MODULATION OF OVULATION

FIELD OF THE INVENTION

The present invention relates to the identification of novel domains of functional significance on GDF-9 and GDF-9B molecules and to agonists and antagonists which interact therewith to modulate the biological activity of these molecules to alter mammalian ovarian function and ovulation rate.

BACKGROUND OF THE INVENTION

GDF-9 and GDF-9B (also known as BMP15) are expressed in the oocyte of the developing follicle and play a role in mammalian fertility (Fitzpatrick *et al*, 1998). GDF9 is a member of the transforming growth factor beta (TGFβ) superfamily (McPherron and Lee, 1993) which is expressed in oocytes from the primary stage of follicular development until ovulation (McGrath *et al.*, 1995; Laitinen *et al.*, 1998). GDF9B is closely related to GDF9 (Dube *et al.*, 1998; Laitinen *et al.*, 1998) and is expressed in mouse oocytes at the same time as GDF9, but in human primary follicles slightly later than GDF9. In the ovary GDF9 and GDF9B have now been shown to be expressed in the developing oocyte in humans (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 GDF9 can be seen in primordial follicles whereas GDF9B is expressed in primary follicles (Bodensteiner *et al.*, 1999; Galloway *et al.*, 2000).

GDF9 and GDF9B, like most other members of the TGFβ 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 furin protease cleavage site. Most members of the TGFβ superfamily are biologically active as dimers, and although GDF9 and GDF9B do not contain the cysteine molecule responsible for covalent interchain disulphide bonding seen in nearly all 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 are homodimers (GDF9-GDF9 and

GDF9B-GDF9B), or heterodimers (GDF9-GDF9B) or whether all three dimer forms play a role. It has been postulated based on the above models that GDF9 homodimers play a more important role in the mouse but in sheep the GDF9B 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 (maturing usually only one egg per cycle) whereas mice are poly-ovulatory. Clearly both GDF9 and GDF9B play crucial roles in controlling and maintaining fertility in mammals, and understanding the nature of their actions is essential for the development of therapies.

Jeffery et al., 2003, used a bioinformatics tool (GoCore) to analyse conserved regions across a number of different TGF-β family members and for GDF-9 and GDF-9B across different species, in an attempt to identify regions of functional significance. However, this study did not correlate regions identified as possible functionally significant sites with effects on modulation of ovulation in vivo.

It is an object of the present invention to identify domains of functional significance on the GDF-9 and/or GDF-9B molecules and to identify agonists and antagonists which interact with such domains or mimic such domains to modulate their biological activity thereby modulating mammalian ovarian function and ovulation rates in vivo; and/or to provide the public with a useful choice.

The term "comprising" as used in this specification and claims means "consisting at least in part of", that is to say when interpreting independent claims including that term, the feature prefaced by that term in each claim all need to be present but other features can also be present.

SUMMARY OF THE INVENTION

The present invention is based on the identification of novel domains of functional significance on the GDF-9 and GDF-9B molecules and to agonists and antagonists that interact therewith.

More specifically, the present invention is based on the identification and characterisation of putative type I and type II receptor binding domains of GDF-9 and/or GDF-9B and putative dimerisation binding domains of GDF-9 and/or GDF-9B, and to agonists and antagonists that interact therewith.

The present invention is directed to a putative type I receptor binding domain of GDF-9 comprising the amino acid sequence:

$(X)_{32}ECE(X)_{10}LKW(X)_2WI(X)_{16}RAVGHRY(X)_6M(X)_2NI(X)_2EK(X)_{33}Y$ (SEQ ID NO: 1); wherein the amino acids (other than X), form the binding domain when the GDF-9 molecule is in a three dimensional tertiary structure.

The present invention further provides the putative type II receptor binding domain of GDF-9 comprising the amino acid sequence:

$(X)_{51}IVAPH(X)Y(X)_{47}SPLSVL(X)_8AY(X)_6I$ (SEQ ID NO: 2);
wherein the amino acids (other than X), form the binding domain when the GDF-9 molecule is in a three dimensional tertiary structure.

The present invention further provides the putative dimerisation binding domain of GDF-9 comprising the amino acid sequence:

$(X)_{26}F(X)_{11}F(X)L(X)_4L(X)W(X)_9Y(X)PRYC(X)_{14}PVHT(X)VQ(X)_2I(X)_7VP(X)PSCVP(X)_2Y(X)_{20}DM(X)A(X)_5R$ (SEQ ID NO: 3);
wherein the amino acids (other than X), form the dimerisation binding domain when the GDF-9 molecule is in a three dimensional tertiary structure.

X in SEQ ID NOs 1, 2 and 3 represents the amino acid residues present in the GDF-9 sequence shown in Figure 1. The X amino acids do not form part of the putative binding domains of the present invention. The amino acid symbols, other than X, correspond to the one letter code as set out in Table 1, below.

The present invention further provides an agonist or antagonist that is capable of interacting with one or more putative GDF-9 binding domains selected from the group comprising SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

The agonist or antagonist is preferably a peptide comprising at least 5 contiguous amino acids of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto. Preferably the peptide is at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or at least 20 contiguous amino acids of SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3, or a functional derivative homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto. The peptides of the invention preferably abut with or include at least one amino acid of the putative binding domains.

Most preferably the agonist or antagonist comprises one or more peptides selected from the group comprising:

C(GG)PRAVGHRYGSPVHTM	(SEQ ID NO: 4);
FSQLKWDNWIVA(C)	(SEQ ID NO: 5);
C(GG)PRAVGHRYGS	(SEQ ID NO: 6);
SVPRPSCVPAKYS	(SEQ ID NO: 7);
GSIAYKEYE(C)	(SEQ ID NO: 8); and
AKYSPLSVLA(C)	(SEQ ID NO:9);

or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

Amino acids in parentheses refer to amino acids which are optionally added for conjugation purposes and do not correspond with the GDF-9 sequence per se.

The present invention is also directed to a putative type I receptor binding domain of GDF-9B comprising the amino acid sequence:

$(X)_{22}QCS(X)_{10}LGW(X)_2WI(X)_{16}RVLHYGL(X)_6I(X)_3L(X)_2EL(X)_{33}Y$ (SEQ ID NO:10)

wherein the amino acids (other than X), form the binding domain when the GDF-9B molecule is in a three dimensional tertiary structure.

The present invention also provides the putative type II receptor binding domain of GDF-9B comprising the amino acid sequence:

$(X)_{41}IIAPH(X)Y(X)_{47}VPISIL(X)_8LY(X)_6I$ (SEQ ID NO:11)

wherein the amino acids (other than X), form the binding domain when the GDF-9B molecule is in a three dimensional tertiary structure.

The present invention also provides the putative dimerisation binding domain of GDF-9B comprising the amino acid sequence:

$(X)_{28}F(X)V(X)_4L(X)W(X)_9Y(X)PNYC(X)_{14}PNHA(X)IQ(X)_2V(X)_7VP(X)PSCVP(X)_2Y(X)_{20}G$ MIA $(X)_5R$ (SEQ ID NO:12);

wherein the amino acids (other than X), form the dimerisation binding domain when the GDF-9B molecule is in a three dimensional tertiary structure.

X in SEQ ID NOs 10, 11 and 12 represents the amino acid residues present in the GDF-9B sequence shown in Figure 2. The X amino acids do not form part of the putative binding domains of the present invention. The amino acid symbols, other than X, correspond to the one letter code as set out in Table 1, below.

The present invention further provides an agonist or antagonist that is capable of interacting with one or more putative GDF-9B binding domains selected from the group comprising SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12.

The agonist or antagonist is preferably a peptide comprising at least 5 contiguous amino acids of SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto. Preferably the peptide comprises at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or at least 20 contiguous amino acids of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative, homology, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto. The peptides of the invention preferably abut with or include amino acid(s) of the putative binding domains.

Most preferably the agonist or antagonist comprises one or more peptides selected from the group comprising

C(GG)PRVLHYGLNSPNHAI	(SEQ ID NO: 13);
SFQQLGWDHWI(C)	(SEQ ID NO: 14);
C(GG)PRVLHYGLNS	(SEQ ID NO: 15);
NVPQPSCVPYKYV(C)	(SEQ ID NO: 16);
PISILLIEANGSIL(C)	(SEQ ID NO: 17);

GSILYKEYE(C) (SEQ ID NO: 18); and

C(GG)VPYKYVPISIL (SEQ ID NO: 19);

or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

Amino acids in parentheses refer to amino acids optionally added for conjugation purposes and do not correspond to the GDF-9B sequence per se.

In a further aspect the present invention provides a method of modulating the ovulation rate of a female mammal, said method comprising the step of administering to said mammal an effective amount of one or more agonists or antagonists that are capable of interacting with one or more putative GDF-9 and/or GDF-9B type I or type II receptor binding domains and/or dimerisation binding domains as defined above, and altering the biological activity thereof.

Preferably, the invention provides a method of modulating the ovulation rate of a female mammal, comprising administering to said mammal an effective amount of one or more peptides selected from SEQ ID NOs 4 to 9 and 13 to 19, or a functional variant thereof, or an antibody or antibody fragment that binds thereto.

In a further aspect the present invention provides a use of one or more agonists or antagonist that are capable of interacting with one or more putative GDF-9 and/or GDF-9B type I or type II receptor binding domains and/or dimerisation binding domains as defined above, in the manufacture of a medicament for modulating the ovulation rate of a female mammal.

Preferably, the invention provides a use of one or more peptides selected from the group comprising SEQ ID NOs 4 to 9 and 13 to 19 or a functional variant thereof, or an antibody or antibody fragment that binds thereto, in the manufacture of a medicament for modulating the ovulation rate of a female mammal.

In a still further aspect, the present invention provides a pharmaceutical composition comprising one or more agonists or antagonists that are capable of interacting with one or more putative GDF-9 and/or GDF-9B type I or type II receptor binding domains and/or dimerisation binding domains defined above, together with a pharmaceutically acceptable carrier or excipient.

Preferably, the composition comprises one or more peptides selected from the group comprising SEQ ID NOs 4 to 9 and 13 to 19 or a functional variant thereof, or an antibody or antibody fragment that binds thereto, together with a pharmaceutically acceptable carrier or excipient.

DESCRIPTION OF THE DRAWINGS

The invention will now be described in detail by reference to the figures of the accompanying drawings in which:

Figure 1 shows the position of peptides of SEQ ID NOs 4-9 on ovine GDF-9;

Figure 2 shows the position of peptides of SEQ ID NOs 13-19 on ovine GDF-9B;

Figure 3 shows the GDF-9 and GDF-9B type I and type II receptor binding complexes;

Figures 4a and 4b show the 3-D molecular structure and putative type I receptor binding domains for GDF-9 in two different orientations;

Figure 4c shows the 3-D molecular structure and putative type II receptor binding domains for GDF-9;

Figure 4d shows the 3-D molecular structure and putative dimerisation binding domain for GDF-9;

Figures 5a and 5b show the 3-D molecular structure and putative type I receptor binding domains for GDF-9B in two different orientations;

Figure 5c shows the 3-D molecular structure and putative type II receptor binding domain for GDF-9B;

Figure 5d shows 3-D molecular structure and putative dimerisation binding domain for GDF-9B;

Figures 6a to 6f show sequence homology of GDF9 and GDF-9B from a number of different species and the putative sequences of the various sites of functional significance;

Figure 7 shows the effect of a polyclonal antibody from sheep immunized against ovine GDF-9B peptide (SEQ ID NO: 14) on ovine (o) GDF-9 and oGDF-9B stimulated ^{3}H-thymidine incorporation of rat granulosa cells when added directly to the bioassay. The horizontal line indicates the level of response of the granulosa cells treated with control media (i.e. media without oGDF-9 or oGDF-9B). Data presented are the mean and standard error of the mean of 3 replicate experiments. *$P<0.05$ versus control media treated cells, $^{a}P<0.05$, $^{b}P<0.01$ versus oGDF-9+oGDF-9B treated cells that did not receive antibodies;

Figure 8 shows the effect of a polyclonal antibody from sheep immunized against ovine GDF-9 peptide (SEQ ID NO 8) on ovine (o) GDF-9 and oGDF-9B stimulated ^{3}H-thymidine incorporation of rat granulosa cells when added directly to the bioassay. The horizontal line

indicates the level of response of the granulosa cells treated with control media (i.e. media without oGDF-9 or oGDF-9B). Data presented are the mean and standard error of the mean of 5 replicate experiments. *P<0.05 versus control media treated cells, [a]P<0.05, [b]P<0.01 versus oGDF-9 and oGDF-9B treated cells that did not receive antibodies;

Figure 9 shows the effect of a polyclonal antibody from sheep immunized against ovine GDF-9B peptide (SEQ ID NO 19) on ovine (o) GDF-9 and oGDF-9B stimulated ^{3}H-thymidine incorporation of rat granulosa cells when added directly to the bioassay. The horizontal line indicates the level of response of the granulosa cells treated with control media (i.e. media without oGDF-9 or oGDF-9B). Data presented are the mean and standard error of the mean of 3 replicate experiments. Results from two independent antibodies samples are shown. *P<0.05 versus control media treated cells, [a]P<0.05, [b]P<0.01 versus oGDF-9+oGDF-9B treated cells that did not receive antibodies;

Figure 10 shows the effect of a polyclonal antibody from sheep immunized against ovine GDF-9 peptide (SEQ ID NO 6) on ovine (o) GDF-9 and oGDF-9B stimulated ^{3}H-thymidine incorporation of rat granulosa cells when added directly to the bioassay. The horizontal line indicates the level of response of the granulosa cells treated with control media (i.e. media without oGDF-9 or oGDF-9B). Data presented are the mean and standard error of the mean of 3 replicate experiments. **P<0.01 versus control media treated cells, [a]P<0.05, [b]P<0.01 versus oGDF-9 and oGDF-9B treated cells that did not receive antibodies; and

Figure 11 shows the effect of a polyclonal antibody from sheep immunized against ovine GDF-9 peptide (SEQ ID NO 8) on murine (m) GDF-9 and ovine (o) GDF-9B stimulated ^{3}H-thymidine incorporation of rat granulosa cells when added directly to the bioassay. The horizontal line indicates the level of response of the granulosa cells treated with control media (i.e. media without mGDF-9 or oGDF-9B). Data presented are the mean and standard error of the mean of 3 replicate experiments. **P<0.01 versus control media treated cells, [a]P<0.05 versus mGDF-9 and oGDF-9B treated cells that did not receive antibodies.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides putative type I, type II and dimerisation binding domains of GDF-9 and GDF-9B. It is postulated that stimulation or inhibition of these domains by agonists or antagonists that interact with these domains will be effective in modulating the ovulation rate of a female mammal.

In work leading up to the present invention, a three dimensional structure of the putative TGF-β type I and type II receptor binding domains and the putative dimerisation binding domain of GDF-9 and GDF-9B was employed in the molecular analysis of agonists and antagonists that are potentially capable of binding to these domains and potentiating or reducing their biological activity in vivo. The 3-D molecular structures and putative type I, type II and dimerisation binding domains are shown in figures 4a to 4d and 5a to 5d for GDF-9 and GDF-9B respectively.

The putative three dimensional structures of GDF9, GDF9B, Alk6 ectodomain and BMPR2 ectodomain were created using the Swiss model comparative protein modelling server (Schwede et al 2003). BMP2 (Kirsch et al 2000) and BMP7 (Griffith and Scott, 1995) were used as templates for BMP15. The Alk3 ectodomain (Kirsch et al 2000) was used as a template for the Alk6 ectodomain. The ActR2 ectodomain (Greenwald et al 2002) was used as a template for the BMPR2 ectodomain. The putative GDF9 and GDF9B type I binding domains were determined by Chimera matchmaker homology (Pettersen et al 2004) using a model of BMP2 binding to its type I receptor ALK3 (Kirsch et al 2000). The putative GDF9 and GDF9B type II binding domains were determined by homology using a model of BMP7 binding its type II receptor ActR2 (Greenwald et al 2002). The putative dimerisation binding domains of GDF9 and GDF9B were determined by homology using a model of BMP2 dimerisation (Kirsch et al 2000). Molecular graphics images were produced using the UCSF Chimera package from the Computer Graphics Laboratory, University of California, San Francisco (supported by NIH P41 RR-01081), (Pettersen et al 2004).

The sequences of the putative binding domains were then compared with the corresponding sequences of the GDF-9 and GDF-9B proteins in a number of different species and a consensus sequence determined as shown in Figures 6a to 6f for the type I, type II and dimerisation binding domains for GDF-9 and GDF-9B respectively.

A number of peptides were then synthesised which corresponded with sequences within or overlapping with the putative binding domains, or which closely abutted the putative binding domains, and which were anticipated to have an agonistic or antagonistic effect on the biological activity of GDF-9 and/or GDF-9B when administered in vivo. In particular, peptides were designed to be on the outside of the molecule according to its three dimensional structure; in a flexible region of the molecule; at least nine amino acids in length; non homologous with other

TGF beta family members; non convergent with other known proteins; in areas that did not contain a glycosylation site; and so that they could be coupled to a carrier protein. It was considered that the combination of these factors would result in peptides that could be used to produce antibodies that would influence biological activity and would not have cross-reactivity problems.

Thus, in a first embodiment, the present invention is directed to putative binding domains of GDF-9 comprising:

1. the putative type I receptor binding domain of GDF-9 comprising the amino acid sequence:

   $(X)_{32}ECE(X)_{10}LKW(X)_2WI(X)_{16}RAVGHRY(X)_6M(X)_2NI(X)_2EK(X)$

   (SEQ ID NO: 1);

2. the putative type II receptor binding domain of GDF-9 comprising the amino acid sequence:

   $(X)_{51}IVAPH(X)Y(X)_{47}SPLSVL(X)_8AY(X)_6I$ (SEQ ID NO: 2); and

3. the putative dimerisation binding domain of GDF-9 comprising the amino acid sequence:

   $(X)_{26}F(X)_{11}F(X)L(X)_4L(X)W(X)_9Y(X)PRYC(X)_{14}PVHT(X)VQ(X)_2I(X)_7VP(X)$ $PSCVP(X)_2Y(X)_{20}DM(X)A(X)_5R$ (SEQ ID NO: 3);

wherein the amino acids (other than X), form the putative binding domains when the GDF-9 molecule is in a three dimensional tertiary structure; and the remaining amino acid symbols correspond to the one letter code set out in Table 1, below.

X in SEQ ID NOs 1, 2 and 3 represents the amino acid residues present in the GDF-9 sequence shown in Figure 1 and do not form part of the putative binding domains of the present invention.

The present invention is further directed to an agonist or antagonist that is capable of interacting with one or more putative GDF-9 binding domains selected from the group comprising SEQ ID NOs 1 to 3.

Preferably the agonist or antagonist is a peptide comprising at least 5 contiguous amino acids of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto. Preferably the peptide is at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or at least 20 contiguous amino acids of SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3, or a functional derivative homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto. The peptides of the invention preferably abut with or include at least one amino acid of the putative binding sites.

Most preferably the agonist or antagonist comprises one or more peptides selected from the group comprising:

C(GG)PRAVGHRYGSPVHTM	(SEQ ID NO: 4);
FSQLKWDNWIVA(C)	(SEQ ID NO: 5);
C(GG)PRAVGHRYGS	(SEQ ID NO: 6);
SVPRPSCVPAKYS	(SEQ ID NO: 7);
GSIAYKEYE(C)	(SEQ ID NO: 8); and
AKYSPLSVLA(C)	(SEQ ID NO:9);

or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

Amino acids in parentheses refer to amino acids optionally added for conjugation purposes and do not correspond to the GDF-9 sequence per se.

In a second embodiment the present invention is directed to putative binding domains of GDF-9B comprising:

1. the putative type I receptor binding domain of GDF-9B comprising the amino acid sequence:
   $(X)_{22}QCS(X)_{10}LGW(X)_2WI(X)_{16}RVLHYGL(X)_6I(X)_3L(X)_2EL(X)_{33}Y$
   (SEQ ID NO: 10);
2. the putative type II receptor binding domain of GDF-9B comprising the amino acid sequence:
   $(X)_{41}IIAPH(X)Y(X)_{47}VPISIL(X)_8LY(X)_6I$ (SEQ ID NO: 11); and

3. the putative dimerisation binding domain of GDF-9B comprising the amino acid sequence:
$(X)_{28}F(X)V(X)_4L(X)W(X)_9Y(X)PNYC(X)_{14}PNHA(X)IQ(X)_2V(X)_7VP(X)PSCVP(X)_2Y(X)_{20}GMIA\ (X)_5R$ (SEQ ID NO: 12);

where the amino acids (other than X) form the putative binding domains when the GDF-9B molecule is in a three dimensional tertiary structure; and the remaining amino acid symbols correspond to the one letter code as set out in Table 1, below.

X in SEQ ID NOs 10, 11 and 12 represents the amino acid residues present in the GDF-9B sequence shown in Figure 2 and do not form part of the putative binding domains of the present invention.

The present invention is also directed to an agonist or antagonist that is capable of interacting with one or more putative binding domains selected from the group comprising SEQ ID NOs: 10 to 12.

Preferably, the agonist or antagonist is a peptide comprising at least 5 contiguous amino acids of SEQ ID NOS: 10-12 or a functional derivative, analog, homolog or mimetic thereof, or an antibody or antibody fragment that binds thereto. Preferably the peptide comprises at least 8, at least 10, at least 12, at least 14, at least 16, at least 18, or at least 20 contiguous amino acids of SEQ ID NO:10, SEQ ID NO: 11 or SEQ ID NO: 12, or a functional derivative homology, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto. The peptides of the invention preferably abut with or include at least one amino acid of the putative binding domains.

Most preferably, the agonist or antagonist comprises one or more peptides selected from the group comprising:

C(GG)PRVLHYGLNSPNHAI (SEQ ID NO: 13);
SFQQLGWDHWI(C) (SEQ ID NO: 14);
C(GG)PRVLHYGLNS (SEQ ID NO: 15);
NVPQPSCVPYKYV(C) (SEQ ID NO: 16);
PISILLIEANGSIL(C) (SEQ ID NO: 17);
GSILYKEYE(C) (SEQ ID NO: 18); and

C(GG)VPYKYVPISIL (SEQ ID NO: 19);

or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

Amino acids in parentheses refer to amino acids optionally added for conjugation purposes.

The peptides of the present invention may be synthesised using known technology.

Analogs, derivatives or variants of the peptides of the invention may include sequence modifications or non-sequence modifications. Non-sequence modifications can include acetylation, methylation, phosphomethylation, carboxilation or glycosylation.

The specific binding site peptides exemplified in the present invention are shown in relation to their position on GDF-9 and GDF-9B molecules as shown in figures 1 and 2, respectively.

In a third embodiment the invention is directed to an isolated peptide selected from the group comprising:

C(GG)PRAVGHRYGSPVHTM (SEQ ID NO: 4);
FSQLKWDNWIVA(C) (SEQ ID NO: 6);
SVPRPSCVPAKYS (SEQ ID NO: 7);
GSIAYKEYE(C) (SEQ ID NO: 8);
AKYSPLSVLA(C) (SEQ ID NO:9);
C(GG)PRVLHYGLNSPNHAI (SEQ ID NO: 13);
SFQQLGWDHWI(C) (SEQ ID NO: 14);
C(GG)PRVLHYGLNS (SEQ ID NO: 15);
NVPQPSCVPYKYV(C) (SEQ ID NO: 16);
PISILLIEANGSIL(C) (SEQ ID NO: 17);
GSILYKEYE(C) (SEQ ID NO: 18); and
C(GG)VPYKYVPISIL (SEQ ID NO: 19);

or a functional derivative, homologue, analog or mimetic thereof.

In a fourth embodiment, the invention is directed to antibodies or antibody fragments which belong to one or more peptides of SEQ ID NOS: 4-9 and 13-19.

Preferred analogs include peptides who's sequence differs from those of the invention by one or more conservative amino acid substitutions, deletions or insertions which do not affect the biological activity of the peptide. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Examples of conservative substitutions can also be found in the sequences of GDF-9 and GDF-9B in Figures 6a to 6f whereby the substitutions in different mammalian species compared to the consensus sequence are shown. Other conservative substitutions can be taken from Table 1 below.

TABLE 1

CONSERVATIVE AMINO ACID REPLACEMENTS

For Amino Acid	Code	Replace with any of
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, .beta.-Ala Acp
Histidine	H	Asp, D-Asp, Lys, D-Lys, Tyr
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Leu, D-Leu, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val

Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, cis-3,4, or 5-phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D- or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

Other analogs include peptides with modifications which increase peptide stability. Such analogs may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are analogs that include residues other than naturally occurring L-amino acids, e.g. D-amino acids or non-naturally occurring synthetic amino acids, e.g. beta or gamma amino acids and cyclic analogs.

In a further aspect, the invention provides a use of the agonists and antagonists of the invention, in a method of modulating the ovulation rate of a female mammal, including both human and non-human mammals. Such non-human mammals include sheep, cattle, goats, deer, pigs, horses, camelids, possums, non-human primates such as marmosets, cats, dogs and other commercially important species.

The method may comprise administering to said mammal an effective amount of one or more of said agonists or antagonists, or a functional variant thereof, or an antibody or antibody fragment that binds thereto.

It is contemplated that equivalent binding site peptides of GDF-9 and/or GDF-9B of a mammal to be treated will be used in the methods of the present invention, as shown in Table 1a, below.

TABLE 1A

GDF-9B	Seq ID No 13	Seq ID No 14	Seq ID No 15
	C(GG)PRVLHYGLNSPNHAI	SFQQLGWDHWI(C)	C(GG)PRVLHYGLNS
cow	CPRVLHYGLNSPNHAI	SFQQLGWDHWI	CPRVLHYGLNS
deer	CPRVLHYGLNSPNHAI	SFQQLGWDHWI	CPRVLHYGLNS
dog	CPRVLHYGLNSPNHAI	SFHQLGWDHWI	CPRVLHYGLNS
cat	CPRVLHYGLNSPNHAI	SFHQLGWDHWI	CPRVLHYGLNS
possum	CPRVLHSGLRSPNHAI	SFHQLGWENWI	CPRVLHSGLRS
chimpanzee	CLRVLRDGLNSPNHAI	SFRQLGWDHWI	CLRVLRDGLNS
human	CLRVLRDGLNSPNHAI	SFRQLGWDHWI	CLRVLRDGLNS
mouse	CTRVLPYGLNSPNHAI	SFHQLGWDHWI	CTRVLPYGLNS
rat	CTGVLPYGLNSPNHAI	SFHQLGWDHWI	CTGVLPYGLNS
rabbit	CPRVLRYGLNSPNHAI	SFRQLGWDHWI	CPRVLRYGLNS
pig	CPRVLHYGLNSPNHAI	SFHQLGWDHWI	CPRVLHYGLNS
goat	CPRVLYYGLNSPNHAI	SFQQLGWDHWI	CPRVLYYGLNS
sheep	CPRVLHYGLNSPNHAI	SFQQLGWDHWI	CPRVLHYGLNS
consensus	CPRVLHYGLNSPNHAI	SF QLGWDHWI	CPRVLHYGLNS

GDF-9B	Seq ID No 16	Seq ID No 17	Seq ID No 18	Seq ID No 19
	NVPQPSCVPYKYV(C)	PISILLIEANGSIL(C)	GSILYKEYE(C)	C(GG)VPYKYVPISIL
cow	SVPQPSCVPYKYV	PISILLIEANGSIL	GSILYKEYE	CVPYKYVPISIL
deer	SVPQPSCVPYKYV	PISILLIEANGSIL	GSILYKEYE	CVPYKYVPISIL
dog	SVPQPSCVPYKYV	PISILLVEANGSIL	GSILYKEYE	CVPYKYVPISIL
cat	SIPQPSCVPYKYV	PISILLVEANGSIL	GSILYKEYE	CVPYKYVPISIL
possum	SIPRPSCVPYKYM	PISVLLIEASGSIL	GSILYKEYE	CVPYKYMPISVL
chimpanzee	SVPRPSCVPYKYV	PISVLMIEASGSIL	GSILYKEYE	CVPYKYVPISVL
human	SVPRPSCVPYKYV	PISVLMIEANGSIL	GSILYKEYE	CVPYKYVPISVL
mouse	SVPQPSCVPYNFL	PMSILLIETNGSIL	GSILYKEYE	CVPYNFLPMSIL
rat	SVPQLSCVPYKFL	PMSILLIEANGSIL	GSILYKEYE	CVPYKFLPMSIL
rabbit	SVPQPSCVPYKYV	PISILLIEANGSIL	GSILYKEYE	CVPYKYVPISIL
pig	SVPQPSCVPYKYV	PISILLIEANGSIL	GSILYKEYE	CVPYKYVPISIL
goat	NVPQPSCVPYKYV	PISILLIEANGSIL	GSILYKEYE	CVPYKYVPISIL
sheep	NVPQPSCVPYKYV	PISILLIEANGSIL	GSILYKEYE	CVPYKYVPISIL
consensus	SVPQPSCVPYKYV	PISILLIEANGSIL	GSILYKEYE	CVPYKYVPISIL

GDF9	Seq ID No 4	Seq ID No 5	Seq ID No 6
	C(GG)PRAVGHRYGSPVHTM	FSQLKWDNWIV(C)	C(GG)PRAVGHRYGS
cow	CPRAVGHRYGSPVHTM	FSQLKWDNWIVA	CPRAVGHRYGS
dog	CPRAVGHRYGSPVHTM	FSQLKWDNWIVA	CPRAVGHRYGS
chimpanzee	CPRAVGHRYGSPVHTM	FSQLKWDNWIVA	CPRAVGHRYGS
cat	CPRALGHRYGSPVHTM	FSQLKWDSWIVA	CPRALGHRYGS
goat	CPRAVGHRYGSPVHTM	FSQLKWDNWIVA	CPRAVGHRYGS
human	CPRAVGHRYGSPVHTM	FSQLKWDNWIVA	CPRAVGHRYGS
mouse	CPRAVRHRYGSPVHTM	FSQLKWDNWIVA	CPRAVRHRYGS
sheep	CPRAVGHRYGSPVHTM	FSQLKWDNWIVA	CPRAVGHRYGS
pig	CPRAVGHRYGSPVHTM	FSQLKWDNWIVA	CPRAVGHRYGS
possum	CPRLVRHRYGSPVHTM	FSQLKWDNWIVA	CPRLVRHRYGS
rabbit	CPRAVGHRYGSPVHTV	FSQLKWDNWIVA	CPRAVGHRYGS

rat	CPRAVRHRYGSPVHTM	FSQLKWDNWIVA	CPRAVRHRYGS
consensus	CPRAVGHRYGSPVHTM	FSQLKWDNWIVA	CPRAVGHRYGS

GDF9	Seq ID No 7	Seq ID No 8	Seq ID No 9
	SVPRPSCVPAKYS	GSIAYKEYE(C)	AKYSPLSVLA(C)
cow	SVPRPSCVPAKYS	GSIAYKEYE	AKYSPLSVLA
dog	SVPRPSCVPAKYS	GSIAYKEYE	AKYSPLSVLT
chimpanzee	SVPRPSCVPAKYS	GSIAYKEYE	AKYSPLSVLT
cat	SVPRPSCVPAKYS	GSIAYKEYE	AKYSPLSVLT
goat	SVPRPSCVPAKYS	GSIAYKEYE	AKYSPLSVLA
human	SVPRPSCVPAKYS	GSIAYKEYE	AKYSPLSVLT
mouse	SVPRPSCVPGKYS	GSIAYKEYE	GKYSPLSVLT
sheep	SVPRPSCVPAKYS	GSIAYKEYE	AKYSPLSVLA
pig	SVPRPSCVPAKYS	GSIAYKEYE	AKYSPLSVLA
possum	SIPRPSCVPAKYS	GSIAYKGYE	AKYSPLSVLT
rabbit	SVPRPSCVPARYS	GSIAYKEYE	ARYSPLSVLT
rat	SVPRPSCVPGKYS	GSIAYKEYE	GKYSPLSVLT
consensus	SVPRPSCVPAKYS	GSIAYKEYE	AKYSPLSVLT

The modulation of the ovulation rate may comprise an increase or decrease in the ovulation rate of the female mammal by the administration of one or more agonists or antagonists of the invention to said animal resulting in antibodies being raised in vivo to said one or more agonists or antagonists which in turn bind to the particular binding domain on GDF-9 and/or GDF-9B to affect the biological activity thereof.

Without being bound by theory, it is considered that binding of a type I, type II and/or dimerisation binding domain of GDF-9 and/or GDF-9B by an agonist/antagonist, and in particular by one or more antibodies raised against one or more peptides selected from the group comprising SEQ ID NOs: 4-10 and 14-20, results in altered circulating concentration of biologically active GDF-9 and/or GDF-9B. Where such a decrease comprises an approximate 50% fall in GDF-9B activity, an increase in ovulation rate has been observed previously, in particular, in Hanna Sheep where a single point mutation in the GDF-9B gene should result in half the amount of active GDF-9B in heterozygous animals and resulting in an increased ovulation rate and twinning. In homozygous animals, where there is no or very little circulating active GDF-9B, the animals were sterile (Galloway *et al; 2000*). Similarly, point mutations in the GDF-9 gene and concomitant modulation in ovulation rates have been observed in sheep (WO 03/102199; Hanrahan *et al.*, 2003).

Thus, it is postulated that an agonist or antagonist of the invention, or combination thereof, that results in a decrease of approximately 50% in the circulating levels of active GDF-9 and/or GDF-9B will result in an increase in ovulation rate, whilst an agonist or antagonist, or combination thereof, that results in a reduction in the circulating concentration of active GDF-9 and/or GDF-9B to approximately zero, will result in a decrease in ovulation and sterilisation in a female mammal.

Preferably the agonist or antagonist of the invention is an antibody which binds to the consensus binding domains of GDF-9 and/or GDF-9B of SEQ ID NOS: 1, 2, 3, 10, 11 and 12. It should be appreciated that the term "antibody" encompasses fragments or analogues of antibodies which retain the ability to bind to a consensus binding domain defined herein, including but not limited to Fv, $F(ab)_2$ fragments, ScFv molecules and the like. The antibody may be polyclonal or monoclonal, but is preferably monoclonal. Such antibodies may be prepared by any technique known in the art for example (Juengel *et al.*, 2002) for administration to an animal, i.e. for use in passive immunisation. Alternatively, such antibodies may be produced in vivo by administration of an antigen in a suitable adjuvant, i.e. for use in active immunisation. Suitable adjuvants include Freund's complete or incomplete adjuvant, DEAE dextran or similar immunostimulatory agent. The antibodies of the present invention may also be produced by genetic engineering methods such as chimeric and humanised monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques.

The present invention further contemplates the use of one or more agonists and/or antagonists of the present invention in combination with one or more active ingredients known to modulate ovulation rate to enhance the effect on ovulation. The active ingredients may be selected from the group comprising GDF-9; GDF-9B; BMPRII; BMPIB receptor (ALK6); ALK 5 and BMP6, or functional fragments or variants thereof. In particular, the invention contemplates the use of BMP1B receptor in combination with an antibody or antibody fragment (Fc) that binds to a peptide of SEQ ID NOs: 13-19, in the modulation of ovulation in a female mammal.

The present invention further provides a pharmaceutical composition comprising at least one agonist or antagonist of the present invention together with a pharmaceutically acceptable carrier useful for the modulation of ovulation rate.

It is contemplated that the agonists or antagonists of the invention will be tested for biological activity in an animal model or in an in vitro model and suitably active compounds formulated into pharmaceutical compositions. The pharmaceutical compositions of the present invention may comprise, in addition to one or more agonists or antagonists of the present invention, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other material well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will be dependent upon the desired nature of the pharmaceutical composition, and the route of administration e.g. oral, intravenous, cutaneous, subcutaneous, intradermal, intramuscular or intraperitoneal.

Pharmaceutical compositions for oral administration may be in tablet, lozenge, capsule, powder, granule or liquid form. A tablet or other solid oral dosage form will usually include a solid carrier such as gelatine, starch, mannitol, crystalline cellulose, or other inert materials generally used in pharmaceutical manufacture. Similarly, liquid pharmaceutical compositions such as a syrup or emulsion, will generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil.

For intravenous, cutaneous, subcutaneous, intradermal or intraperitoneal injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability.

In a further embodiment, the invention contemplates the use of one or more additional modulators of ovulation to be co-administered with the pharmaceutical composition of the present invention to give an additive or synergistic effect to the treatment regime. Examples of such additional modulators of ovulation include follicle stimulating hormone, Androvax (an androsteindione protein vaccine conjugate), and steroid hormone. Such modulators may be administered either separately, sequentially or simultaneously with at least one agonist or antagonist of the present invention depending upon whether ovulation is to be increased or decreased as will be appreciated by a skilled worker.

Administration of the pharmaceutical composition of the invention is preferably in a "therapeutically effective amount", this being sufficient to show the desired benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the female mammals underlying condition. Prescription

of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A. (ed), 1980.

The invention will now be described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

EXAMPLES

Example 1

Use of peptides and antibodies to putative type I and type II receptor domains and to putative dimerisation domains on GDF-9 and GDF-9B to manipulate ovulation.

Twelve 10 to 18mer peptides were synthesised corresponding to the putative TGF-β type I, type II and dimerisation binding domains on the GDF-9 and GDF-9B 3-D protein sequences and including, where necessary, additional residues to facilitate conjugation to Keyhole Limpet Haemocyanin (KLH) to generate an antigen. The peptide sequences were:

C(GG)PRAVGHRYGSPVHTM	(SEQ ID NO: 4);
FSQLKWDNWIVA(C)	(SEQ ID NO: 5);
C(GG)PRAVGHRYGS	(SEQ ID NO: 6);
SVPRPSCVPAKYS	(SEQ ID NO: 7);
GSIAYKEYE(C)	(SEQ ID NO: 8);
AKYSPLSVLA(C)	(SEQ ID NO:9);
C(GG)PRVLHYGLNSPNHAI	(SEQ ID NO: 13);
SFQQLGWDHWI(C)	(SEQ ID NO: 14);
C(GG)PRVLHYGLNS	(SEQ ID NO: 15);
NVPQPSCVPYKYV(C)	(SEQ ID NO: 16);
GSILYKEYE(C)	(SEQ ID NO: 18); and
C(GG)VPYKYVPISIL	(SEQ ID NO: 19).

In this study, groups of 10 anoestrous Romney ewes were injected with 0.4 mg/ewe of each peptide-KLH conjugate antigen in Freunds complete adjuvant and 10 anoestrous Romney ewes were injected with 0.4 mg/ewe KLH antigen as a control group. Subsequently at monthly intervals on 4 occasions, the animals were boosted with further antigen (0.2 mg/ewe on each occasion) in a Span/Tween/Oil mixture (sc) and oestrous activity was monitored using vasectomised rams during the breeding season. The ovulation rate was assessed by laparoscopy over four successive oestrous cycles and again at the termination of the experiment approximately 20-30 days after the last laparoscopy. All of the control ewes displayed cyclical oestrous activity. The results of the effects of peptides of SEQ ID NOs: 4-9 and 13-16, 18 and 19 on ovulation rate are shown in Table 2 below:

TABLE 2 Number of ewes showing altered ovulation rate (inactive ovary or ovulation rate $\geq$ 3/number of ewes examined

Treatment	Observation					Target
	1	2	3	4	5	
KLH	0/10	0/8	1/9	0/9	0/10	
KLH-Peptide (SEQ ID NO:4)	5/9 **	5/9 *	7/9 **	8/10 ***	8/10 ***	I
KLH-Peptide (SEQ ID NO:5)	5/10 **	3/10	4/10	7/9 ***	8/10 ***	I
KLH-Peptide (SEQ ID NO:6)	4/10 *	4/10 *	7/10 **	7/10 **	10/10 ***	I
KLH-Peptide (SEQ ID NO:7)	0/10	0/10	1/10	1/9	1/10	D
KLH-Peptide (SEQ ID NO:8)	1/9	1/10	2/9	4/10 *	5/10 **	II
KLH-Peptide (SEQ ID NO:9)	0/9	3/9	1/10	2/10	1/10	II
KLH-Peptide (SEQ ID NO:13)	1/10	3/10	4/10	9/10 **	9/10 ***	I
KLH-Peptide (SEQ ID NO:14)	2/9	0/10	7/9 **	5/9 **	6/9 **	I
KLH-Peptide (SEQ ID NO 15)	2/10	4/9 *	6/9 **	6/10 **	6/10 **	I
KLH-Peptide (SEQ ID NO:16)	2/10	0/10	2/9	3/10	5/10 **	D
KLH-Peptide (SEQ ID NO:18)	2/10	3/10	2/9	4/10 *	7/10 **	II
KLH-Peptide (SEQ ID NO:19)	4/9 *	5/9 *	5/10	7/10 **	9/10 ***	II

* $P<0.05$; ** $P<0.01$; *** $P<0.001$ using χ^2
I = Type I receptor binding site; II = Type II receptor binding site; D = dimerisation site

The peptides directed to the type I receptor binding site of GDF-9 (SEQ ID NOs: 4-6) and GDF-9B (SEQ ID NOs: 13-15) showed significant alteration of ovulation rate compared to the control (KHL) ewes.

The peptide directed to the dimerisation site of GDF-9 (SEQ ID NO: 7) and the peptide directed to the type II receptor binding site of GDF-9 (SEQ ID NO: 9) did not appear to have any significant effect on the ovulation rate of the treated ewes compared to controls (KHL) ewes. However, peptide of SEQ ID NO: 8 (directed to the type II receptor binding site of GDF-9) showed a significant alteration in ovulation rate in ewes at the 4^{th} and 5^{th} observation (i.e. after 3 booster injections).

The peptides directed to the type II receptor binding site of GDF-9B (SEQ ID NOs: 18 and 19) showed a significant alteration on ovulation rate. Peptide of SEQ ID NO: 19 appeared to be more effective at modulation of ovulation of the type II receptor of GDF-9B than the peptide of SEQ ID NO: 18 in that it significantly affected ovulation rate after the first booster injection.

The peptide directed to the dimerisation site of GDF-9B (SEQ ID NO: 16) had no significant effect on ovulation rate until after the final observation when 50% of the treated ewes showed an altered ovulation rate.

Example 2

Effect of antibodies raised against GDF-9 and GDF-9B peptides on ^{3}H thymidine incorporation by rat granulosa cells in vitro.

Various antibody preparations were tested for their ability to neutralize the effects of ovine or murine GDF-9 and ovine GDF-9B on rat granulosa cells when added directly to the granulosa cell culture using a previously described method (McNatty et al., 2005). A total of 100 μg/ml of IgG was added for each treatment which was comprised of IgG purified from a sheep immunized with the GDF-9 or GDF-9B peptide specified with the balance of IgG purified from sheep immunized with KLH. The antibodies were able to neutralize the effects of the growth factor that they were directed against (see Figures 7-11). In Figure 9, even though a small suppressive effect was observed with the control antibody, thymidine incorporation was further suppressed by the GDF-9B antibody samples and both GDF-9B antibody samples were able to completely suppress the stimulatory effect of oGDF-9 and oGDF-9B on thymidine incorporation indicating that this antibody was specifically blocking the effects of

GDF-9B. Likewise, in Figure 10, even though a small suppressive effect was observed with the control antibody, thymidine incorporation was further suppressed by the GDF-9 antibody and the GDF-9 antibody sample was able to completely suppress the stimulatory effect of oGDF-9 and oGDF-9B on thymidine incorporation indicating that this antibody was specifically blocking the effects of GDF-9.

Neutralization of the effects of ovine and murine GDF-9 and ovine GDF-9B by antibodies directed against specific regions of the protein (e.g. SEQ ID NO: 6, SEQ ID NO 8; SEQ ID NO: 14; SEQ ID NO: 19) on incorporation of thymidine uptake by rat granulosa cells in vitro, taken together with the effects of active immunization with these peptides in vivo in sheep (See example 1), indicates that targeting of these regions of the protein can block biological activity of the protein in multiple species. These results also indicate that the antibodies against these regions of the mature region of GDF-9 and GDF-9B are effective in passively neutralizing GDF-9 and GDF-9B.

In summary, peptides directed to the type I and type II receptor binding sites of GDF-9 and GDF-9B disclosed herein are useful in altering ovulation rate in female mammals. It does not appear from this study, that peptides targeted to the dimerisation site are effective at interfering with ovulation in vivo.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alternatives to the embodiments and methods described herein may be made without deporting from the scope of the invention disclosed herein.

References are listed in the following page and are incorporated herein by this reference.

INDUSTRIAL APPLICATION

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

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WHAT WE CLAIM IS:

1. A putative type I receptor binding domain of GDF-9 comprising the amino acid sequence:

$(X)_{32}ECE(X)_{10}LKW(X)_2WI(X)_{16}RAVGHRY(X)_6M(X)_2NI(X)_2EK(X)_{33}Y$ (SEQ ID NO: 1), wherein the amino acids (other than X), form the binding domain when the GDF-9 molecule is in a three dimensional tertiary structure.

2. A putative type II receptor binding domain of GDF-9 comprising the amino acid sequence:

$(X)_{51}IVAPH(X)Y(X)_{47}SPLSVL(X)_8AY(X)_6I$ (SEQ ID NO: 2), wherein the amino acids (other than X), form the binding domain when the GDF-9 molecule is in a three dimensional tertiary structure.

3. A putative dimerisation binding domain of GDF-9 comprising the amino acid sequence:

$(X)_{26}F(X)_{11}F(X)L(X)_4L(X)W(X)_9Y(X)PRYC(X)_{14}PVHT(X)VQ(X)_2I(X)_7VP(X)PSCVP(X)_2Y(X)_{20}DM(X)A(X)_5R$ (SEQ ID NO: 3), wherein the amino acids (other than X), form the dimerisation binding domain when the GDF-9 molecule is in a three dimensional tertiary structure.

4. A putative binding domain as claimed in any one of claims 1 to 3, wherein X represents the amino acid residues present in the GDF-9 sequence shown in Figure 1.

5. An agonist or antagonist that is capable of interacting with one or more of the putative GDF-9 binding domains of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, as claimed in any one of claims 1 to 3.

6. An agonist or antagonist as claimed in claim 5, comprising a peptide of at least 5 contiguous amino acids of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

7. An agonist or antagonist as claimed in claim 6, comprising a peptide of at least 8 contiguous amino acids of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

8. An agonist or antagonist as claimed in claim 6, comprising a peptide of at least 10 contiguous amino acids of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

9. An agonist or antagonist as claimed in claim 6, comprising a peptide of at least 12 contiguous amino acids of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

10. An agonist or antagonist as claimed in claim 6, comprising a peptide of at least 14 contiguous amino acids of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

11. An agonist or antagonist as claimed in claim 6, comprising a peptide of at least 16 contiguous amino acids of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

12. An agonist or antagonist as claimed in claim 6, comprising a peptide of at least 18 contiguous amino acids of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

13. An agonist or antagonist as claimed in claim 6, comprising a peptide of at least 20 contiguous amino acids of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

14. An agonist or antagonist as claimed in any one of claims 5-13, comprising a peptide wherein the peptide abuts with or includes at least one amino acid of a putative binding domain as claimed in any one of claims 1-4.

15. An agonist or antagonist as claimed in any one of claims 5-14, comprising one or more peptides selected from the group comprising:

C(GG)PRAVGHRYGSPVHTM (SEQ ID NO: 4);
FSQLKWDNWIVA(C) (SEQ ID NO: 5);
C(GG)PRAVGHRYGS (SEQ ID NO: 6);
SVPRPSCVPAKYS (SEQ ID NO: 7);
GSIAYKEYE(C) (SEQ ID NO: 8); and
AKYSPLSVLA(C) (SEQ ID NO:9);

or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

16. An isolated peptide useful as an agonist or antagonist of one or more of the putative binding domains of any one of claims 1-4, selected from the group comprising:

C(GG)PRAVGHRYGSPVHTM (SEQ ID NO: 4);
FSQLKWDNWIVA(C) (SEQ ID NO: 5);
C(GG)PRAVGHRYGS (SEQ ID NO: 6);
SVPRPSCVPAKYS (SEQ ID NO: 7);
GSIAYKEYE(C) (SEQ ID NO: 8); and
AKYSPLSVLA(C) (SEQ ID NO:9);

or a functional derivative, homolog, analog or mimetic thereof.

17. An antibody or antibody fragment that binds to one or more peptides of claim 16 and is useful as an agonist or antagonist of one or more putative binding domains of any one of claims 1-4.

18. A putative type I receptor binding domain of GDF-9B comprising the amino acid sequence:

$(X)_{22}QCS(X)_{10}LGW(X)_2WI(X)_{16}RVLHYGL(X)_6I(X)_3L(X)_2EL(X)_{33}Y$ (SEQ ID NO:10), wherein the amino acids (other than X), form the binding domain when the GDF-9B molecule is in a three dimensional tertiary structure.

19. A putative type II receptor binding domain of GDF-9B comprising the amino acid sequence:
$(X)_{41}$IIAPH(X)Y$(X)_{47}$VPISIL$(X)_8$LY$(X)_6$I (SEQ ID NO:11), wherein the amino acids (other than X), form the binding domain when the GDF-9B molecule is in a three dimensional tertiary structure.

20. A putative dimerisation binding domain of GDF-9B comprising the amino acid sequence:
$(X)_{28}$F(X)V$(X)_4$L(X)W$(X)_9$Y(X)PNYC$(X)_{14}$PNHA(X)IQ$(X)_2$V$(X)_7$VP(X)PSCVP$(X)_2$Y$(X)_{20}$G MIA $(X)_5$R (SEQ ID NO:12), wherein the amino acids (other than X), form the dimerisation binding domain when the GDF-9B molecule is in a three dimensional tertiary structure.

21. A putative binding domain as claimed in any one of claims 18-20, wherein X represents the amino acid residues present in the GDF-9B sequence shown in Figure 2.

22. An agonist or antagonist that is capable of interacting with one or more of the putative GDF-9B binding domains of SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12 as claimed in any one of claims 18-20.

23. An agonist or antagonist as claimed in claim 22, comprising a peptide of at least 5 contiguous amino acids of SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

24. An agonist or antagonist as claimed in claim 23, comprising a peptide of at least 8 contiguous amino acids of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative, homology, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

25. An agonist or antagonist as claimed in claim 23, comprising a peptide of at least 10 contiguous amino acids of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative, homology, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

26. An agonist or antagonist as claimed in claim 23, comprising a peptide of at least 12 contiguous amino acids of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative, homology, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

27. An agonist or antagonist as claimed in claim 23, comprising a peptide of at least 14 contiguous amino acids of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative, homology, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

28. An agonist or antagonist as claimed in claim 23, comprising a peptide of at least 16 contiguous amino acids of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative, homology, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

29. An agonist or antagonist as claimed in claim 23, comprising a peptide of at least 18 contiguous amino acids of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative, homology, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

30. An agonist or antagonist as claimed in claim 23, comprising a peptide of at least 20 contiguous amino acids of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative, homology, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

31. An agonist or antagonist as claimed in any one of claims 22-30, comprising a peptide, wherein the peptide abuts with or includes at least one amino acid of a putative binding domain as claimed in any one of claims 18-21.

32. An agonist or antagonist as claimed in any one of claims 22-31, comprising one or more peptides selected from the group comprising:

C(GG)PRVLHYGLNSPNHAI (SEQ ID NO: 13);

SFQQLGWDHWI(C) (SEQ ID NO: 14);

C(GG)PRVLHYGLNS (SEQ ID NO: 15);
NVPQPSCVPYKYV(C) (SEQ ID NO: 16);
PISILLIEANGSIL(C) (SEQ ID NO: 17);
GSILYKEYE(C) (SEQ ID NO: 18); and
C(GG)VPYKYVPISIL (SEQ ID NO: 19);
or a functional derivative, homolog, analog or mimetic thereof, or an antibody or antibody fragment that binds thereto.

33. An isolated peptide useful as an agonist or antagonist of one or more of the putative binding domain of any one of claims 18-21, selected from the group comprising:
C(GG)PRVLHYGLNSPNHAI (SEQ ID NO: 13);
SFQQLGWDHWI(C) (SEQ ID NO: 14);
C(GG)PRVLHYGLNS (SEQ ID NO: 15);
NVPQPSCVPYKYV(C) (SEQ ID NO: 16);
PISILLIEANGSIL(C) (SEQ ID NO: 17);
GSILYKEYE(C) (SEQ ID NO: 18); and
C(GG)VPYKYVPISIL (SEQ ID NO: 19);
or a functional derivative, homolog, analog or mimetic thereof.

34. An antibody or antibody fragment that binds to one or more peptides of claim 33 and is useful as an agonist or antagonist of one or more putative binding domains of any one of claims 18-21.

35. A method of modulating the ovulation rate of a female mammal, said method comprising the step of administering to said mammal an effective amount of one or more agonists or antagonists of any one of claims 5-15 and 22-32, that are capable of interacting with one or more putative GDF-9 and/or GDF-9B type I or type II receptor binding domains and/or dimerisation binding domains of any one of claims 1-4 and 18-21, and altering the biological activity thereof.

36. A method of modulating the ovulation rate of a female mammal, comprising administering to said mammal an effective amount of one or more peptides selected from SEQ ID NOs 4 to 9 and 13 to 19, or a functional variant thereof, or an antibody or antibody fragment that binds thereto.

37. A use of one or more agonists or antagonist as claimed in any one of claims 5-18 and 22-32 in the manufacture of a medicament for modulating the ovulation rate of a female mammal.

38. A use of one or more peptides as claimed in claim 16 or 33, in the manufacture of a medicament for modulating the ovulation rate of a female mammal.

39. A use of one or more antibodies or antibody fragments as claimed in claim 17 or 34 , in the manufacture of a medicament for modulating the ovulation rate of a female mammal.

40. A use of a) one or more peptides as claimed in claim 16 or 33 and b) one or more antibodies or antibody fragments as claimed in claim 17 or 34, in the manufacture of a medicament for modulating the ovulation rate of a female mammal, wherein the medicament is formulated for separate, sequential or simultaneous administration of a) and b).

41. A pharmaceutical composition comprising one or more agonists or antagonists as claimed in any one of claims 5-15 and 22-32 together with a pharmaceutically acceptable carrier or excipient.

42. A pharmaceutical composition comprising one or more peptides as claimed in claim 16 or 33 together with a pharmaceutically acceptable carrier or excipient.

43. A pharmaceutical composition comprising one or more antibodies or antibody fragments as claimed in claim 17 or 34, together with a pharmaceutically acceptable carrier or excipient.

44. A pharmaceutical composition comprising a) one or more peptides as claimed in claim 16 or 33 and b) one or more antibodies or antibody fragments as claimed in claim 17 or 34, together with a pharmaceutically acceptable carrier or excipient.