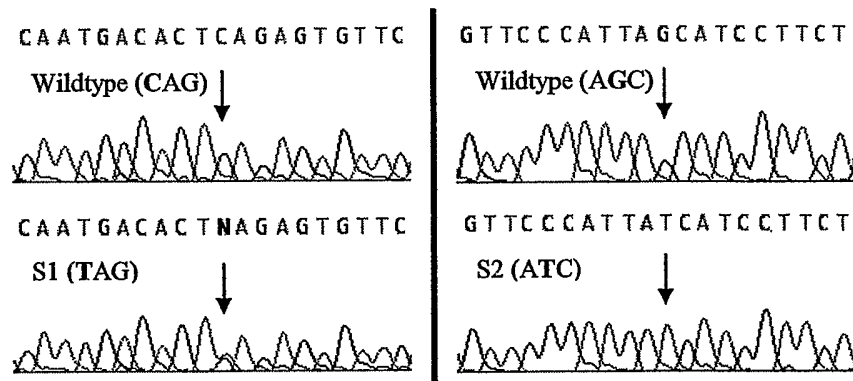
Figure 2b

Figure 3a

Animal		R 8 3 0	9 3 0 4 5 8	9 3 0 4 5 9	9 7 0 4	9 3 0 8 1 1	9 3 0 8 1 2	8 7 8 3	9 3 0 8 1 0	9 4 8 3 0 2	7 8 1 0
Phenotype Sterile (S) Fertile (F)		n/a	S	S	F	S	S	F	F	F	F
G E N O T Y P E	S1	+/Y	S1/+	S1/+	S1/+	+/+	+/+	+/+	+/+	+/+	+/+
	S2	S2/Y	S2/+	S2/+	+/+	S2/+	S2/+	+/+	S2/+	S2/+	+/+
	787	T/+	T/+	+/+	+/+	T/T	T/T	T/+	+/+	+/+	+/+

Figure 3b

Animal		9 6 2 1 0 1	9 9 7 6 3 4	9 9 7 6 3 5	9 6 2 1 5 2	9 9 7 5 5 2	9 9 7 5 5 3	9 7 6 2 3 4	9 3 0 1 4 2	9 4 8 0 9 3	8 8 7 4
Phenotype Sterile (S) Fertile (F)		n/a	S	S	F	F	S	F	n/a	S	F
G E N O T Y P E	S1	S1/Y	S1/S1	S1/S1	S1/+	S1/+	S1/S1	S1/+	S1/Y	S1/+	+/+
	S2	+/Y	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
	787	T/+	T/T	T/T	T/+	T/+	T/+	+/+	T/T	T/T	T/+

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Figure 4

Sheep GDF9															
atg	gcg	ctt	ccc	aac	aaa	ttc	ttc	ctt	tgg	ttt	tgc	tgc	ttt	gcc	45
Met	Ala	Leu	Pro	Asn	Lys	Phe	Phe	Leu	Trp	Phe	Cys	Cys	Phe	Ala	
			-315					-310					-305		
tgg	ctc	tgt	ttt	cct	att	agc	ctt	gat	tct	ctg	cct	tct	agg	gga	90
Trp	Leu	Cys	Phe	Pro	Ile	Ser	Leu	Asp	Ser	Leu	Pro	Ser	Arg	Gly	
			-300					-295					-290		
gaa	gct	cag	att	gta	gct	agg	act	gcg	ttg	gaa	tct	gag	gct	gag	135
Glu	Ala	Gln	Ile	Val	Ala	Arg	Thr	Ala	Leu	Glu	Ser	Glu	Ala	Glu	
			-285					-280					-275		
act	tgg	tcc	ttg	ctg	aac	cat	tta	ggt	ggg	aga	cac	aga	cct	ggt	180
Thr	Trp	Ser	Leu	Leu	Asn	His	Leu	Gly	Gly	Arg	His	Arg	Pro	Gly	
			-270					-265					-260		
ctc	ctt	tcc	cct	ctc	tta	gag	gtt	ctg	tat	gat	ggg	cac	ggg	gaa	225
Leu	Leu	Ser	Pro	Leu	Leu	Glu	Val	Leu	Tyr	Asp	Gly	His	Gly	Glu	
			-255					-250					-245		
[E1]															
ccc	ccc	agg	ctg	cag	cca	gat	gac	aga	gct	ttg	cgc	tac	atg	aag	270
Pro	Pro	Arg	Leu	Gln	Pro	Asp	Asp	Arg	Ala	Leu	Arg	Tyr	Met	Lys	
			-240					-235					-230		
agg	ctc	tat	aag	gca	tac	gct	acc	aag	gag	ggg	acc	cct	aaa	tcc	315
Arg	Leu	Tyr	Lys	Ala	Tyr	Ala	Thr	Lys	Glu	Gly	Thr	Pro	Lys	Ser	
			-225					-220					-215		
aac	aga	cgc	cac	ctc	tac	aac	act	gtt	cgg	ctc	ttc	acc	ccc	tgt	360
Asn	Arg	Arg	His	Leu	Tyr	Asn	Thr	Val	Arg	Leu	Phe	Thr	Pro	Cys	
			-210					-205					-200		
Intron position															
gct	cag	cac	aag	cag	gct	cct	ggg	gac	ctg	gcg	gca	gga	acc	ttt	405
Ala	Gln	His	Lys	Gln	Ala	Pro	Gly	Asp	Leu	Ala	Ala	Gly	Thr	Phe	
			-195					-190					-185		
cca	tca	gtg	gat	ctg	ctg	ttt	aac	ctg	gat	cgt	gtt	act	gtt	gtg	450
Pro	Ser	Val	Asp	Leu	Leu	Phe	Asn	Leu	Asp	Arg	Val	Thr	Val	Val	
			-180					-175					-170		
[74] [80]															
gaa	cat	tta	ttc	aag	tca	gtt	ttg	ctg	tat	act	ttc	aac	aac	tcc	495
Glu	His	Leu	Phe	Lys	Ser	Val	Leu	Leu	Tyr	Thr	Phe	Asn	Asn	Ser	
			-165					-160					-155		
att	tct	ttt	ccc	ttt	cct	gtt	aaa	tgt	ata	tgc	aac	ctg	gtg	ata	540
Ile	Ser	Phe	Pro	Phe	Pro	Val	Lys	Cys	Ile	Cys	Asn	Leu	Val	Ile	
			-150					-145					-140		
aaa	gag	cca	gag	ttt	tct	agc	aag	act	ctc	cct	aga	gct	cca	tac	585
Lys	Glu	Pro	Glu	Phe	Ser	Ser	Lys	Thr	Leu	Pro	Arg	Ala	Pro	Tyr	
			-135					-130					-125		
tca	ttt	acc	tat	aac	tca	cag	ttt	gaa	ttt	aga	aag	aaa	tac	aaa	630
Ser	Phe	Thr	Tyr	Asn	Ser	Gln	Phe	Glu	Phe	Arg	Lys	Lys	Tyr	Lys	
			-120					-115					-110		
tgg	atg	gag	att	gat	gtg	acg	gct	cct	ctt	gag	cct	ctg	gtg	gcc	678
Trp	Met	Glu	Ile	Asp	Val	Thr	Ala	Pro	Leu	Glu	Pro	Leu	Val	Ala	
			-105					-100					-95		
[324]															

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Figure 4 continued

cac aag agg aat att cac atg tct gta aat ttt aca tgt gcg <u>gaa</u> gac His Lys Arg Asn Ile His Met Ser Val Asn Phe Thr Cys Ala <u>Glu</u> Asp -90 -85 -80	726
cag ctg cag cat cct tca gcg cgg gac agc ctg ttt aac atg act ctt Gln Leu Gln His Pro Ser Ala Arg Asp Ser Leu Phe Asn Met Thr Leu -75 -70 -65	774
ctc gta gcg ccc tca ctg ctt ttg tat ctg aac gac aca agt gct cag Leu Val Ala Pro Ser Leu Leu Leu Tyr Leu Asn Asp Thr Ser Ala Gln -60 -55 -50 -45	822
gct ttt cac agg tgg cat tcc ctc cac cct aaa agg aag cct tca cag Ala Phe His Arg Trp His Ser Leu His Pro Lys Arg Lys Pro Ser Gln -40 -35 -30	870
ggt cct gac cag aag aga ggg cta tct gcc tac ccc gtg gga gaa gaa Gly Pro Asp Gln Lys Arg Gly Leu Ser Ala Tyr Pro Val Gly Glu Glu -25 -20 -15	918
gct gct gag ggt gta aga tcg tcc cgt cac cgc aga gac cag gag agt Ala Ala Glu Gly Val Arg Ser Ser Arg His Arg Arg Asp Gln Glu Ser -10 -5 -1 1	966
[581] gcc agc tct ga <u>a</u> ttg aag aag cct ctg [597] cca gct tca gtc aat ctg Ala Ser Ser Glu Leu Lys Lys Pro Leu <u>Val</u> Pro Ala Ser Val Asn Leu 5 10 15 20	1014
agt gaa tac ttc aaa cag ttt ctt ttt ccc cag aat gaa tgt gag ctc Ser Glu Tyr Phe Lys Gln Phe Leu Phe Pro Gln Asn Glu Cys Glu Leu 25 30 35	1062
cat gac ttt aga ctt agc ttt agt cag ctg aag tgg gac aac tgg att His Asp Phe Arg Leu Ser Phe Ser Gln Leu Lys Trp Asp Asn Trp Ile 40 45 50	1110
[714] <u>g</u> tg gcc cca cac aaa tac aac cct cga tac tgt aaa ggg gac tgt ccc <u>Val</u> Ala Pro His Lys Tyr Asn Pro Arg Tyr Cys Lys Gly Asp Cys Pro 55 60 65	1158
agg gcg gtc gga cat cgg tat ggc t <u>ct</u> ccg gtt cac acc atg gtg cag Arg Ala Val Gly His Arg Tyr Gly <u>Ser</u> Pro Val His Thr Met Val Gln 70 75 80	1206
aac atc atc cat gag aaa ctt gac tcc tca gtg cca aga cca tcc tgt Asn Ile Ile His Glu Lys Leu Asp Ser Ser Val Pro Arg Pro Ser Cys 85 90 95 100	1254
gta cct gcc aag tat agc cct ttg agt gtt ttg gcc atc gag cct gat Val Pro Ala Lys Tyr Ser Pro Leu Ser Val Leu Ala Ile Glu Pro Asp 105 110 115	1302
ggc tca atc gct tat aaa gaa tat gaa gat atg ata gcc act aag tgt Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp Met Ile Ala Thr Lys Cys 120 125 130	1350
acc tgt cgt taa cagactc ctgtcaagta aaaccatgag tgtcctggcc Thr Cys Arg STOP 135	1399
agtgtaaatg ccgcgcc	1416

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Figure 5

Sheep BMP15 full

.	.atg gtc ctc ctg	agc atc ctt aga atc	27
.	.Met Val Leu Leu	Ser Ile Leu Arg Ile	
	-265	-260	
[Leu] deletion			
ctt ctt tgg gga ctg	gtg ctt ttt atg gaa	cat agg gtc caa atg	72
Leu Leu Trp Gly	Leu Val Leu Phe Met Glu	His Arg Val Gln Met	
	-255	-250	-245
aca cag gta ggg cag	ccc tct att gcc cac	ctg cct gag gcc cct	117
Thr Gln Val Gly Gln	Pro Ser Ile Ala His	Leu Pro Glu Ala Pro	
	-240	-235	-230
acc ttg ccc ctg att	cag gag ctg cta gaa	gaa gcc cct ggc aag	162
Thr Leu Pro Leu Ile	Gln Glu Leu Leu Glu	Glu Ala Pro Gly Lys	
	-225	-220	-215
cag cag agg aag ccg	cgg gtc tta ggg cat	ccc tta cgg tat atg	207
Gln Gln Arg Lys Pro	Arg Val Leu Gly His	Pro Leu Arg Tyr Met	
	-210	-205	-200
ctg gag ctg tac cag	cgt tca gct gac gca	agt gga cac cct agg	252
Leu Glu Leu Tyr Gln	Arg Ser Ala Asp Ala	Ser Gly His Pro Arg	
	-195	-190	-185
gaa aac cgc acc att	ggg gcc acc atg gtg	agg ctg gtg agg ccg	297
Glu Asn Arg Thr Ile	Gly Ala Thr Met Val	Arg Leu Val Arg Pro	
	-180	-175	-170
Intron position			
ctg gct agt gta gca	agg cct ctc aga g-----gc		327
Leu Ala Ser Val Ala	Arg Pro Leu Arg	Gly	
	-165	-160	
tcc tgg cac ata cag	acc ctg gac ttt cct	ctg aga cca aac cgg	372
Ser Trp His Ile Gln	Thr Leu Asp Phe Pro	Leu Arg Pro Asn Arg	
	-155	-150	-145
gta gca tac caa cta	gtc aga gcc act gtg	gtt tac cgc cat cag	417
Val Ala Tyr Gln Leu	Val Arg Ala Thr Val	Val Tyr Arg His Gln	
	-140	-135	-130
ctt cac cta act cat	tcc cac ctc tcc tgc	cat gtg gag ccc tgg	462
Leu His Leu Thr His	Ser His Leu Ser Cys	His Val Glu Pro Trp	
	-125	-120	-115
gtc cag aaa agc cca	acc aat cac ttt cct	tct tca gga aga ggc	507
Val Gln Lys Ser Pro	Thr Asn His Phe Pro	Ser Ser Gly Arg Gly	
	-110	-105	-100
tcc tca aag cct tcc	ctg ttg ccc aaa act	tgg aca gag atg gat atc	555
Ser Ser Lys Pro Ser	Leu Leu Pro Lys Thr	Trp Thr Glu Met Asp Ile	
	-95	-90	-85
atg gaa cat gtt ggg	caa aag ctc tgg aat	cac aag ggg cgc agg gtt	603
Met Glu His Val Gly	Gln Lys Leu Trp Asn	His Lys Gly Arg Arg Val	
	-80	-75	-70

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Figure 5 continued

```

cta cga ctc cgc ttc gtg tgt cag cag cca aga ggt agt gag gtt ctt      651
Leu Arg Leu Arg Phe Val Cys Gln Gln Pro Arg Gly Ser Glu Val Leu
      -65                      -60                      -55

gag ttc tgg tgg cat ggc act tca tca ttg gac act gtc ttc ttg tta      699
Glu Phe Trp Trp His Gly Thr Ser Ser Leu Asp Thr Val Phe Leu Leu
      -50                      -45                      -40

          [S1]                      [422]
ctg tat ttc aat gac act cag agt gtt cag aag acc aaa cct ctc cct      747
Leu Tyr Phe Asn Asp Thr Gln Ser Val Gln Lys Thr Lys Pro Leu Pro
-35                      -30                      -25                      -20

aaa ggc ctg aaa gag ttt aca gaa aaa gac cct tct ctt ctc ttg agg      795
Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser Leu Leu Leu Arg
      -15                      -10                      -5

agg gct cgt caa gca ggc agt att gca tcg gaa gtt cct ggc ccc tcc      843
Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val Pro Gly Pro Ser
      -1  1                      5                      10

agg gag cat gat ggg cct gaa agt aac cag tgt tcc ctc cac cct ttt      891
Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser Leu His Pro Phe
      15                      20          ***          25

caa gtc agc ttc cag cag ctg ggc tgg gat cac tgg atc att gct ccc      939
Gln Val Ser Phe Gln Gln Leu Gly Trp Asp His Trp Ile Ile Ala Pro
30  ***          35                      40          45

cat ctc tat acc cca aac tac tgt aag gga gta tgt cct cgg gta cta      987
His Leu Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys Pro Arg Val Leu
      50                      55                      60

cac tat ggt ctc aat tct ccc aat cat gcc atc atc cag aac ctt gtc      1035
His Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile Gln Asn Leu Val
      65                      70                      75

agt gag ctg gtg gat cag aat gtc cct cag cct tcc tgt gtc cct tat      1083
Ser Glu Leu Val Asp Gln Asn Val Pro Gln Pro Ser Cys Val Pro Tyr
      80                      85                      90

          [S2]
aag tat gtt ccc att agc atc ctt ctg att gag gca aat ggg agt atc      1131
Lys Tyr Val Pro Ile Ser Ile Leu Leu Ile Glu Ala Asn Gly Ser Ile
      95                      100                      105

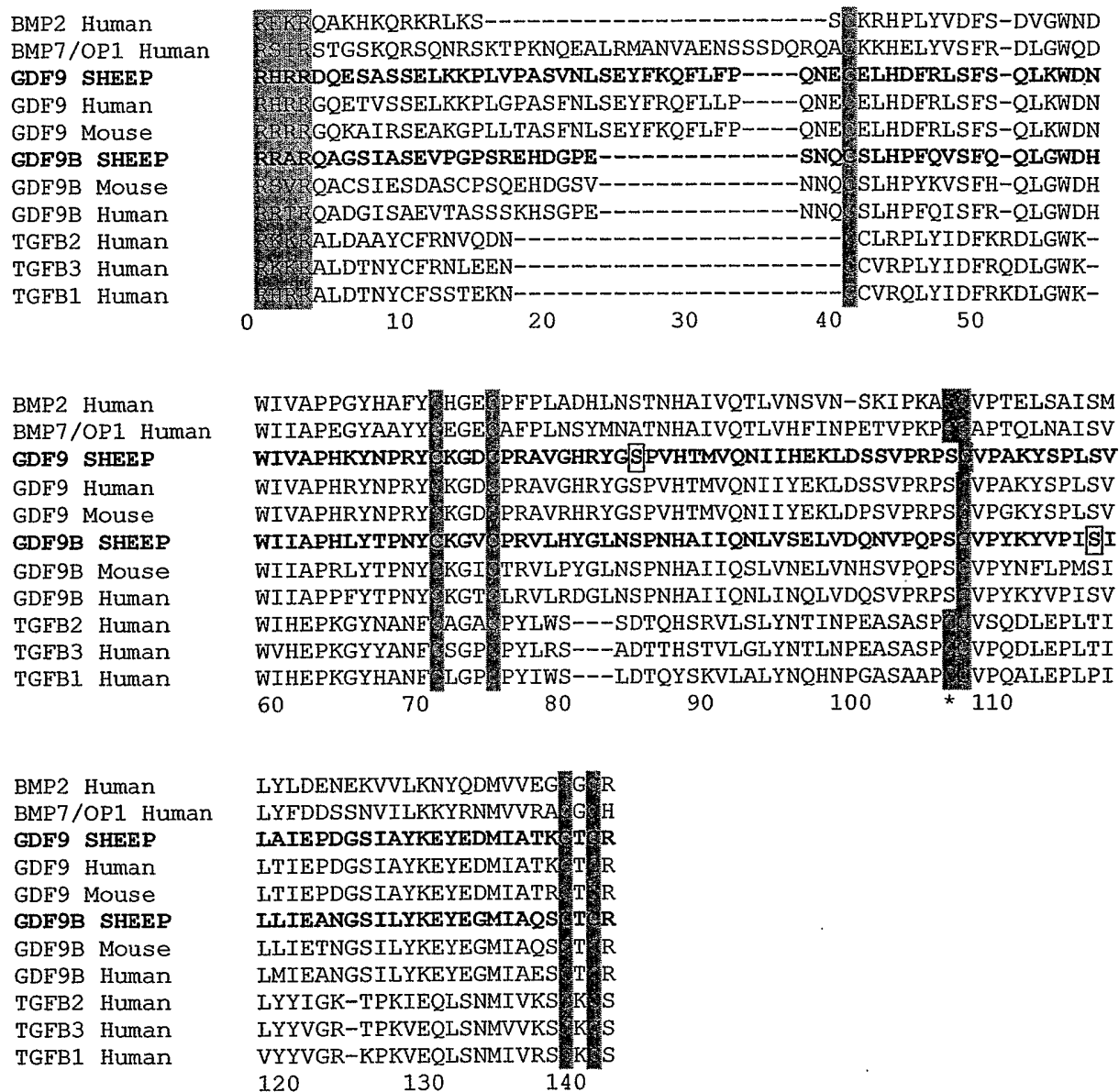
ttg tac aag gag tat gag ggt atg att gcc cag tcc tgc aca tgc agg      1179
Leu Tyr Lys Glu Tyr Glu Gly Met Ile Ala Gln Ser Cys Thr Cys Arg
110                      115                      120                      125

tga cggcaaaggtgca
STOP

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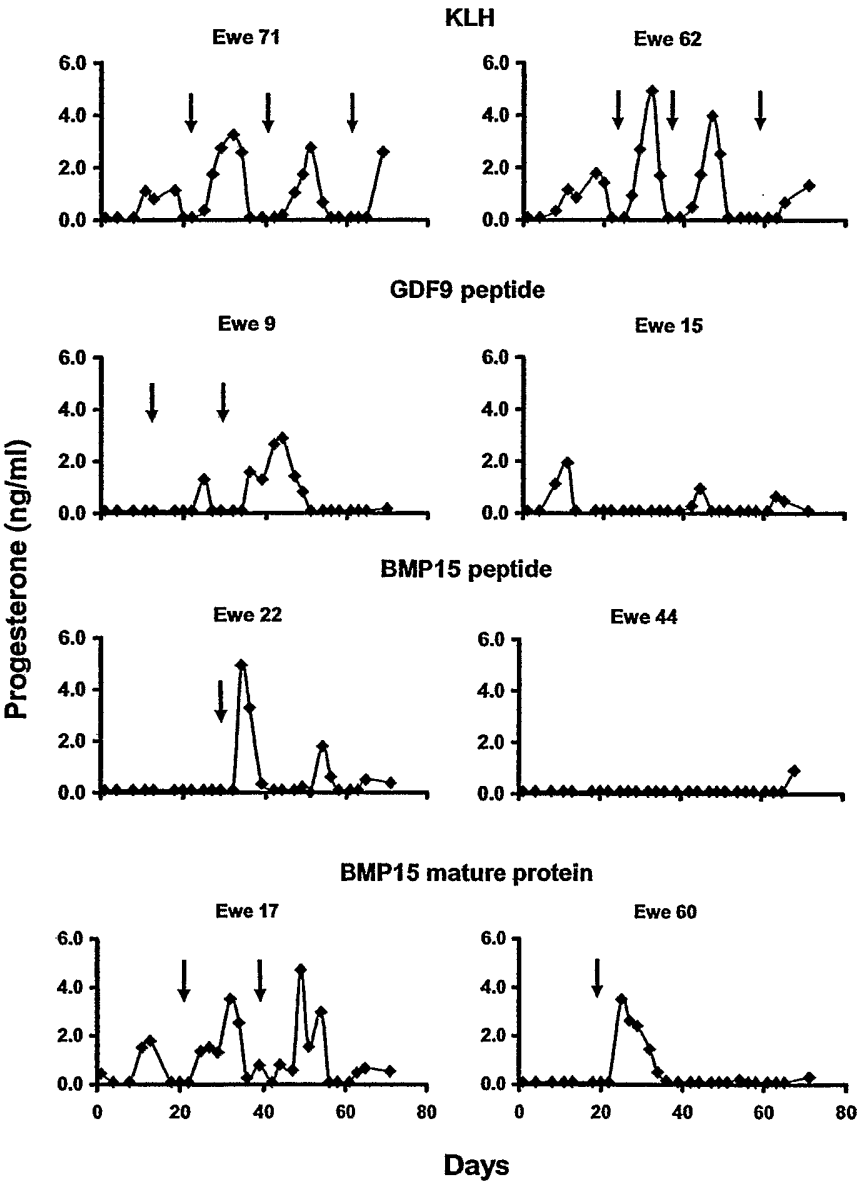
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Figure 6

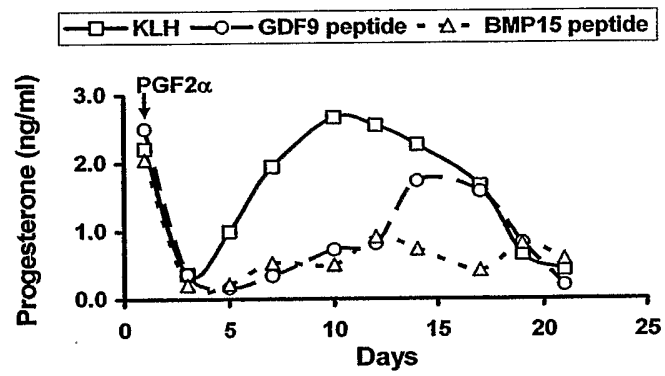


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Figure 7



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Figure 8

SEQUENCE LISTING

5 <110> Galloway, Susan May
Davis, George Henry
Gregan, Scott Michael

10 Hanrahan, James Patrick
Juengel, Jennifer Lee
McNatty, Kenneth Pattrick

15 Mulsant, Philippe
Powell, Richard Patrick

20

<120> New sequences for altering mammalian ovarian function and
ovulation rate
<170> PatentIn version 3.0

25 <210> 1: GDF-9 [787] Full
<211> 1879
<212> DNA
<213> Ovis aries

30 <221> 5'UTR
<222> (1)..(121)
<221> misc feature
<222> (122)..(124)
<223> atg start codon.

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<221> CDS
<221> Intron
<222> (519)..(838)

40 <223> n at 709 represents remainder of approx 900 bp unsequenced of
the approx 1.1 kb intron
<221> mat_peptide
<222> (1396)..()
<221> misc feature

45 <222> (709)..()
<223> n represents approximately 900 bp of unsequenced intron.
<221> misc feature
<222> (1801)..(1803)
<223> taa stop codon.

50 <221> 3'UTR
<222> (1804)..(1879)
<221> mutation
<222> (1624)..(1626)
<223> c to t at 1625 in [787] sheep changing tct serine codon to ttt
phenylalanine

55

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gaattgaacc tagccacccc acacacctaa agtttatatta agagaccaac cgaggctctt
60
cctgggttttt aggaagaaga ctggtatggg gaaatgtgtt ccttgctaatt tcttccaagc
120

2

c atg gcg ctt ccc aac aaa ttc ttc ctt tgg ttt tgc tgc ttt gcc
 166
 Met Ala Leu Pro Asn Lys Phe Phe Leu Trp Phe Cys Cys Phe Ala
 -315 -310 -305

5 tgg ctc tgt ttt cct att agc ctt gat tct ctg cct tct agg gga
 211
 Trp Leu Cys Phe Pro Ile Ser Leu Asp Ser Leu Pro Ser Arg Gly
 -300 -295 -290

10 gaa gct cag att gta gct agg act gcg ttg gaa tct gag gct gag
 256
 Glu Ala Gln Ile Val Ala Arg Thr Ala Leu Glu Ser Glu Ala Glu
 -285 -280 -275

15 act tgg tcc ttg ctg aac cat tta ggt ggg aga cac aga cct ggt
 301
 Thr Trp Ser Leu Leu Asn His Leu Gly Gly Arg His Arg Pro Gly
 -270 -265 -260

20 ctc ctt tcc cct ctc tta gag gtt ctg tat gat ggg cac ggg gaa
 346
 Leu Leu Ser Pro Leu Leu Glu Val Leu Tyr Asp Gly His Gly Glu
 -255 -250 -245

25 ccc ccc agg ctg cag cca gat gac aga gct ttg cgc tac atg aag
 391
 Pro Pro Arg Leu Gln Pro Asp Asp Arg Ala Leu Arg Tyr Met Lys
 -240 -235 -230

30 agg ctc tat aag gca tac gct acc aag gag ggg acc cct aaa tcc
 436
 Arg Leu Tyr Lys Ala Tyr Ala Thr Lys Glu Gly Thr Pro Lys Ser
 -225 -220 -215

35 aac aga cgc cac ctc tac aac act gtt cgg ctc ttc acc ccc tgt
 481
 Asn Arg Arg His Leu Tyr Asn Thr Val Arg Leu Phe Thr Pro Cys
 -210 -205 -200

40 gct cag cac aag cag gct cct ggg gac ctg gcg gca g gtgtgtagga
 528
 Ala Gln His Lys Gln Ala Pro Gly Asp Leu Ala Ala
 -195 -190

45 gcagattggt taatgggtgg agggaagaag aaagaccttt ttgcatttca gttacataaa
 588

50 ggagttggcc ctgctccttg acttgcatatt tactttgcat ggtactcaat atccaaacaa
 648

acctgggtgct tgatcttact tactgtttat tcctaattggc ctcatgggtt gatgtaggct
 708

55 natcccaccc tgacgttttaa ggcttgagaa tgtggggaga aaagggacag aagcacattc
 768

tgagggtactg attccttgat ttgacttcct gttacatatg gcattactgt tggattgttt
 828

60 ttcttctcag ga acc ttt cca tca gtg gat ctg ctg ttt aac ctg gat
 876
 Gly Thr Phe Pro Ser Val Asp Leu Leu Phe Asn Leu Asp

3

	-185	-180	-175
	cgt gtt act gtt	gtg gaa cat tta ttc	aag tca gtc ttg ctg tat
5	921 Arg Val Thr Val -170	Val Glu His Leu Phe -165	Lys Ser Val Leu Leu Tyr -160
	act ttc aac aac	tcc att tct ttt ccc	ttt cct gtt aaa tgt ata
10	966 Thr Phe Asn Asn -155	Ser Ile Ser Phe Pro -150	Phe Pro Val Lys Cys Ile -145
	tgc aac ctg gtg	ata aaa gag cca gag	ttt tct agc aag act ctc
15	1011 Cys Asn Leu Val -140	Ile Lys Glu Pro Glu -135	Phe Ser Ser Lys Thr Leu -130
	cct aga gct cca	tac tca ttt acc tat	aac tca cag ttt gaa ttt
20	1056 Pro Arg Ala Pro -125	Tyr Ser Phe Thr Tyr -120	Asn Ser Gln Phe Glu Phe -115
	aga aag aaa tac	aaa tgg atg gag att	gat gtg acg gct cct ctt
25	1101 Arg Lys Lys Tyr -110	Lys Trp Met Glu Ile -105	Asp Val Thr Ala Pro Leu -100
	gag cct ctg gtg gcc tcc cac aag agg aat att cac atg tct gta aat		
30	1149 Glu Pro Leu Val Ala Ser His Lys Arg Asn Ile His Met Ser Val Asn -95	-90	-85
	ttt aca tgt gcg gaa gac cag ctg cag cat cct tca gcg cgg gac agc		
35	1197 Phe Thr Cys Ala Glu Asp Gln Leu Gln His Pro Ser Ala Arg Asp Ser -80	-75	-70
	ctg ttt aac atg act ctt ctc gta gcg ccc tca ctg ctt ttg tat ctg		
40	1245 Leu Phe Asn Met Thr Leu Leu Val Ala Pro Ser Leu Leu Leu Tyr Leu -65	-60	-55
	aac gac aca agt gct cag gct ttt cac agg tgg cat tcc ctc cac cct		
45	1293 Asn Asp Thr Ser Ala Gln Ala Phe His Arg Trp His Ser Leu His Pro -50	-45	-40
	aaa agg aag cct tca cag ggt cct gac cag aag aga ggg cta tct gcc		
50	1341 Lys Arg Lys Pro Ser Gln Gly Pro Asp Gln Lys Arg Gly Leu Ser Ala -30	-25	-20
	tac ccc gtg gga gaa gaa gct gct gag ggt gta aga tcg tcc cgt cac		
55	1389 Tyr Pro Val Gly Glu Glu Ala Ala Glu Gly Val Arg Ser Ser Arg His -15	-10	-5
	cgc aga gac cag gag agt gcc agc tct gaa ttg aag aag cct ctg gtt		
60	1437 Arg Arg Asp Gln Glu Ser Ala Ser Ser Glu Leu Lys Lys Pro Leu Val -1 1	5	10

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cca gct tca gtc aat ctg agt gaa tac ttc aaa cag ttt ctt ttt ccc
1485
Pro Ala Ser Val Asn Leu Ser Glu Tyr Phe Lys Gln Phe Leu Phe Pro
15          20          25          30
5
cag aat gaa tgt gag ctc cat gac ttt aga ctt agc ttt agt cag ctg
1533
Gln Asn Glu Cys Glu Leu His Asp Phe Arg Leu Ser Phe Ser Gln Leu
35          40          45
10
aag tgg gac aac tgg att gtg gcc cca cac aaa tac aac cct cga tac
1581
Lys Trp Asp Asn Trp Ile Val Ala Pro His Lys Tyr Asn Pro Arg Tyr
50          55          60
15
tgt aaa ggg gac tgt ccc agg gcg gtc gga cat cgg tat ggc ttt ccg
1629
Cys Lys Gly Asp Cys Pro Arg Ala Val Gly His Arg Tyr Gly Phe Pro
65          70          75
20
gtt cac acc atg gtg cag aac atc atc cat gag aaa ctt gac tcc tca
1677
Val His Thr Met Val Gln Asn Ile Ile His Glu Lys Leu Asp Ser Ser
80          85          90
25
gtg cca aga cca tcc tgt gta cct gcc aag tat agc cct ttg agt gtt
1725
Val Pro Arg Pro Ser Cys Val Pro Ala Lys Tyr Ser Pro Leu Ser Val
95          100          105          110
30
ttg gcc atc gag cct gat ggc tca atc gct tat aaa gaa tat gaa gat
1773
Leu Ala Ile Glu Pro Asp Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp
115          120          125
35
atg ata gcc act aag tgt acc tgt cgt taacagactc ctgtcaagta
1820
Met Ile Ala Thr Lys Cys Thr Cys Arg
130          135
40
aaaccatgag tgtcctggcc agtgtaaag cgcgcgccct gtctatgcct ttgggagga
1879

45 <210> 2: protein GDF-9[787] full
    <211> 453
    <212> PRT
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50 <221> misc feature
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55 <223> n represents approximately 900 bp of unsequenced intron
    <221> misc feature
    <222> (1801)..(1803)
    <223> taa stop codon.

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5

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

6

His Lys Arg Asn Ile His Met Ser Val Asn Phe Thr Cys Ala Glu Asp
 -90 -85 -80

5 Gln Leu Gln His Pro Ser Ala Arg Asp Ser Leu Phe Asn Met Thr Leu
 -75 -70 -65

10 Leu Val Ala Pro Ser Leu Leu Leu Tyr Leu Asn Asp Thr Ser Ala Gln
 -60 -55 -50 -45

15 Ala Phe His Arg Trp His Ser Leu His Pro Lys Arg Lys Pro Ser Gln
 -40 -35 -30

20 Gly Pro Asp Gln Lys Arg Gly Leu Ser Ala Tyr Pro Val Gly Glu Glu
 -25 -20 -15

25 Ala Ala Glu Gly Val Arg Ser Ser Arg His Arg Arg Asp Gln Glu Ser
 -10 -5 -1 1

30 Ala Ser Ser Glu Leu Lys Lys Pro Leu Val Pro Ala Ser Val Asn Leu
 5 10 15 20

35 Ser Glu Tyr Phe Lys Gln Phe Leu Phe Pro Gln Asn Glu Cys Glu Leu
 25 30 35

40 His Asp Phe Arg Leu Ser Phe Ser Gln Leu Lys Trp Asp Asn Trp Ile
 40 45 50

45 Val Ala Pro His Lys Tyr Asn Pro Arg Tyr Cys Lys Gly Asp Cys Pro
 55 60 65

50 Arg Ala Val Gly His Arg Tyr Gly Phe Pro Val His Thr Met Val Gln
 70 75 80

55 Asn Ile Ile His Glu Lys Leu Asp Ser Ser Val Pro Arg Pro Ser Cys
 85 90 95 100

60 Val Pro Ala Lys Tyr Ser Pro Leu Ser Val Leu Ala Ile Glu Pro Asp
 105 110 115

65 Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp Met Ile Ala Thr Lys Cys
 120 125 130

70 Thr Cys Arg
 135

<210> 3: GDF-9 [787] coding
 <211> 1362
 <212> DNA
 <213> Ovis aries
 5 <221> misc_feature
 <222> (1)..(3)
 <223> atg start codon.
 <221> CDS
 10 <222> (1)..(1359)
 <221> mat_peptide
 <222> (955)..()
 <221> mutation
 <222> (1183)..(1185)
 15 <223> c to t 1184 in [787] sheep changing serine tct codon to ttt
 phenylalanine
 <221> misc_feature
 <222> (1360)..(1362)
 <223> taa stop codon.
 20 <400> 3
 atg gcg ctt ccc aac aaa ttc ttc ctt tgg ttt tgc tgc ttt gcc
 45
 Met Ala Leu Pro Asn Lys Phe Phe Leu Trp Phe Cys Cys Phe Ala
 -315 -310 -305
 25 tgg ctc tgt ttt cct att agc ctt gat tct ctg cct tct agg gga
 90
 Trp Leu Cys Phe Pro Ile Ser Leu Asp Ser Leu Pro Ser Arg Gly
 -300 -295 -290
 30 gaa gct cag att gta gct agg act gcg ttg gaa tct gag gct gag
 135
 Glu Ala Gln Ile Val Ala Arg Thr Ala Leu Glu Ser Glu Ala Glu
 -285 -280 -275
 35 act tgg tcc ttg ctg aac cat tta ggt ggg aga cac aga cct ggt
 180
 Thr Trp Ser Leu Leu Asn His Leu Gly Gly Arg His Arg Pro Gly
 -270 -265 -260
 40 ctc ctt tcc cct ctc tta gag gtt ctg tat gat ggg cac ggg gaa
 225
 Leu Leu Ser Pro Leu Leu Glu Val Leu Tyr Asp Gly His Gly Glu
 -255 -250 -245
 45 ccc ccc agg ctg cag cca gat gac aga gct ttg cgc tac atg aag
 270
 Pro Pro Arg Leu Gln Pro Asp Asp Arg Ala Leu Arg Tyr Met Lys
 -240 -235 -230
 50 agg ctc tat aag gca tac gct acc aag gag ggg acc cct aaa tcc
 315
 Arg Leu Tyr Lys Ala Tyr Ala Thr Lys Glu Gly Thr Pro Lys Ser
 -225 -220 -215
 55 aac aga cgc cac ctc tac aac act gtt cgg ctc ttc acc ccc tgt
 360
 Asn Arg Arg His Leu Tyr Asn Thr Val Arg Leu Phe Thr Pro Cys
 -210 -205 -200
 60 gct cag cac aag cag gct cct ggg gac ctg gcg gca gga acc ttt
 405
 Ala Gln His Lys Gln Ala Pro Gly Asp Leu Ala Ala Gly Thr Phe

			-195					-190						-185	
	cca	tca	gtg	gat	ctg	ctg	ttt	aac	ctg	gat	cgt	gtt	act	gtt	gtg
5	450	Pro	Ser	Val	Asp	Leu	Leu	Phe	Asn	Leu	Asp	Arg	Val	Thr	Val
				-180						-175				-170	
	gaa	cat	tta	ttc	aag	tca	gtc	ttg	ctg	tat	act	ttc	aac	aac	tcc
10	495	Glu	His	Leu	Phe	Lys	Ser	Val	Leu	Leu	Tyr	Thr	Phe	Asn	Ser
				-165						-160				-155	
	att	tct	ttt	ccc	ttt	cct	gtt	aaa	tgt	ata	tgc	aac	ctg	gtg	ata
15	540	Ile	Ser	Phe	Pro	Phe	Pro	Val	Lys	Cys	Ile	Cys	Asn	Leu	Ile
				-150						-145				-140	
	aaa	gag	cca	gag	ttt	tct	agc	aag	act	ctc	cct	aga	gct	cca	tac
20	585	Lys	Glu	Pro	Glu	Phe	Ser	Ser	Lys	Thr	Leu	Pro	Arg	Ala	Tyr
				-135						-130				-125	
	tca	ttt	acc	tat	aac	tca	cag	ttt	gaa	ttt	aga	aag	aaa	tac	aaa
25	630	Ser	Phe	Thr	Tyr	Asn	Ser	Gln	Phe	Glu	Phe	Arg	Lys	Lys	Lys
				-120						-115				-110	
	tgg	atg	gag	att	gat	gtg	acg	gct	cct	ctt	gag	cct	ctg	gtg	gcc
30	678	Trp	Met	Glu	Ile	Asp	Val	Thr	Ala	Pro	Leu	Glu	Pro	Leu	Ser
				-105						-100				-95	
	cac	aag	agg	aat	att	cac	atg	tct	gta	aat	ttt	aca	tgt	gcg	gaa
35	726	His	Lys	Arg	Asn	Ile	His	Met	Ser	Val	Asn	Phe	Thr	Cys	Ala
				-90					-85					-80	
	cag	ctg	cag	cat	cct	tca	gcg	cgg	gac	agc	ctg	ttt	aac	atg	act
40	774	Gln	Leu	Gln	His	Pro	Ser	Ala	Arg	Asp	Ser	Leu	Phe	Asn	Met
				-75				-70					-65		
	ctc	gta	gcg	ccc	tca	ctg	ctt	ttg	tat	ctg	aac	gac	aca	agt	gct
45	822	Leu	Val	Ala	Pro	Ser	Leu	Leu	Tyr	Leu	Asn	Asp	Thr	Ser	Ala
		-60					-55				-50				-45
	gct	ttt	cac	agg	tgg	cat	tcc	ctc	cac	cct	aaa	agg	aag	cct	tca
50	870	Ala	Phe	His	Arg	Trp	His	Ser	Leu	His	Pro	Lys	Arg	Lys	Pro
					-40					-35					-30
	ggt	cct	gac	cag	aag	aga	ggg	cta	tct	gcc	tac	ccc	gtg	gga	gaa
55	918	Gly	Pro	Asp	Gln	Lys	Arg	Gly	Leu	Ser	Ala	Tyr	Pro	Val	Gly
				-25						-20				-15	
	gct	gct	gag	ggt	gta	aga	tcg	tcc	cgt	cac	cgc	aga	gac	cag	gag
60	966	Ala	Ala	Glu	Gly	Val	Arg	Ser	Ser	Arg	His	Arg	Arg	Asp	Gln
				-10				-5				-1	1		

gcc agc tct gaa ttg aag aag cct ctg gtt cca gct tca gtc aat ctg
 1014
 Ala Ser Ser Glu Leu Lys Lys Pro Leu Val Pro Ala Ser Val Asn Leu
 5 5 10 15 20
 5 agt gaa tac ttc aaa cag ttt ctt ttt ccc cag aat gaa tgt gag ctg
 1062
 Ser Glu Tyr Phe Lys Gln Phe Leu Phe Pro Gln Asn Glu Cys Glu Leu
 25 30 35
 10 cat gac ttt aga ctt agc ttt agt cag ctg aag tgg gac aac tgg att
 1110
 His Asp Phe Arg Leu Ser Phe Ser Gln Leu Lys Trp Asp Asn Trp Ile
 40 45 50
 15 gtg gcc cca cac aaa tac aac cct cga tac tgt aaa ggg gac tgt ccc
 1158
 Val Ala Pro His Lys Tyr Asn Pro Arg Tyr Cys Lys Gly Asp Cys Pro
 55 60 65
 20 agg gcg gtc gga cat cgg tat ggc ttt ccg gtt cac acc atg gtg cag
 1206
 Arg Ala Val Gly His Arg Tyr Gly Phe Pro Val His Thr Met Val Gln
 70 75 80
 25 aac atc atc cat gag aaa ctt gac tcc tca gtg cca aga cca tcc tgt
 1254
 Asn Ile Ile His Glu Lys Leu Asp Ser Ser Val Pro Arg Pro Ser Cys
 85 90 95 100
 30 gta cct gcc aag tat agc cct ttg agt gtt ttg gcc atc gag cct gat
 1302
 Val Pro Ala Lys Tyr Ser Pro Leu Ser Val Leu Ala Ile Glu Pro Asp
 105 110 115
 35 ggc tca atc gct tat aaa gaa tat gaa gat atg ata gcc act aag tgt
 1350
 Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp Met Ile Ala Thr Lys Cys
 120 125 130
 40 acc tgt cgt taa
 1362
 Thr Cys Arg
 135
 45
 <210> 4: Protein GDF-9 [787] coding
 <211> 453
 <212> PRT
 50 <213> Ovis aries
 <221> misc_feature
 <222> (1)..(3)
 <223> atg start codon.
 <221> misc_feature
 55 <222> (1360)..(1362)
 <223> taa stop codon.
 <400> 4
 60 Met Ala Leu Pro Asn Lys Phe Phe Leu Trp Phe Cys Cys Phe Ala
 -315 -310 -305

10

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

11

Leu Val Ala Pro Ser Leu Leu Leu Tyr Leu Asn Asp Thr Ser Ala Gln
 -60 -55 -50 -45
 5
 Ala Phe His Arg Trp His Ser Leu His Pro Lys Arg Lys Pro Ser Gln
 -40 -35 -30
 10
 Gly Pro Asp Gln Lys Arg Gly Leu Ser Ala Tyr Pro Val Gly Glu Glu
 -25 -20 -15
 15
 Ala Ala Glu Gly Val Arg Ser Ser Arg His Arg Arg Asp Gln Glu Ser
 -10 -5 -1 1
 20
 Ala Ser Ser Glu Leu Lys Lys Pro Leu Val Pro Ala Ser Val Asn Leu
 5 10 15 20
 Ser Glu Tyr Phe Lys Gln Phe Leu Phe Pro Gln Asn Glu Cys Glu Leu
 25 30 35
 His Asp Phe Arg Leu Ser Phe Ser Gln Leu Lys Trp Asp Asn Trp Ile
 40 45 50
 30
 Val Ala Pro His Lys Tyr Asn Pro Arg Tyr Cys Lys Gly Asp Cys Pro
 55 60 65
 35
 Arg Ala Val Gly His Arg Tyr Gly Phe Pro Val His Thr Met Val Gln
 70 75 80
 40
 Asn Ile Ile His Glu Lys Leu Asp Ser Ser Val Pro Arg Pro Ser Cys
 85 90 95 100
 Val Pro Ala Lys Tyr Ser Pro Leu Ser Val Leu Ala Ile Glu Pro Asp
 105 110 115
 45
 Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp Met Ile Ala Thr Lys Cys
 120 125 130
 50
 Thr Cys Arg
 135
 55 <210> 5: GDF-9 [787] mutation
 <211> 168
 <212> DNA
 <213> Ovis aries
 <221> CDS
 60 <222> (1)..(168)
 <221> mutation
 <222> (82)..(84)

12

<223> c to t at 83 in [787] sheep changing tct serine codon to ttt phenylalanine

<400> 5
 5 aac tgg att gtg gcc cca cac aaa tac aac cct cga tac tgt aaa ggg
 48
 Asn Trp Ile Val Ala Pro His Lys Tyr Asn Pro Arg Tyr Cys Lys Gly
 1 5 10 15

10 gac tgt ccc agg gcg gtc gga cat cgg tat ggc ttt ccg gtt cac acc
 96
 Asp Cys Pro Arg Ala Val Gly His Arg Tyr Gly Phe Pro Val His Thr
 20 25 30

15 atg gtg cag aac atc atc cat gag aaa ctt gac tcc tca gtg cca aga
 144
 Met Val Gln Asn Ile Ile His Glu Lys Leu Asp Ser Ser Val Pro Arg
 35 40 45

20 cca tcc tgt gta cct gcc aag tat
 168
 Pro Ser Cys Val Pro Ala Lys Tyr
 50 55

25
 <210> 6: protein GDF-9 [787] mutation
 <211> 56
 <212> PRT
 <213> Ovis aries

30
 <400> 6
 Asn Trp Ile Val Ala Pro His Lys Tyr Asn Pro Arg Tyr Cys Lys Gly
 1 5 10 15

35
 Asp Cys Pro Arg Ala Val Gly His Arg Tyr Gly Phe Pro Val His Thr
 20 25 30

40
 Met Val Gln Asn Ile Ile His Glu Lys Leu Asp Ser Ser Val Pro Arg
 35 40 45

45
 Pro Ser Cys Val Pro Ala Lys Tyr
 50 55

50
 <210> 7: GDF-9B [S1] full
 <211> 1665
 <212> DNA
 <213> Ovis aries
 <221> 5'UTR
 <222> (1)..(252)

55
 <221> misc feature
 <222> (253)..(255)
 <223> atg start codon.
 <221> CDS
 <222> (253)..(577)

60
 <221> CDS
 <222> (774)..(1165)
 <221> Intron
 <222> (578)..(773)

13

<223> n at 685 represents remainder of approx 5.2 kb intron.
 <221> misc_feature
 <222> (1253)..(1255)
 <223> position of first codon of mature peptide in wildtype sheep.
 5 <221> misc_feature
 <222> (685)..()
 <223> n represents approx 5.2 kb of intron.
 <221> misc_feature
 <222> (1628)..(1630)
 10 <223> tga stop codon in wildtype sheep.
 <221> 3'UTR
 <222> (1631)..(1665)
 <221> mutation
 <222> (1166)..(1168)
 15 <223> c to t at 1166 of [S1] sheep changes cag glutamine codon to tag
 STOP

 <400> 7
 20 catgctgcct tgtccacact gctgtttctg tttgtttgat gcaaagagga caatttagaa
 60

 gacctctttt tggttcagga gacacctacca gaggaagaaa cataggacct gcctgccagc
 120

 25 ctttcatttt tccttgccct atcctttgtg gtagtggagc ctggatgctg ttacccatgt
 180

 aaaaggaaag gtttaaagcg ttatcctttg ggcttttatc agaacatggt gctgaacacc
 240
 30 aagcttttca ag atg gtc ctc ctg agc atc ctt aga atc ctt ctt tgg gga
 291

 Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly
 1 5 10
 35 ctg gtg ctt ttt atg gaa cat agg gtc caa atg aca cag gta ggg cag
 339
 Leu Val Leu Leu Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln
 15 20 25
 40 ccc tct att gcc cac ctg cct gag gcc cct acc ttg ccc ctg att cag
 387
 Pro Ser Ile Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln
 30 35 40 45
 45 gag ctg cta gaa gaa gcc cct ggc aag cag cag agg aag ccg cgg gtc
 435
 Glu Leu Leu Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg Val
 50 55 60
 50 tta ggg cat ccc tta cgg tat atg ctg gag ctg tac cag cgt tca gct
 483
 Leu Gly His Pro Leu Arg Tyr Met Leu Glu Leu Tyr Gln Arg Ser Ala
 65 70 75
 55 gac gca agt gga cac cct agg gaa aac cgc acc att ggg gcc acc atg
 531
 Asp Ala Ser Gly His Pro Arg Glu Asn Arg Thr Ile Gly Ala Thr Met
 80 85 90
 60 gtg agg ctg gtg agg ccg ctg gct agt gta gca agg cct ctc aga g
 577

14

Val Arg Leu Val Arg Pro Leu Ala Ser Val Ala Arg Pro Leu Arg
95 100 105

5 gtgagttatc atactatatt gttctggtgg gaggggggga gaaaatgggg aagaaaagtg
637

tagaaaaaag tggatctgtc agttttctgt caggcttcac attgcctnca gtttgactg
697

10 agcaggtctg ttagagagac taaggctagg atataagaag ctaacgcttt gctcttgctc
757

cctcttacta atgcag gc tcc tgg cac ata cag acc ctg gac ttt cct ctg
808

15 Gly Ser Trp His Ile Gln Thr Leu Asp Phe Pro Leu
110 115 120

aga cca aac cgg gta gca tac caa cta gtc aga gcc act gtg gtt tac
856

20 Arg Pro Asn Arg Val Ala Tyr Gln Leu Val Arg Ala Thr Val Val Tyr
125 130 135

cgc cat cag ctt cac cta act cat tcc cac ctc tcc tgc cat gtg gag
904

25 Arg His Gln Leu His Leu Thr His Ser His Leu Ser Cys His Val Glu
140 145 150

ccc tgg gtc cag aaa agc cca acc aat cac ttt cct tct tca gga aga
952

30 Pro Trp Val Gln Lys Ser Pro Thr Asn His Phe Pro Ser Ser Gly Arg
155 160 165

ggc tcc tca aag cct tcc ctg ttg ccc aaa act tgg aca gag atg gat
1000

35 Gly Ser Ser Lys Pro Ser Leu Leu Pro Lys Thr Trp Thr Glu Met Asp
170 175 180

atc atg gaa cat gtt ggg caa aag ctc tgg aat cac aag ggg cgc agg
1048

40 Ile Met Glu His Val Gly Gln Lys Leu Trp Asn His Lys Gly Arg Arg
185 190 195 200

gtt cta cga ctc cgc ttc gtg tgt cag cag cca aga ggt agt gag gtt
1096

45 Val Leu Arg Leu Arg Phe Val Cys Gln Gln Pro Arg Gly Ser Glu Val
205 210 215

ctt gag ttc tgg tgg cat ggc act tca tca ttg gac act gtc ttc ttg
1144

50 Leu Glu Phe Trp Trp His Gly Thr Ser Ser Leu Asp Thr Val Phe Leu
220 225 230

tta ctg tat ttc aat gac act tagagtgttc agaagaccaa acctctccct
1195

55 Leu Leu Tyr Phe Asn Asp Thr
235

aaaggcctga aagagtttac agaaaaagac ccttctcttc tcttgaggag ggctcgtcaa
1255

60 gcaggcagta ttgcatcgga agttcctggc ccctccaggg agcatgatgg gcctgaaagt
1315

15

aaccagtgtt ccctccaccc ttttcaagtc agcttccagc agctgggctg ggatcactgg
 1375

5 atcattgctc cccatctcta taccctaaac tactgtaagg gagtatgtcc tcgggtacta
 1435

cactatggctc tcaattctcc caatcatgcc atcatccaga accttgtcag tgagctgggtg
 1495

10 gatcagaatg tccctcagcc ttctgtgtc ccttataagt atgttcccat tagcatcctt
 1555

ctgattgagg caaatgggag tatcttgtac aaggagtatg agggatgat tgcccagtc
 1615

15 tgcacatgca ggtgacggca aagggtgcagc tagctcaggt ttcccaagaa
 1665

20 <210> 8: protein GDF-9B [S1] full
 <211> 239
 <212> PRT
 <213> Ovis aries
 <221> misc feature

25 <222> (253)..(255)
 <223> atg start codon.
 <221> misc feature
 <222> (1253)..(1255)
 <223> position of first codon of mature peptide in wildtype sheep.

30 <221> misc feature
 <222> (685)..()
 <223> n represents approx 5.2 kb of intron.
 <221> misc feature
 <222> (1628)..(1630)

35 <223> tga stop codon in wildtype sheep.
 <400> 8

40 Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val Leu
 1 5 10 15

Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln Pro Ser Ile
 20 25 30

45 Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln Glu Leu Leu
 35 40 45

50 Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg Val Leu Gly His
 50 55 60

55 Pro Leu Arg Tyr Met Leu Glu Leu Tyr Gln Arg Ser Ala Asp Ala Ser
 65 70 75 80

60 Gly His Pro Arg Glu Asn Arg Thr Ile Gly Ala Thr Met Val Arg Leu
 85 90 95

16

Val Arg Pro Leu Ala Ser Val Ala Arg Pro Leu Arg Gly Ser Trp His
100 105 110

5 Ile Gln Thr Leu Asp Phe Pro Leu Arg Pro Asn Arg Val Ala Tyr Gln
115 120 125

10 Leu Val Arg Ala Thr Val Val Tyr Arg His Gln Leu His Leu Thr His
130 135 140

15 Ser His Leu Ser Cys His Val Glu Pro Trp Val Gln Lys Ser Pro Thr
145 150 155 160

Asn His Phe Pro Ser Ser Gly Arg Gly Ser Ser Lys Pro Ser Leu Leu
165 170 175

20 Pro Lys Thr Trp Thr Glu Met Asp Ile Met Glu His Val Gly Gln Lys
180 185 190

25 Leu Trp Asn His Lys Gly Arg Arg Val Leu Arg Leu Arg Phe Val Cys
195 200 205

30 Gln Gln Pro Arg Gly Ser Glu Val Leu Glu Phe Trp Trp His Gly Thr
210 215 220

35 Ser Ser Leu Asp Thr Val Phe Leu Leu Leu Tyr Phe Asn Asp Thr
225 230 235

<210> 9: GDF-9B [S1] coding
<211> 1182
<212> DNA
40 <213> Ovis areis
<221> misc_feature
<222> (1)..(3)
<223> atg start codon
<221> mutation
45 <222> (718)..(720)
<223> c to t at 718 of [S1] sheep changes cag glutamine codon to tag
STOP.
<221> CDS
<222> (1)..(717)
50 <221> misc_feature
<222> (805)..(807)
<223> first codon of mature peptide in wildtype sheep.
<221> misc_feature
<222> (1180)..(1182)
55 <223> tga stop codon.

<400> 9
atg gtc ctc ctg agc atc ctt aga atc ctt ctt tgg gga ctg gtg ctt
48
60 Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val Leu
1 5 10 15

17

ttt atg gaa cat agg gtc caa atg aca cag gta ggg cag ccc tct att
 96
 Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln Pro Ser Ile
 20 25 30
 5
 gcc cac ctg cct gag gcc cct acc ttg ccc ctg att cag gag ctg cta
 144
 Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln Glu Leu Leu
 35 40 45
 10
 gaa gaa gcc cct ggc aag cag cag agg aag ccg cgg gtc tta ggg cat
 192
 Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg Val Leu Gly His
 50 55 60
 15
 ccc tta cgg tat atg ctg gag ctg tac cag cgt tca gct gac gca agt
 240
 Pro Leu Arg Tyr Met Leu Glu Leu Tyr Gln Arg Ser Ala Asp Ala Ser
 65 70 75 80
 20
 gga cac cct agg gaa aac cgc acc att ggg gcc acc atg gtg agg ctg
 288
 Gly His Pro Arg Glu Asn Arg Thr Ile Gly Ala Thr Met Val Arg Leu
 85 90 95
 25
 gtg agg ccg ctg gct agt gta gca agg cct ctc aga ggc tcc tgg cac
 336
 Val Arg Pro Leu Ala Ser Val Ala Arg Pro Leu Arg Gly Ser Trp His
 100 105 110
 30
 ata cag acc ctg gac ttt cct ctg aga cca aac cgg gta gca tac caa
 384
 Ile Gln Thr Leu Asp Phe Pro Leu Arg Pro Asn Arg Val Ala Tyr Gln
 115 120 125
 35
 cta gtc aga gcc act gtg gtt tac cgc cat cag ctt cac cta act cat
 432
 Leu Val Arg Ala Thr Val Val Tyr Arg His Gln Leu His Leu Thr His
 130 135 140
 40
 tcc cac ctc tcc tgc cat gtg gag ccc tgg gtc cag aaa agc cca acc
 480
 Ser His Leu Ser Cys His Val Glu Pro Trp Val Gln Lys Ser Pro Thr
 145 150 155 160
 45
 aat cac ttt cct tct tca gga aga ggc tcc tca aag cct tcc ctg ttg
 528
 Asn His Phe Pro Ser Ser Gly Arg Gly Ser Ser Lys Pro Ser Leu Leu
 165 170 175
 50
 ccc aaa act tgg aca gag atg gat atc atg gaa cat gtt ggg caa aag
 576
 Pro Lys Thr Trp Thr Glu Met Asp Ile Met Glu His Val Gly Gln Lys
 180 185 190
 55
 ctc tgg aat cac aag ggg cgc agg gtt cta cga ctc cgc ttc gtg tgt
 624
 Leu Trp Asn His Lys Gly Arg Arg Val Leu Arg Leu Arg Phe Val Cys
 195 200 205
 60
 cag cag cca aga ggt agt gag gtt ctt gag ttc tgg tgg cat ggc act
 672

18

Gln Gln Pro Arg Gly Ser Glu Val Leu Glu Phe Trp Trp His Gly Thr
 210 215 220

5 tca tca ttg gac act gtc ttc ttg tta ctg tat ttc aat gac act
 717
 Ser Ser Leu Asp Thr Val Phe Leu Leu Leu Tyr Phe Asn Asp Thr
 225 230 235

10 tagagtgttc agaagaccaa acctctccct aaaggcctga aagagtttac agaaaaagac
 777
 ccttctcttc tcttgaggag ggctcgtcaa gcaggcagta ttgcatcgga agttcctggc
 837

15 ccctccaggg agcatgatgg gcctgaaagt aaccagtgtt ccctccaccc ttttcaagtc
 897
 agcttccagc agctgggctg ggatcactgg atcattgctc cccatctcta taccctaaac
 957

20 tactgtaagg gagtatgtcc tcgggtacta cactatgggc tcaattctcc caatcatgcc
 1017
 atcatccaga accttgtcag tgagctggcg gatcagaatg tccctcagcc ttcctgtgtc
 1077

25 ccttataagt atgttcccat tagcatcctt ctgattgagg caaatgggag tatcttgtac
 1137
 aaggagtatg agggatatgat tgcccagtc tgcacatgca ggtga
 1182

35 <210> 10: protein GDF-9B [S1] coding
 <211> 239
 <212> PRT
 <213> Ovis areis
 <221> misc_feature
 <222> (1)..(3)
 40 <223> atg start codon.
 <221> misc_feature
 <222> (805)..(807)
 <223> first codon of mature peptide in wildtype sheep.
 <221> misc_feature
 45 <222> (1180)..(1182)
 <223> tga stop codon.
 <400> 10

50 Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val Leu
 1 5 10 15
 Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln Pro Ser Ile
 20 25 30

55 Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln Glu Leu Leu
 35 40 45

60 Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg Val Leu Gly His
 50 55 60

5 Pro Leu Arg Tyr Met Leu Glu Leu Tyr Gln Arg Ser Ala Asp Ala Ser
 65 70 75 80
 Gly His Pro Arg Glu Asn Arg Thr Ile Gly Ala Thr Met Val Arg Leu
 85 90 95
 10 Val Arg Pro Leu Ala Ser Val Ala Arg Pro Leu Arg Gly Ser Trp His
 100 105 110
 15 Ile Gln Thr Leu Asp Phe Pro Leu Arg Pro Asn Arg Val Ala Tyr Gln
 115 120 125
 20 Leu Val Arg Ala Thr Val Val Tyr Arg His Gln Leu His Leu Thr His
 130 135 140
 25 Ser His Leu Ser Cys His Val Glu Pro Trp Val Gln Lys Ser Pro Thr
 145 150 155 160
 Asn His Phe Pro Ser Ser Gly Arg Gly Ser Ser Lys Pro Ser Leu Leu
 165 170 175
 30 Pro Lys Thr Trp Thr Glu Met Asp Ile Met Glu His Val Gly Gln Lys
 180 185 190
 35 Leu Trp Asn His Lys Gly Arg Arg Val Leu Arg Leu Arg Phe Val Cys
 195 200 205
 40 Gln Gln Pro Arg Gly Ser Glu Val Leu Glu Phe Trp Trp His Gly Thr
 210 215 220
 45 Ser Ser Leu Asp Thr Val Phe Leu Leu Leu Tyr Phe Asn Asp Thr
 225 230 235
 50 <210> 11: GDF-9B [S1] mutation
 <211> 168
 <212> DNA
 <213> Ovis aries
 <221> CDS
 <222> (1)..(84)
 <221> mutation
 <222> (85)..(87)
 55 <223> c to t at 85 of [S1] sheep changes glutamine cag codon to tag
 STOP
 60 <400> 11
 aga ggt agt gag gtt ctt gag ttc tgg tgg cat ggc act tca tca ttg
 48
 Arg Gly Ser Glu Val Leu Glu Phe Trp Trp His Gly Thr Ser Ser Leu
 1 5 10 15

20

gac act gtc ttc ttg tta ctg tat ttc aat gac act tagagtgttc
94

Asp Thr Val Phe Leu Leu Leu Tyr Phe Asn Asp Thr
20 25

5

agaagaccaa acctctccct aaaggcctga aagagtttac agaaaaagac ctttctcttc
154

10

tcttgaggag ggct
168

<210> 12: protein GDF-9B [S1] mutation

<211> 28

15

<212> PRT

<213> Ovis aries

<400> 12

20

Arg Gly Ser Glu Val Leu Glu Phe Trp Trp His Gly Thr Ser Ser Leu
1 5 10 15

25

Asp Thr Val Phe Leu Leu Leu Tyr Phe Asn Asp Thr
20 25

30

<210> 13: GDF-9B [S2] full

<211> 1665

<212> DNA

<213> Ovis aries

<221> 5'UTR

<222> (1)..(252)

<221> misc_feature

35

<222> (253)..(255)

<223> atg start codon.

<221> CDS

<222> (253)..(577)

<221> CDS

40

<222> (774)..(1627)

<221> Intron

<222> (578)..(773)

<223> n at 685 represents approx 5.2 kb intron.

<221> mat_peptide

45

<222> (1253)..()

<221> misc_feature

<222> (685)..()

<223> n represents approx 5.2 kb of intron

<221> misc_feature

50

<222> (1628)..(1630)

<223> tga stop codon.

<221> 3'UTR

<222> (1628)..(1665)

<221> mutation

55

<222> (1547)..(1549)

<223> g to t at 1548 of [S2] sheep changes agc serine codon to atc
isoleucine codon

<400> 13

60

catgctgcct tgtccacct gctgtttctg tttgtttgat gcaaagagga caatttagaa
60

21

gacctctttt tggttcagga gacacctacca gaggaagaaa cataggacct gcctgccagc
 120

5 ctttcatttt tccttgcctt atcctttgtg gtagtggagc ctggatgctg ttacccatgt
 180

aaaaggaaaag gtttaaagcg ttatcctttg ggcttttata agaacatgtt gctgaacacc
 240

10 aagctttttca ag atg gtc ctc ctg agc atc ctt aga atc ctt ctt tgg
 288
 Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp
 -265 -260

15 gga ctg gtg ctt ttt atg gaa cat agg gtc caa atg aca cag gta
 333
 Gly Leu Val Leu Phe Met Glu His Arg Val Gln Met Thr Gln Val
 -255 -250 -245

20 ggg cag ccc tct att gcc cac ctg cct gag gcc cct acc ttg ccc
 378
 Gly Gln Pro Ser Ile Ala His Leu Pro Glu Ala Pro Thr Leu Pro
 -240 -235 -230

25 ctg att cag gag ctg cta gaa gaa gcc cct ggc aag cag cag agg
 423
 Leu Ile Gln Glu Leu Leu Glu Glu Ala Pro Gly Lys Gln Gln Arg
 -225 -220 -215

30 aag ccg cgg gtc tta ggg cat ccc tta cgg tat atg ctg gag ctg
 468
 Lys Pro Arg Val Leu Gly His Pro Leu Arg Tyr Met Leu Glu Leu
 -210 -205 -200

35 tac cag cgt tca gct gac gca agt gga cac cct agg gaa aac cgc
 513
 Tyr Gln Arg Ser Ala Asp Ala Ser Gly His Pro Arg Glu Asn Arg
 -195 -190 -185

40 acc att ggg gcc acc atg gtg agg ctg gtg agg ccg ctg gct agt
 558
 Thr Ile Gly Ala Thr Met Val Arg Leu Val Arg Pro Leu Ala Ser
 -180 -175 -170

45 gta gca agg cct ctc aga g gtgagttatc atactatatt gttctggtg
 607
 Val Ala Arg Pro Leu Arg
 -165

50 gaggggggga gaaaatgggg aagaaaagt tagaaaaaag tggatctgtc agttttctgt
 667

caggcttcac attgcctnca gtttgtactg agcaggtctg ttagagagac taaggctagg
 727

55 atataagaag ctaacgcttt gctcttgttc cctcttacta atgcag gc tcc tgg
 781
 Gly Ser Trp
 -160

60 cac ata cag acc ctg gac ttt cct ctg aga cca aac cgg gta gca
 826

22

His Ile Gln Thr Leu Asp Phe Pro Leu Arg Pro Asn Arg Val Ala
 -155 -150 -145
 5 tac caa cta gtc aga gcc act gtg gtt tac cgc cat cag ctt cac
 871
 Tyr Gln Leu Val Arg Ala Thr Val Val Tyr Arg His Gln Leu His
 -140 -135 -130
 10 cta act cat tcc cac ctc tcc tgc cat gtg gag ccc tgg gtc cag
 916
 Leu Thr His Ser His Leu Ser Cys His Val Glu Pro Trp Val Gln
 -125 -120 -115
 15 aaa agc cca acc aat cac ttt cct tct tca gga aga ggc tcc tca
 961
 Lys Ser Pro Thr Asn His Phe Pro Ser Ser Gly Arg Gly Ser Ser
 -110 -105 -100
 20 aag cct tcc ctg ttg ccc aaa act tgg aca gag atg gat atc atg gaa
 1009
 Lys Pro Ser Leu Leu Pro Lys Thr Trp Thr Glu Met Asp Ile Met Glu
 -95 -90 -85
 25 cat gtt ggg caa aag ctc tgg aat cac aag ggg cgc agg gtt cta cga
 1057
 His Val Gly Gln Lys Leu Trp Asn His Lys Gly Arg Arg Val Leu Arg
 -80 -75 -70
 30 ctc cgc ttc gtg tgt cag cag cca aga ggt agt gag gtt ctt gag ttc
 1105
 Leu Arg Phe Val Cys Gln Gln Pro Arg Gly Ser Glu Val Leu Glu Phe
 -65 -60 -55 -50
 35 tgg tgg cat ggc act tca tca ttg gac act gtc ttc ttg tta ctg tat
 1153
 Trp Trp His Gly Thr Ser Ser Leu Asp Thr Val Phe Leu Leu Leu Tyr
 -45 -40 -35
 40 ttc aat gac act cag agt gtt cag aag acc aaa cct ctc cct aaa ggc
 1201
 Phe Asn Asp Thr Gln Ser Val Gln Lys Thr Lys Pro Leu Pro Lys Gly
 -30 -25 -20
 45 ctg aaa gag ttt aca gaa aaa gac cct tct ctt ctc ttg agg agg gct
 1249
 Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser Leu Leu Leu Arg Arg Ala
 -15 -10 -5
 50 cgt caa gca ggc agt att gca tcg gaa gtt cct ggc ccc tcc agg gag
 1297
 Arg Gln Ala Gly Ser Ile Ala Ser Glu Val Pro Gly Pro Ser Arg Glu
 -1 1 5 10 15
 55 cat gat ggg cct gaa agt aac cag tgt tcc ctc cac cct ttt caa gtc
 1345
 His Asp Gly Pro Glu Ser Asn Gln Cys Ser Leu His Pro Phe Gln Val
 20 25 30
 60 agc ttc cag cag ctg ggc tgg gat cac tgg atc att gct ccc cat ctc
 1393
 Ser Phe Gln Gln Leu Gly Trp Asp His Trp Ile Ile Ala Pro His Leu
 35 40 45

23

tat acc cca aac tac tgt aag gga gta tgt cct cgg gta cta cac tat
 1441
 Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys Pro Arg Val Leu His Tyr
 50 55 60
 5 ggt ctc aat tct ccc aat cat gcc atc atc cag aac ctt gtc agt gag
 1489
 Gly Leu Asn Ser Pro Asn His Ala Ile Ile Gln Asn Leu Val Ser Glu
 65 70 75
 10 ctg gtg gat cag aat gtc cct cag cct tcc tgt gtc cct tat aag tat
 1537
 Leu Val Asp Gln Asn Val Pro Gln Pro Ser Cys Val Pro Tyr Lys Tyr
 80 85 90 95
 15 gtt ccc att atc atc ctt ctg att gag gca aat ggg agt atc ttg tac
 1585
 Val Pro Ile Ile Ile Leu Leu Ile Glu Ala Asn Gly Ser Ile Leu Tyr
 100 105 110
 20 aag gag tat gag ggt atg att gcc cag tcc tgc aca tgc agg
 1627
 Lys Glu Tyr Glu Gly Met Ile Ala Gln Ser Cys Thr Cys Arg
 115 120 125
 25 tgacggcaaa ggtgcagcta gctcaggttt cccaagaa
 1665
 30 <210> 14: protein GDF-9B [S1] mutation
 <211> 393
 <212> PRT
 <213> Ovis aries
 <221> misc feature
 35 <222> (253)..(255)
 <223> atg start codon.
 <221> misc feature
 <222> (685)..()
 <223> n represents approx 5.2 kb of intron
 40 <221> misc feature
 <222> (1628)..(1630)
 <223> tga stop codon.
 <400> 14
 45 Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val
 -265 -260 -255
 50 Leu Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln Pro
 -250 -245 -240
 55 Ser Ile Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln
 -235 -230 -225
 Glu Leu Leu Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg
 -220 -215 -210
 60 Val Leu Gly His Pro Leu Arg Tyr Met Leu Glu Leu Tyr Gln Arg
 -205 -200 -195

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

25

Ile Ile Ala Pro His Leu Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys
45 50 55

5 Pro Arg Val Leu His Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile
60 65 70

10 Gln Asn Leu Val Ser Glu Leu Val Asp Gln Asn Val Pro Gln Pro Ser
75 80 85

15 Cys Val Pro Tyr Lys Tyr Val Pro Ile Ile Ile Leu Leu Ile Glu Ala
90 95 100 105

20 Asn Gly Ser Ile Leu Tyr Lys Glu Tyr Glu Gly Met Ile Ala Gln Ser
110 115 120

Cys Thr Cys Arg
125

25 <210> 15: GDF-9B [S2] coding
<211> 1182
<212> DNA
<213> Ovis aries
30 <221> misc_feature
<222> (1)..(3)
<223> atg start codon.
<221> mutation
<222> (1099)..(1101)
35 <223> g to to at 1100 of [S2] sheep changes agc serine codon to atc
isoleucine codon
<221> CDS
<222> (1)..(1179)
<221> mat_peptide
40 <222> (805)..()
<221> misc_feature
<222> (1180)..(1182)
<223> tga stop codon.

45 <400> 15
atg gtc ctc ctg agc atc ctt aga atc ctt ctt tgg gga ctg gtg
45
Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val
-265 -260 -255

50 ctt ttt atg gaa cat agg gtc caa atg aca cag gta ggg cag ccc
90
Leu Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln Pro
-250 -245 -240

55 tct att gcc cac ctg cct gag gcc cct acc ttg ccc ctg att cag
135
Ser Ile Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln
-235 -230 -225

60 gag ctg cta gaa gaa gcc cct ggc aag cag cag agg aag ccg cgg
180
Glu Leu Leu Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg

26

	-220	-215	-210
	gtc tta ggg cat	ccc tta cgg tat atg	ctg gag ctg tac cag cgt
	225		
5	Val Leu Gly His	Pro Leu Arg Tyr Met	Leu Glu Leu Tyr Gln Arg
	-205	-200	-195
	tca gct gac gca	agt gga cac cct agg	gaa aac cgc acc att ggg
	270		
10	Ser Ala Asp Ala	Ser Gly His Pro Arg	Glu Asn Arg Thr Ile Gly
	-190	-185	-180
	gcc acc atg gtg	agg ctg gtg agg ccg	ctg gct agt gta gca agg
	315		
15	Ala Thr Met Val	Arg Leu Val Arg Pro	Leu Ala Ser Val Ala Arg
	-175	-170	-165
	cct ctc aga ggc	tcc tgg cac ata cag	acc ctg gac ttt cct ctg
	360		
20	Pro Leu Arg Gly	Ser Trp His Ile Gln	Thr Leu Asp Phe Pro Leu
	-160	-155	-150
	aga cca aac cgg	gta gca tac caa cta	gtc aga gcc act gtg gtt
	405		
25	Arg Pro Asn Arg	Val Ala Tyr Gln Leu	Val Arg Ala Thr Val Val
	-145	-140	-135
	tac cgc cat cag	ctt cac cta act cat	tcc cac ctc tcc tgc cat
	450		
30	Tyr Arg His Gln	Leu His Leu Thr His	Ser His Leu Ser Cys His
	-130	-125	-120
	gtg gag ccc tgg	gtc cag aaa agc cca	acc aat cac ttt cct tct
	495		
35	Val Glu Pro Trp	Val Gln Lys Ser Pro	Thr Asn His Phe Pro Ser
	-115	-110	-105
	tca gga aga ggc	tcc tca aag cct tcc	ctg ttg ccc aaa act tgg aca
	543		
40	Ser Gly Arg Gly	Ser Ser Lys Pro Ser	Leu Leu Pro Lys Thr Trp Thr
	-100	-95	-90
	gag atg gat atc	atg gaa cat gtt ggg	caa aag ctc tgg aat cac aag
	591		
45	Glu Met Asp Ile	Met Glu His Val Gly	Gln Lys Leu Trp Asn His Lys
	-85	-80	-75
	ggg cgc agg gtt	cta cga ctc cgc ttc	gtg tgt cag cag cca aga ggt
	639		
50	Gly Arg Arg Val	Leu Arg Leu Arg Phe	Val Cys Gln Gln Pro Arg Gly
	-70	-65	-60
	agt gag gtt ctt	gag ttc tgg tgg cat	ggc act tca tca ttg gac act
	687		
55	Ser Glu Val Leu	Glu Phe Trp Trp His	Gly Thr Ser Ser Leu Asp Thr
	-55	-50	-45
	gtc ttc ttg tta	ctg tat ttc aat gac	act cag agt gtt cag aag acc
	735		
60	Val Phe Leu Leu	Leu Tyr Phe Asn Asp	Thr Gln Ser Val Gln Lys Thr
	-35	-30	-25

27

aaa cct ctc cct aaa ggc ctg aaa gag ttt aca gaa aaa gac cct tct
 783
 Lys Pro Leu Pro Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser
 -20 -15 -10
 5
 ctt ctc ttg agg agg gct cgt caa gca ggc agt att gca tcg gaa gtt
 831
 Leu Leu Leu Arg Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val
 -5 -1 1 5
 10
 cct ggc ccc tcc agg gag cat gat ggg cct gaa agt aac cag tgt tcc
 879
 Pro Gly Pro Ser Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser
 10 15 20 25
 15
 ctc cac cct ttt caa gtc agc ttc cag cag ctg ggc tgg gat cac tgg
 927
 Leu His Pro Phe Gln Val Ser Phe Gln Gln Leu Gly Trp Asp His Trp
 30 35 40
 20
 atc att gct ccc cat ctc tat acc cca aac tac tgt aag gga gta tgt
 975
 Ile Ile Ala Pro His Leu Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys
 45 50 55
 25
 cct cgg gta cta cac tat ggt ctc aat tct ccc aat cat gcc atc atc
 1023
 Pro Arg Val Leu His Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile
 60 65 70
 30
 cag aac ctt gtc agt gag ctg gtg gat cag aat gtc cct cag cct tcc
 1071
 Gln Asn Leu Val Ser Glu Leu Val Asp Gln Asn Val Pro Gln Pro Ser
 75 80 85
 35
 tgt gtc cct tat aag tat gtt ccc att atc atc ctt ctg att gag gca
 1119
 Cys Val Pro Tyr Lys Tyr Val Pro Ile Ile Ile Leu Leu Ile Glu Ala
 90 95 100 105
 40
 aat ggg agt atc ttg tac aag gag tat gag ggt atg att gcc cag tcc
 1167
 Asn Gly Ser Ile Leu Tyr Lys Glu Tyr Glu Gly Met Ile Ala Gln Ser
 110 115 120
 45
 tgc aca tgc agg tga
 1182
 Cys Thr Cys Arg
 125
 50
 <210> 16: protein GDF-9B [S2] coding
 <211> 393
 <212> PRT
 55 <213> Ovis aries
 <221> misc_feature
 <222> (1)..(3)
 <223> atg start codon.
 <221> misc_feature
 60 <222> (1180)..(1182)
 <223> tga stop codon.
 <400> 16

28

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

5 Lys Pro Leu Pro Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser
 -20 -15 -10

10 Leu Leu Leu Arg Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val
 -5 -1 1 5

15 Pro Gly Pro Ser Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser
 10 15 20 25

20 Leu His Pro Phe Gln Val Ser Phe Gln Gln Leu Gly Trp Asp His Trp
 30 35 40

25 Ile Ile Ala Pro His Leu Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys
 45 50 55

30 Pro Arg Val Leu His Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile
 60 65 70

35 Gln Asn Leu Val Ser Glu Leu Val Asp Gln Asn Val Pro Gln Pro Ser
 75 80 85

40 Cys Val Pro Tyr Lys Tyr Val Pro Ile Ile Ile Leu Leu Ile Glu Ala
 90 95 100 105

45 Asn Gly Ser Ile Leu Tyr Lys Glu Tyr Glu Gly Met Ile Ala Gln Ser
 110 115 120

50 Cys Thr Cys Arg
 125

55 <210> 17: GDF-9B [S2] mutation
 <211> 168
 <212> DNA
 <213> Ovis aries
 <221> CDS
 <222> (1)..(168)
 <221> mutation
 <222> (85)..(87)
 <223> g to t at 86 of GDF9B sheep changes agc serine codon to atc
 isoleucine codon

60 <400> 17
 gcc atc atc cag aac ctt gtc agt gag ctg gtg gat cag aat gtc cct
 48
 Ala Ile Ile Gln Asn Leu Val Ser Glu Leu Val Asp Gln Asn Val Pro
 1 5 10 15

60 cag cct tcc tgt gtc cct tat aag tat gtt ccc att atc atc ctt ctg
 96
 Gln Pro Ser Cys Val Pro Tyr Lys Tyr Val Pro Ile Ile Ile Leu Leu
 20 25 30

```

att gag gca aat ggg agt atc ttg tac aag gag tat gag ggt atg att
144
Ile Glu Ala Asn Gly Ser Ile Leu Tyr Lys Glu Tyr Glu Gly Met Ile
5      35      40      45

gcc cag tcc tgc aca tgc agg tga
168
Ala Gln Ser Cys Thr Cys Arg
10     50      55

<210> 18: protein GDF-9B [S2] coding
15 <211> 55
    <212> PRT
    <213> Ovis aries

<400> 18

20 Ala Ile Ile Gln Asn Leu Val Ser Glu Leu Val Asp Gln Asn Val Pro
    1      5      10      15

25 Gln Pro Ser Cys Val Pro Tyr Lys Tyr Val Pro Ile Ile Ile Leu Leu
    20      25      30

Ile Glu Ala Asn Gly Ser Ile Leu Tyr Lys Glu Tyr Glu Gly Met Ile
30     35      40      45

Ala Gln Ser Cys Thr Cys Arg
35     50      55

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ03/00109

A. CLASSIFICATION OF SUBJECT MATTERInt. Cl. ⁷: C12N 015/12; A61K 038/16, 039/395; A61P 015/00

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)

SEE ELECTRONIC DATABASE BOX BELOW

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

SEE ELECTRONIC DATABASE BOX BELOW

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

AGRICOLA CA MEDLINE WPIDS: GDF-9? GDF9? BMP15 BMP-15 FERTIL? INFERTIL? OVULAT? BREED?
 IMMUNIS? VACC? OOCYTE? FOLLIC? FECUND? PROLIFACITY OVAR? CORPUS LUTEUM CONTRACEPTI?
 SEQ ID NOS 6, 12 AND 18

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/85926 A2 (AGRESEARCH LIMITED) 15 November 2001. See the entire document.	2, 7-11, 13, 14, 17, 19-37, 41-43
Y		1, 2, 11-22, 34-37, 41-43
X	WO 01/96393 A2 (AGRESEARCH LIMITED) 20 December 2001. See the entire document.	2, 7-11, 13, 14, 17, 19-38, 41-43
Y		1, 2, 11-22, 34-37, 41-43

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"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
 1 September 2003

Date of mailing of the international search report 08 SEP 2003

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ03/00109

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	Juengel JL et al (2002). Growth differentiation factor 9 and bone morphogenetic protein 15 are essential for ovarian follicular development in sheep. <i>Biology of Reproduction</i> 67: 1777–1789. See the entire document.	23–33, 38 1, 12, 15, 16, 18–22, 34–37, 41–43
X	WO 99/17797 (THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE) 15 April 1999. See the entire document.	23, 24, 26–29, 31–33
X	Vitt UA et al (2000). <i>In vivo</i> treatment with GDF-9 stimulates primordial and primary follicle progression and theca cell marker CYP17 in ovaries of immature rats. <i>Endocrinology</i> 141: 3814–3820. See the entire document.	23, 24, 27
X Y	WO 00/66620 (CREATIVE BIOMOLECULES INC) 9 November 2000. See the entire document.	38 1, 12, 15, 16, 18–22, 34–37, 41–43
X Y	Yan C et al (2001). Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. <i>Molecular Endocrinology</i> 15: 854–866. See the entire document.	39, 40 1, 12, 15, 16, 18–22, 34–37, 41–43
X Y	Montgomery GW et al (2001). Genes controlling ovulation rate in sheep. <i>Reproduction</i> 121: 843–852. See the entire document, and in particular the abstract and page 849–851.	1, 3–6, 12, 15, 16, 18–22, 34–37, 41, 43 2, 11, 13, 14, 17, 19–22, 34–37, 41–43
	Note: for the Y indications, either WO 01/85926 or WO 01/96393 may be combined with any one of Juengel et al (2002), WO 00/66620 or Yan et al (2001) (with relevance to claims 2, 11, 13, 14, 17, 19–22, 34–37 and 41–43) or with Montgomery et al (2001) (with relevance to claims 1, 12, 15, 16, 18–22, 34–37 and 41–43).	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ03/00109

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos : **38 (in part)**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The claim does not comply with Rule 6.3 of the PCT, because this claim recites analogues or antagonists of GDF-9 or GDF-9B, which owe nothing to the applicant's invention and do not define the technical features of the invention. The search on this claim has been restricted to analogues and antagonists derived specifically using GDF-9 or GDF-9B. The full scope of these claims has not been searched.

3. ☐ Claims Nos :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/NZ03/00109

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	01/85926	AU	608210	CA	2408051	EP	1292674
		NZ	500844				
WO	01/96393	AU	6795001	CA	2410532	EP	1294755
		NZ	502796				
WO	99/17797	AU	1071499	AU	757563	CA	2306452
		EP	1021201	JP	2001518516T		
WO	00/66620	AU	4676600	CA	2371695	EP	1181041