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Highly Prolific Booroola Sheep Have a Mutation in the Intracellular Kinase Domain of Bone Morphogenetic Protein IB Receptor (ALK-6) That Is Expressed in Both Oocytes and Granulosa Cells[1]

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ABSTRACT

The Booroola fecundity gene (*FecB*) increases ovulation rate and litter size in sheep and is inherited as a single autosomal locus. The effect of *FecB* is additive for ovulation rate (increasing by about 1.6 corpora lutea per cycle for each copy) and has been mapped to sheep chromosome 6q23–31, which is syntenic to human chromosome 4q21–25. Bone morphogenetic protein IB (BMP-IB) receptor (also known as ALK-6), which binds members of the transforming growth factor-β (TGF-β) superfamily, is located in the region containing the *FecB* locus. Booroola sheep have a mutation (Q249R) in the highly conserved intracellular kinase signaling domain of the BMP-IB receptor. The mutation segregated with the *FecB* phenotype in the Booroola backcross and half-sib flocks of sheep with no recombinants. The mutation was not found in individuals from a number of sheep breeds not derived from the Booroola strain. BMPR-IB was expressed in the ovary and in situ hybridization revealed its specific location to the oocyte and the granulosa cell. Expression of mRNA encoding the BMP type II receptor was widespread throughout the ovary. The mutation in BMPR-IB found in Booroola sheep is the second reported defect in a gene from the TGF-β pathway affecting fertility in sheep following the recent discovery of mutations in the growth factor, GDF9b/BMP15.

granulosa cells, kinases, ovulation, signal transduction

INTRODUCTION

The Booroola phenotype of increased litter size was originally selected in specific lines of Australian Merino sheep. Subsequent studies of sheep carrying the Booroola gene (*FecB*) confirmed segregation as a single autosomal locus [1, 2]. *FecB* is located on sheep chromosome 6 [3], which is syntenic with human chromosome 4 [4], and was the first major gene for prolificacy identified in sheep. On average, this gene increases litter size by one to two extra lambs with each copy of the *FecB* mutation. Another major gene (*FecX*) affecting the ovulation rate has been found in Romney sheep [5]. The Inverdale gene (*FecX*), located on the X chromosome, carries an inactivating mutation in the growth differentiation factor, GDF9b/BMP15 [6].

The bone morphogenetic proteins (BMPs) are members of the transforming growth factor-β (TGF-β) superfamily. They are multifunctional proteins that regulate growth and differentiation in many cell types. Members of this family play essential roles during embryogenesis in mammals, amphibians, and insects as well as in bone development, wound healing, hematopoiesis, and immune and inflammatory responses [7–10]. They also play critical roles in the fertility of mammals with the growth factors, GDF9 and GDF9b, localized in the oocyte [11–14], and BMP receptors expressed in the ovary [15]. Several members of this family initiate signaling from the cell surface through distinct complexes of type I and type II serine/threonine receptors [16, 17]. The type I receptor acts downstream of the type II receptor and determines signal specificity [18]. After ligand binding, the type II receptor phosphorylates the type I receptor and activates this kinase. The activated type I receptor then propagates the signal to downstream substrates, using the Smad proteins as carriers of the signal [19, 20]. Signal specificity is determined both by the specific ligand and the different Smad proteins used in the signal transduction.

The identification of the *FecB* mutation is of significance to the sheep industry and is of interest to those involved in the study of mammalian fertility. We describe the mapping and study of a mutation in the BMP-IB receptor that we propose is responsible for the *FecB* phenotype. Moreover, we report on the ontogeny and cellular localization of BMPR-IB and BMPR-II mRNA in the ovaries from sheep with and without the Booroola gene, and the effects of the gene on oocyte growth.

MATERIALS AND METHODS

Animals

The experimental procedures reported in this study were carried out in accordance with the 1987 Animal Protection (Codes of Ethical Conduct) Regulations of New Zealand after approval was granted by the AgResearch Invermay Animal Ethics Committee and the Wallaceville Animal Ethics Committee. The animals used in the mapping study were from the AgResearch Booroola half-sib and backcross flock. Fifteen B+ rams were mated with ++ ewes, which generated 540 half-sib daughters. For the backcross families, BB rams were mated with ++ ewes and their B+ daughters were mated with ++ rams to follow the inheri-

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tance through 3–4 generations (249 animals in total). Female progeny were measured by laparoscopy twice at consecutive cycles at approximately 19 and 31 mo of age. If a measurement could not be taken or no corpora lutea were observed the ovulation rate was recorded as missing. The mean residual deviation from the population mean over all four traits were used for quantitative trait locus analysis.

Mapping

For initial mapping studies, markers from chromosome 6 were typed-in DNA samples from the half-sib and backcross flocks. *FecB* genotypes were assigned on the basis of records of ovulation rate as previously described [3], except that an additional constraint was placed on the half-sib family members before a genotype was assigned. This constraint required that the mean ovulation rate was not in the central 10% of mean ovulation rates for that family, and was used to account for the differences in mean ovulation rate across families. The *FecB* genotype was mapped onto the OOV6 map using the ''all'' option of CRI-MAP, as previously described [21] to find the intervals with lod 3 support. Orders that had been physically determined previously using yeast artificial chromosome (YAC) contigs (see below) were held fixed.

Quantitative Trait Locus Analysis

Further mapping was carried out treating the ovulation rate observations for each as a quantitative trait locus (QTL). The statistical approach adopted was a simple extension of the method by Haley [22], which had been developed for use in multiple generations [23]. The probability of each of the offspring being a carrier of the Booroola allele is calculated conditional upon the marker genotypes at steps along the chromosome. The trait of interest is then regressed on these conditional probabilities in a model that included the fixed effects of founder sire donating the Booroola allele, and contemporary group. At each location, an F ratio was calculated comparing the model that included a QTL with that of the identical model containing no QTL. The location with the largest F ratio estimates the location of the Booroola gene. A model for two QTLs and a model allowing differing effects within each founder family were also tested. The chromosome was searched for the presence of two QTLs using a grid search method, fixing one QTL and fitting the other putative QTL at 1-cM intervals along the length of the chromosomes.

Haplotype Analysis

Markers flanking the critical region for the *FecB* locus were screened in all daughters from the half-sib families. Individuals with a genetic recombination in a 20 cM region around the *FecB* locus were identified for subsequent analysis. Additional markers identified within the critical region were typed in the families, or in the recombinant panel, or both. The critical region was further defined by linkage and haplotype analysis to lie between PDHA2 and JP27.

Yeast Artificial Chromosome Walking

A sheep YAC library was constructed as described [24] and YACs were isolated using both an Amplified Fragment Length Polymorphism (AFLP) approach and specific genes as probes for pools of YACs [25]. Markers from the region and AFLP markers were used to assemble the YACs into contiguous sets [25].

Mutation Detection

Total RNA was isolated from sheep ovaries (both Booroola and non-Booroola) using Trizol (Gibco BRL, Auckland, New Zealand) and reverse transcribed with Expand Reverse Transcriptase (Roche, Auckland, New Zealand) and oligo(dT) according to manufacturer instructions. The BMP-IB receptor was amplified from cDNA using primers designed from regions of homology between human and mouse sequences and the polymerase chain reaction (PCR) products sequenced. The primers used were as follows: F3, 5′-GTGGGCACCAAGAAAGAGGATG; R1, 5′-GGTGAAGAACACTTTCACAGCT; F2, 5′-AGCTGTGAAAGTGTTCTTCACC; R3, 5′-GCTGTCAGCCTTGATGCAGGAT; F10, 5′-AGTGTTCTTCACCACAGAG; R10, 5′-CATGCCTCATCAACACCG; F11, 5′-GCTGGTTCAGAGAGACAG; R11, 5′-GTCCCTTTGATATCTGCAG; F12, 5′-GTCGCTATGGGGAAGTTTGGATG; F13, 5′-AGCAAGCCTGTCATACGTAGAAG; R13, 5′-CATCGCAAGATCTTTGGACGAG.

Forced Restriction Fragment Length Polymorphism

To screen the mutation through the flocks of sheep, a method that deliberately introduces a point mutation into one of the primers was used so that the PCR product will contain an *Ava*II restriction site. PCR products from non-carrier animals contain no restriction site. Primers 5′-GTCGCTATGGGGAAGTTTGGATG and 5′-CAAGATGTTTTCATGCCTCATCAACACGGTC amplify a 140 base pair (bp) band, after digestion with *Ava*II the BB animals will have a 110 bp band, B+ animals will have 140 and 110 bp, and the ++ animals will have a 140 bp band. The fragments were amplified using 35 cycles at 94°C for 15 sec, 60° for 30 sec, and 72°C for 30 sec, followed by 72°C for 5 min and 99°C for 15 min. The fragments were then electrophoresed on a 2.5% agarose gel and scored for the presence of the mutation.

Reverse Transcriptase-PCR

The expression of the BMP-IB receptor in different tissues was determined by PCR from cDNA produced from 0.1 μg of total RNA isolated from tissues from BB ewes or BB rams. Primers 5′-AGCTGTGAAAGTGTTCTTCACC and 5′-TCTTTTGCTCTGCCCACAAAC amplify across the 1.2 kilobase (kb) intron of BMP-IB receptor to produce an 880 bp fragment from cDNA. β-Actin primers 5′-GCATGGGCCAGAAGGACTCC and 5′-CGTAGATGGGCACCGTGTGG were used as a control.

In Situ Hybridization

Sheep fetuses were recovered at 135 days of gestation following transfer of 4- to 5-day-old embryos to Romney ewes (gestation = 147 days). The fetuses were the results of matings of Romney ewes (with or without the Booroola gene), superovulated using eCG (Folligon; Intervet, Lane Cove, New Zealand) and Ovagen (Immuno-Chemicals Products Ltd., Auckland, New Zealand) as described [26]. The superovulated ewes were mated either individually or in pairs with a ram (with or without the Booroola gene). Four or 5 days after mating, embryos were recovered and transferred into estrous-synchronized recipient ewes (three embryos per ewe using a laparoscopic technique). Fetuses with no (++, n = 2) or two copies of the Booroola gene (BB, n = 3) were recovered after a barbiturate overdose (sodium pentobarbitone, 20 ml i.v. of a 500 mg/ml solution

w/v; South Island Chemicals, New Zealand) was administered to their mothers. Ovaries were also collected from 4-wk-old lambs (++, n = 3 and BB, n = 2) and adult ewes (++ only; mid-luteal phase, n = 4). Ovaries were fixed in 4% (w/v) phosphate-buffered paraformaldehyde and embedded in paraffin wax.

Because of conservation among members of the BMPR family, cDNAs encoding a portion of the BMPR-IB (330 bp) and BMPR-II (713 bp) gene were isolated to determine the ovarian sites of expression of BMPR-IB and BMPR-II. First-strand cDNA was produced using 4–5 μg of total cellular RNA isolated from ovaries collected from 4-wk-old lambs using the SuperScript (Gibco BRL) preamplification system for first-strand cDNA synthesis. Primers used were 5′-CGATGTAAATGCCACCACC and 5′-GACCAAGAGCAAACTACAC for BMPR-IB; and 5′-CCTCAAGGAAAGTTCTGATG and 5′-CTTGCTCAGAACATGGATTGC for BMPR-II based on the ovine BMPR-IB sequence obtained as described above and the known sequence for human BMPR-II [27] as well as preliminary analysis of the ovine BMPR-II cDNA. Specificity of generated cDNAs was tested with Northern blot analysis as previously described [28]. Cellular localization of mRNAs were determined using the in situ hybridization protocol described previously [29], with minor modifications. Sense and antisense RNA probes were generated with T7 or SP6 RNA polymerase using the Riboprobe Gemini system (Promega, Madison, WI).

Measurement of Follicles and Oocytes

The ovaries used for the morphometric studies were obtained from nonpregnant homozygous carriers (BB, n = 9) or noncarriers (++, n = 12) of the Booroola gene. Both ovaries were fixed in Bouins fluid, cut in half, and embedded in plastic (Technovit 7100; Kulzer and Co., Weirheim, Germany). These ovaries were then serially sectioned at a thickness of 30 μm, and stained with hematoxylin and eosin.

Only nonatretic follicles larger than 130 μm and smaller than 3000 μm were considered in this study. A follicle was considered atretic as previously described [30]. Measurements were performed using an Olympus BH-2 microscope linked to a video camera, color monitor, and computer with National Institutes of Health image analysis software system (version 1.6). Between 10–20 healthy follicles per size range (250-μm intervals up to 1500 μm and then 500 μm intervals up to 3000 μm) were counted. The diameter of the oocyte was obtained by averaging the two diameters at right angles to each other in the section in which the oocyte was at its largest. The volume of the follicle was estimated from point-counting over a series of sections through the follicle using the Cavalieri Principle as described [31].

Classification of Follicles

Ovarian follicles in the ++ and BB ovaries were classified from a section through the oocyte nucleolus by the configuration of granulosa cells around the oocyte. The classification system of Lundy et al. [32] was used. Type 1 (primordial follicle), one layer of flattened granulosa cells; type 1a (transitory follicles), one layer of a mixture of flattened and cuboidal granulosa cells; type 2 (primary follicles), from one to less than two complete layers of cuboidal granulosa cells; type 3 (small preantral follicles), from two to less than four complete layers of cuboidal granulosa cells; and type 4 (large preantral follicles), from four to less than six complete layers of cuboidal granulosa cells. For in situ hybridization, because follicles of types 3 and 4 were not often observed in the sections, these follicle types were classified using a section through the oocyte nucleus. In addition, any follicle with an antrum was classified as a type 5+ follicle regardless of whether an oocyte was present in the section.

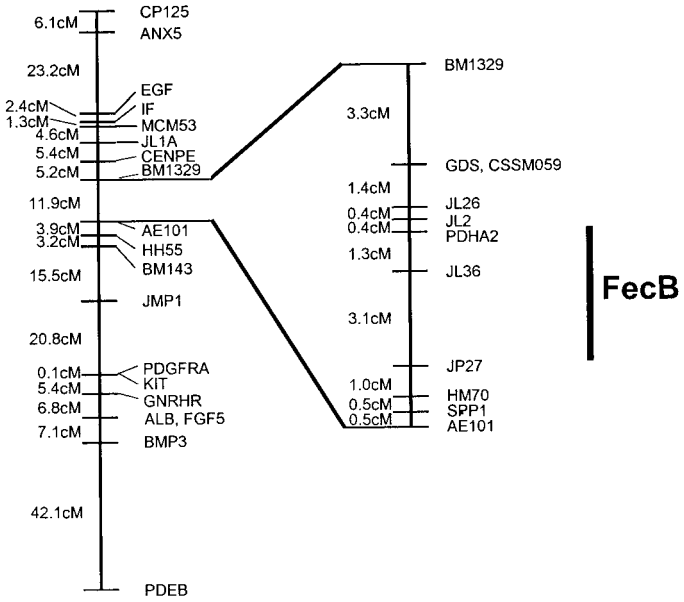

FIG. 1. The comprehensive map of sheep chromosome 6 (OOV6) and the lod 3 support region for *FecB*. Intermarker distances are also shown in Kosambi cM. Chromosome 6 marker data from the Booroola half-sib families, backcross families, and from the international mapping flock were combined, and a comprehensive linkage map created using CRI-MAP.

RESULTS

Mapping of FecB

The fecundity gene, *FecB*, derived from the Booroola Merino, was mapped to a narrow region (around 4 cM) on sheep chromosome 6 using polymorphic microsatellites and known gene markers (Fig. 1). QTL analysis of combined data from individual observations of ovulation rate taken at four time points over a 2-yr period showed strong linkage to the critical region (Fig. 2). The 99.9% confidence interval spanned a 1.6-cM-wide region. The data did not support a model with a second QTL. Haplotype analysis of animals that had undergone a recombination event around the *FecB* region indicated that microsatellite marker JL36 was the closest linked marker to *FecB* (Fig. 3) with no recombinations between JL36 and *FecB*.

Candidate for FecB

A search for genes on human chromosome 4 around the region 4q21–24 identified a likely candidate gene for *FecB* in BMP-IB receptor (BMPR-IB). Sequencing of the BMPR-IB cDNA from two Booroola and two non-Booroola sheep revealed a point mutation (an A to G transition) at position 830 (GenBank accession number AF312016). This translates to a change from a glutamine (neutral/polar amino acid) to an arginine (basic amino acid) at position 249 of the protein (Fig. 4) in the intracellular kinase signaling domain of the BMPR-IB. Glutamine is conserved at this position in most of the type I receptors. There is high

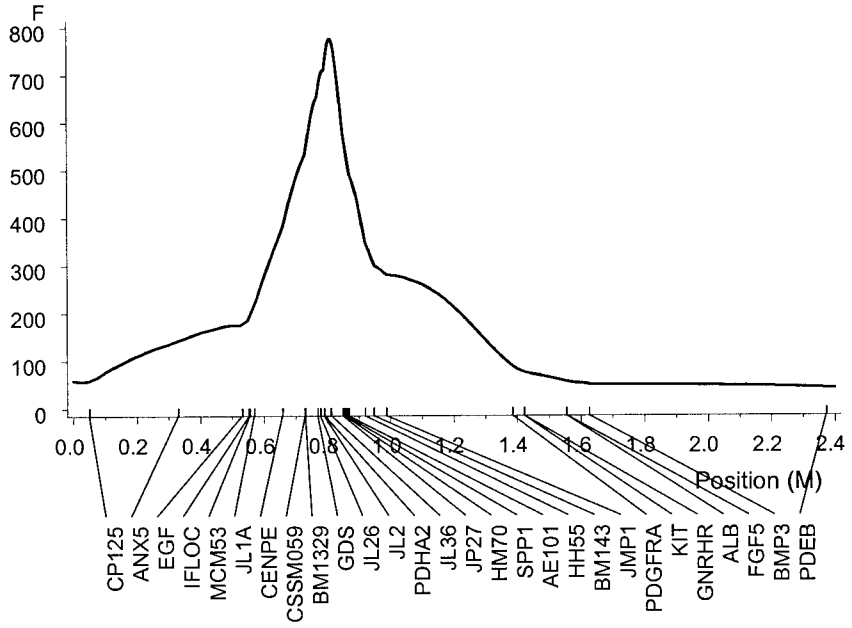

FIG. 2. QTL analysis of the distribution of the test statistic (F ratio) along chromosome 6 for the trait analyzed. Ovulation rate was measured in early and late April at 2.5 yr of age and at equivalent times at 3.5 yr of age. These four traits were combined and the mean residual deviation from the population mean over all four traits was used in the analysis. Positions of markers are indicated along the x axis.

amino acid conservation among different species for this receptor, with sheep and human having 98.4% identity.

A partial physical map of the critical region was developed by chromosome walking from marker JL2 toward JP27 using a sheep YAC library [24]. More than 36 sequentially overlapping YACs (average insert size 700 kb) were isolated. To examine where the BMPR-IB physically mapped on these YACs, a region of BMPR-IB was amplified from these YACs. Marker JL36 and the BMPR-IB were located on the same YACs (data not shown).

Screening Through Families

To screen for the mutation through our sheep families, a forced restriction fragment length polymorphism (RFLP) approach was developed using primers that will produce an *Ava*II restriction site in animals carrying the BMPR-IB mutation. We screened 300 representative animals from our backcross and half-sib families (Fig. 5, A and B) and our recombinant panel. The mutation was followed through 3–4 generations for the backcross flock, and for the half-sib flock 17B+ rams and representatives of their daughters were screened. The mutation segregated with the *FecB* phenotype and no recombinants were observed. We screened 65 animals representing 6 different breeds of sheep not derived from Booroola strains and none carried the mutation. The breeds used were Coopworths, Finns, Gotland, Perindale, Romney, and Texel. To show that this was not a Merino breed-specific mutation we tested for the mutation in four non-Booroola Merinos each from France, Germany, Spain, and New Zealand and found it was absent from all four (Fig. 5C). Sheep from Saudi Arabia, the Netherlands, and the United States in which the Booroola phenotype had been introgressed into their breeds of sheep were tested (80 animals) and the mutation was found segregating in their flocks.

Characterization of cDNAs Encoding a Portion of the Ovine BMPR-IB and BMPR-II Gene

The nucleotide sequence of the cDNA encoding a portion of BMPR-IB was identical to that observed in the initial sequence analysis of ++ animals used to identify the mutation in BB animals. The ovine partial cDNA hybridized to a band at approximately 6.2 kb in adrenal, pituitary, kidney, and ovarian total cellular RNA. In addition, a weaker signal was observed at approximately 4.4 kb in ovarian total cellular RNA (data not shown). No hybridization was observed in total cellular RNA isolated from adult liver or heart (data not shown). Previously, a primary transcript of a similar size has been shown in mouse [33] and human [34] tissues and cells following Northern blot analysis. In addition, minor transcripts of approximately 4.0 kb and 2.4 kb were observed in human tissues that strongly expressed BMPR-IB. The high degree of homology between the generated ovine BMPR-IB cDNA and the characterized human and mouse BMPR-IB cDNAs, as well as the hybridization of this cDNA to a primary transcript in ovine tissues giving a very similar size to that observed in human and mouse tissues, indicates that this cDNA is specifically recognizing the BMPR-IB mRNA.

The nucleotide sequence of the generated BMPR-II cDNA was 91% and 90% identical to the human [27] and mouse [35] ovine BMPR-II cDNA, respectively. The ovine BMPR-II cDNA hybridized to transcripts at greater than 9.5 kb and approximately 6.9 and 4.2 kb in RNA isolated from all tissues examined (data not shown). Previously, mRNA greater than 10 kb and at approximately 6–7.6 kb and 4–5 kb have been shown in human [27, 34] and mouse [33] tissues and cells following Northern blot analysis. Thus, the generated partial cDNA encoding ovine BMPR-II specifically detects mRNA encoding BMPR-II.

Expression of BMPR-IB

BMPR-IB was expressed in both male and female reproductive tissues of sheep (Fig. 6A). Moderate levels were also found in the brain, skeletal muscle, and kidney, similar to the pattern found in human tissue [34]. Expression of mRNA encoding BMPR-IB was first observed in oocytes of type 1 follicles in both ++ and BB genotypes (Fig. 7, Table 1). The oocyte continued to express mRNA encoding

Animal	BM1329	GDS	CSSMO59	JL26	JL2	PDHA2	JL36	Phenotype	JP27	HM70	AE101	HH55	BM143
	Markers												
880257	-	-	-	□	□	-	□	+	●	●	-	●	-
870019	□	-	□	□	□	-	□	+	●	●	-	-	-
890156	□	□	-	□	□	-	□	+	●	●	-	-	●
911023	□	-	-	□	□	□	□	+	-	●	●	-	-
850096	●	-	-	●	●	-	●	B	□	□	-	□	□
900007	-	-	●	●	●	-	●	B	□	□	-	□	□
890037	●	-	-	●	●	-	●	B	□	-	□	-	□
900028	●	-	●	●	●	-	●	B	□	□	-	□	□
890124	●	-	-	●	●	●	-	B	□	□	-	-	□
890173	●	-	●	●	●	●	●	B	-	□	□	□	□
900085	-	-	●	●	●	-	-	B	□	□	-	-	-
890011	-	-	□	-	□	-	●	B	●	●	-	-	●
880261	-	-	□	□	□	-	●	B	●	●	-	●	-
850055	□	□	□	□	□	□	●	B	●	●	-	●	●
880086	-	-	□	□	□	□	●	B	●	●	●	-	●
850087	-	□	□	□	□	□	-	+	●	●	-	●	●
911003	●	-	-	●	●	-	-	+	□	-	□	□	□
880457	-	●	●	●	●	-	-	+	□	-	□	-	□

FIG. 3. Booroola haplotype analysis. The recombinant animals listed were analyzed for inheritance of alleles from either the Booroola (●) or non-Booroola (□) chromosome. − Indicates where the markers were not informative for these animals. The breakpoint is indicated by the column for phenotype, where the carrier (B), or noncarrier (+) status of the *FecB* for each animal is shown.

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          1                                                  50
sheep MLLRSSGKLS VGTKKEDGES TAPTPRPKIL RCKCHHHCPE DSVNNICSTD
human MLLRSAGKLN VGTKKEDGES TAPTPRPKVL RCKCHHHCPE DSVNNICSTD
mouse MLLRSSGKLN VGTKKEDGES TAPTPRPKIL RCKCHHHCPE DSVNNICSTD
chick MPLLSSSKLS MESRKEDSEG TAPAPPQKKL SCQCHHHCPR DSVNSTCSTD

          51                                                 100
sheep GYCFTMIEED DSGMPVVTSG CLGLEGSDFQ CRDTPIPHQR RSIECCTERN
human GYCFTMIEED DSGLPVVTSG CLGLEGSDFQ CRDTPIPHQR RSIECCTERN
mouse GYCFTMIEED DSGMPVVTSG CLGLEGSDFQ CRDTPIPHQR RSIECCTERN
chick GYCFTIIEED DSGGHLVTKG CLGLEGSDFQ CRDTPIPHQR RSIECCTGQD

          101                                                150
sheep ECNKDLHPTL PPLKNRDFVD GPIHHKALLI SVTVCSLLLV LIILFCYFRY
human ECNKDLHPTL PPLKNRDFVD GPIHHRALLI SVTVCSLLLV LIILFCYFRY
mouse ECNKDLHPTL PPLKDRDFVD GPIHHKALLI SVTVCSLLLV LIILFCYFRY
chick YCNKHLHPTL PPLKNRDFAE GNIHHKALLI SVTVCSILLV LIIIFCYFRY

          151                                                200
sheep KRQEARPRYS IGLEQDETYI PPGESLRDLI EQSQSSGSGS GLPLLVQRTI
human KRQETRPRYS IGLEQDETYI PPGESLRDLI EQSQSSGSGS GLPLLVQRTI
mouse KRQEARPRYS IGLEQDETYI PPGESLRDLI EQSQSSGSGS GLPLLVQRTI
chick KRQEARPRYS IGLEQDETYI PPGESLKDLI EQSQSSGSGS GLPLLVQRTI

          201                                                250
                                                  Booroola R
                                                             ▼
sheep AKQIQMVKQI GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT
human AKQIQMVKQI GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT
mouse AKQIQMVKQI GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT
chick AKQIQMVKQI GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT

          251                                                300
sheep VLMRHENILG FIAADIKGTG SWTQLYLITD YHENGSLYDY LKSTTLDTKS
human VLMRHENILG FIAADIKGTG SWTQLYLITD YHENGSLYDY LKSTTLDAKS
mouse VLMRHENILG FIAADIKGTG SWTQLYLITD YHENGSLYDY LKSTTLDAKS
chick VLMRHENILG FIAADIKGTG SWTQLYLITD YHENGSLYDY LKSTTLDTKG

          301                                                350
sheep MLKLAYSAVS GLCHLHTEIF STQGKPAIAH RDLKSKNILV KKNGTCCIAD
human MLKLAYSSVS GLCHLHTEIF STQGKPAIAH RDLKSKNILV KKNGTCCIAD
mouse MLKLAYSSVS GLCHLHTEIF STQGKPAIAH RDLKSKNILV KKNGTCCIAD
chick MLKLAYSSVS GLCHLHTGIF STQGKPAIAH RDLKSKNILV KKNGTCCIAD

          351                                                400
sheep LGLAVKFISD TNEVDIPPNT RVGTKRYMPP EVLDESLNRN HFQSYIMADM
human LGLAVKFISD TNEVDIPPNT RVGTKRYMPP EVLDESLNRN HFQSYIMADM
mouse LGLAVKFISD TNEVDIPPNT RVGTKRYMPP EVLDESLNRN HFQSYIMADM
chick LGLAVKFISD TNEVDIPPNT RVGTKRYMPP EVLDESLNRN HFQSYIMADM

          401                                                450
sheep YSFGLILWEV ARRCVSGGIV EEYQLPYHDL VPSDPSYEDM REIVCIKKLR
human YSFGLILWEV ARRCVSGGIV EEYQLPYHDL VPSDPSYEDM REIVCIKKLR
mouse YSFGLILWEI ARRCVSGGIV EEYQLPYHDL VPSDPSYEDM REIVCMKKLR
chick YSFGLILWEI ARRCVSGGIV EEYQLPYHDL VPSDPSYEDM REIVCIKRLR

          451                                                500
sheep PSFPNRWSSD ECLRQMGKLM TECWAHNPAS RLTALRVKKT LAKMSESQDI
human PSFPNRWSSD ECLRQMGKLM TECWAHNPAS RLTALRVKKT LAKMSESQDI
mouse PSFPNRWSSD ECLRQMGKLM TECWAQNPAS RLTALRVKKT LAKMSESQDI
chick PSFPNRWSSD ECLRQMGKLM MECWAHNPAS RLTALRVKKT LAKMSESQDI

          501
sheep KL
human KL
mouse KL
chick KL
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FIG. 4. Amino acid sequence comparison of sheep, human, mouse, and chick BMPR-IB. Identical regions are indicated by the shading. The predicted membrane spanning domain is indicated by the boxed region and the intracellular kinase domain is underlined. The amino acid residue at the mutation is indicated by the arrow (▼) at position 249. GenBank accession numbers for BMPR-IB were AF312016 for sheep, U89326 for human, U78048 for mouse, and Q05438 for chick.

the BMPR-IB through to the type 5 stage in ++ ewes. Oocytes of type 4 follicles in BB ewes expressed the mRNA encoding BMPR-IB; however, no small antral follicles with oocytes were present in the sections examined for the BB animals. Granulosa cells of type 1 and type 1a follicles did not express mRNA encoding BMPR-IB in either genotype, but expression of this mRNA was first observed in granulosa cells of type 2 and greater follicles in both genotypes (Fig. 7, Table 1). The mRNA for BMPR-IB was also observed in the intraovarian rete and corpus luteum (data not shown).

Expression of mRNA encoding the type II receptor for BMP was widespread throughout the ovary and included follicular, stromal, rete, vascular, and luteal cells (Fig. 8 and data not shown). Oocytes as well as granulosa cells of all types of follicles examined expressed BMPR-II mRNA in both genotypes (Fig. 8, Table 1). In addition, in both ++ and BB sheep, cuboidal cells within the theca interna expressed BMPR-II.

Effects of Booroola Genotype on Oocyte Diameter

The individual data for oocyte diameter with respect to follicular volume are shown in Figure 9. For each Booroola genotype, the best statistically fitting model had different straight lines ($P < 0.01$) before reaching different inflection points followed by parallel lines having a different slope from the earlier ones. The overall R^2 value for this model was 0.732. Moreover, the parameters for this model were as follows: the slopes (se) for the lines before the inflection points were 0.2684 (0.0352) and 0.1727 (0.0125) for BB and ++ sheep, respectively; the mean (se) inflection points

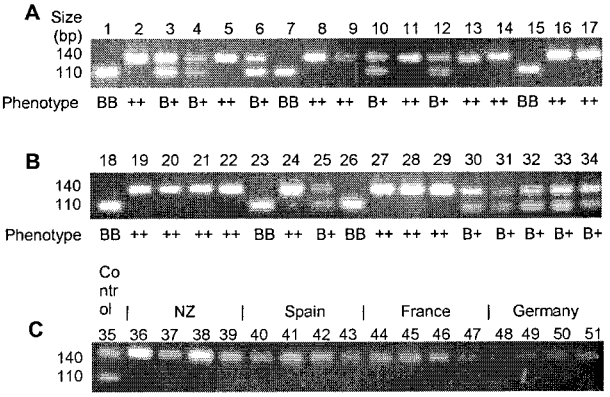

FIG. 5. Mutation detection and cosegregation of BMPR-IB mutations with the *FecB* phenotype. Forced restriction fragment length analysis showing the A ➡ G transition, resulting in an *Ava*II site being generated for animals that contain a 'G'. The phenotype assignment of the animals is shown. In the case of BB rams, the phenotype was determined from ovulation rates of their daughters. Panels **A** and **B** show representative animals from the backcross flock with the phenotype assigned shown underneath. Panel **C** represents non-Booroola merinos from the countries shown with lane 35 containing a B+ control.

with respect to Ln follicle volume were −4.992 (0.137) and −3.539 (0.127) mm^3 for the BB and ++ animals, respectively; the mean inflection point (se) with respect to Ln oocyte diameter was 4.8718 (0.0111) μm; and the slope (se) of the lines after the inflection point for the BB and ++ animals was 0.01269 (0.00312). The oocytes in each genotype reached the same diameter (i.e., 130.5 μm) at the inflection point. The geometric mean (and 95% confidence limits) for the calculated diameters of the follicles at the inflection points were 236 (215, 258) and 383 (352, 412) μm for the BB and ++ animals, respectively; these values are significantly different from each other ($P < 0.01$). For the BB and ++ genotypes, the size of the follicles at the inflection points corresponded to the growth stages for types 4 and 5, respectively.

DISCUSSION

The present study demonstrates that the Booroola sheep carry a mutation in the BMP-IB receptor. BMPR-IB maps within the critical region for the *FecB* locus. Our QTL mapping data and haplotype analysis have reduced the critical region to less than 1.64 cM. A model with two putative QTLs on chromosome 6 influencing ovulation rate was rejected, which supports the view that a mutation at a single locus is responsible for the *FecB* phenotype. From both our mapping data and haplotype analysis, the closest microsatellite marker was JL36, which showed no recombinations with the *FecB* locus. Physical mapping in our YAC contig places BMPR-IB in the same YAC as JL36, probably within 300 kb of this marker. The mutation was not detected in a number of unrelated sheep breeds and was only found segregating in sheep derived from the Booroola Merino.

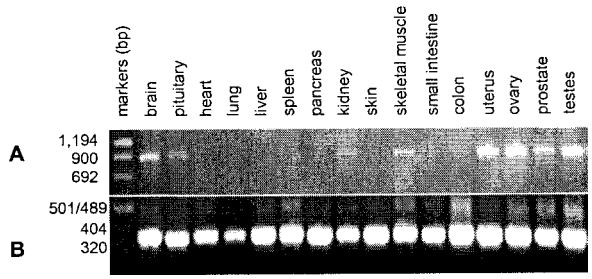

FIG. 6. Tissue distribution and relative expression of BMPR-IB in sheep tissues. BMPR-IB was amplified by reverse transcriptase-PCR from equal amounts of total RNA (**A**) and β-actin was used as a positive control to determine the quality of total RNA (**B**).

TABLE 1. Ontogeny of expression of BMPR-IB and BMPR-II during follicular development in ++ (135 days of gestation [n = 2], 4 wk of age [n = 3] and adults during the luteal phase [n = 4]) and BB (135 days of gestation [n = 3] and 4 wk of age [n = 2]) female sheep.

	Number of animals with positive gene expression/number of animals studies, with respect to follicle type							
	Oocyte				Granulosa cells			
Follicle type	BMPR-IB		BMPR-II		BMPR-IB		BMPR-II	
	++	BB	++	BB	++	BB	++	BB
1/1a	7/7	5/5	6/6	5/5	0/7	0/5	6/6	5/5
2	6/6	5/5	7/7	5/5	7/7	5/5	7/7	5/5
3	6/6	5/5	5/5	4/4	6/6	5/5	5/5	4/4
4	3/3	1/1	3/3	—*	5/5	1/1	3/3	—*
5†	5/5	—*	4/4	—*	7/7	2/2	4/4	2/2

* No follicles of this type observed in sections examined.
† Small antral follicles.

BMPR-IB, in conjunction with the type II receptor, is responsible for interacting with Smad1. Mutations in the intracellular domain have been found in both TGFbR-1 and BMP-IB receptors. Mutations in TGFbR-1 were identified in cell lines that were defective in TGF-β signaling, yet they retain ligand binding activity [36]. One disrupts the kinase activity of TGFbR-1 (G217E), the other two mutations (G261E and G322D) prevent ligand-induced phosphorylation and thus activation, by the type II receptor. A mutation in human BMPR-IB (Q203D) at the penultimate amino acid before the kinase domain resulted in increased Smad1 kinase activity [19]. It also affected the interaction with Smad5 [37], whereby the mutant receptor phosphorylated Smad5 but the wild-type did not. The mutation found in BMPR-IB in Booroola sheep (Q249R) might similarly alter its ability (either positively or negatively) to send a signal through the Smad pathway to the nucleus to regulate the expression of certain genes.

Despite the widespread expression of BMPR-IB, mice carrying a targeted disruption of BMPR-IB are viable and the defects exhibited are largely restricted to multiple abnormalities of the appendicular skeleton with effects on fertility not being reported [38]. Knockouts of the BMPR-IA however, have a severe phenotype and are embryonically lethal [39]. A similar phenotype was observed for the BMPR-II [40]. The BMP receptors form heteromeric and homomeric complexes that allow variety and flexibility in their responses to various ligands [41]. There may be a level of redundancy tolerated within the BMP family that allows BMPR-IB knockouts and mutations with only a small number of tissues being severely affected.

The mRNAs encoding both BMPR-IB and BMPR-II were observed in the granulosa cells of ovine follicles after they had entered the growing phase (type 2). In addition, both mRNAs were observed in oocytes of type 1 follicles through to the small antral stage. Whether expression of these genes in the oocyte persists throughout antral follicle development was not determined in this study. However, the intensity of the signal for BMPR-IB in the oocyte appeared to decrease as the follicle developed and was not observed in some small antral follicles. The intraovarian rete and luteal tissue expressed both mRNAs with expres-

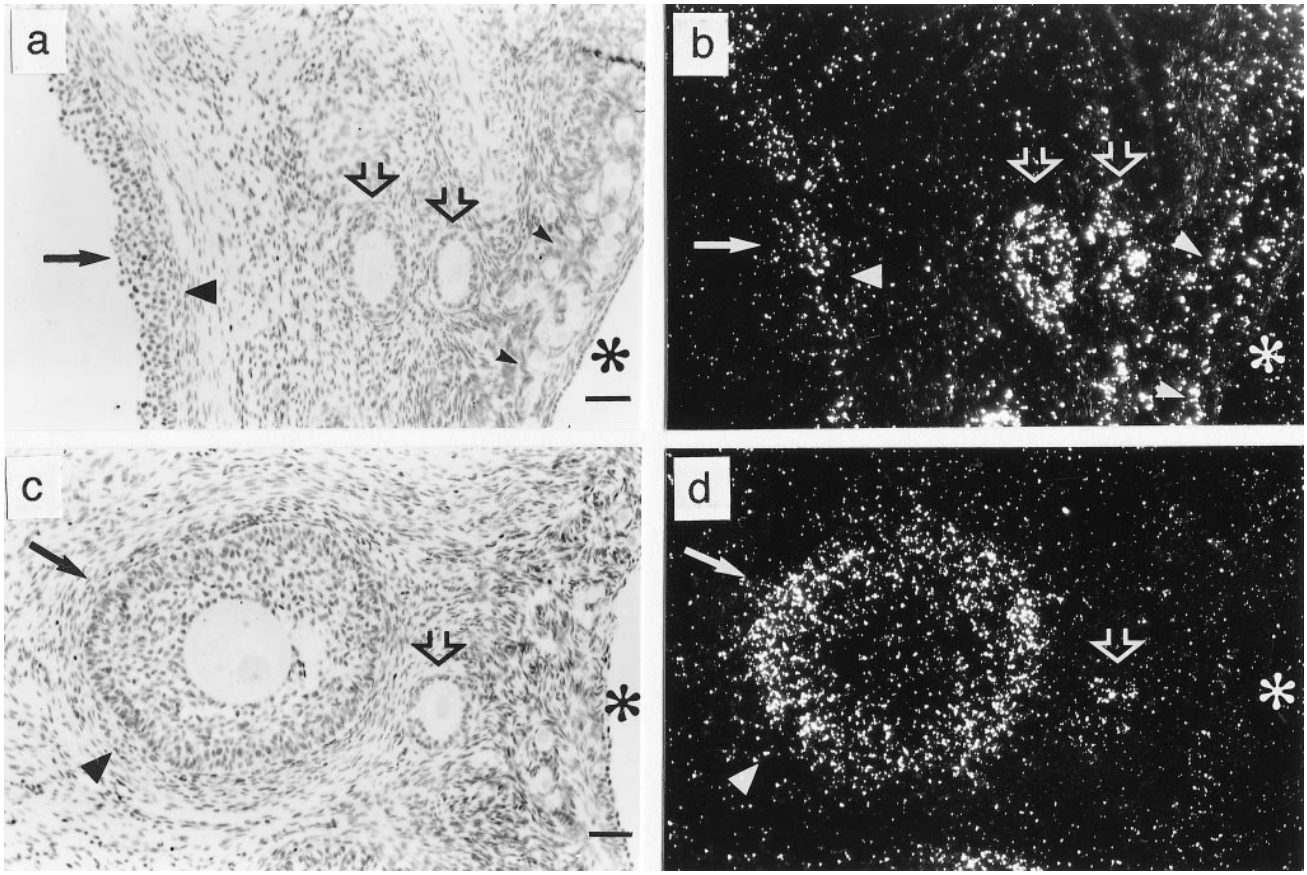

FIG. 7. Corresponding brightfield (**a**, **c**) and darkfield (**b**, **d**) views of ovarian tissues of BB (**a**, **b**) and ++ (**c**, **d**) sheep after hybridization to the BMPR-IB cRNA. The BMPR-IB mRNA was detectable in the oocytes of types 1–1a (**a**, **b**; small arrowheads) and types 2–3 (**a**–**d**, open arrows) and type 5 (**c**, **d**; arrow) follicles. Very low expression was also observed in some type 5 follicles (**c** and **d**, arrow; note the slight increase in the number of silver grains over the oocyte verses the connective tissue of the ovary). Hybridization of BMPR-IB cRNA was also evident in granulosa cells of types 2–3 (**a**–**d**, open arrows) and type 5 (**a**–**d**, solid arrows) follicles. Signal was not observed in theca (**a**–**d**, large arrowheads), surface epithelium (**a**–**d**, asterisks), or stromal cells (**a**–**d**). Bar = 50 μm for all panels.

sion of BMPR-II being widespread throughout the ovary. Thus, functional receptors for BMPs are likely to exist in a number of cell types within the ovary.

Where examined, ontogeny and ovarian cellular sites of expression of the mRNAs encoding BMPR-IB and BMPR-II were not different between BB and ++ ewes. Although intensity of hybridization signal was not determined, there was no evidence for gross differences between the genotypes in level of expression for either gene.

The pattern of gene expression found for ewes in the current study was similar in many respects to that found in the rat [15] with the granulosa cells of growing follicles being the primary site of expression of these receptors in the ovary. However, in the rat ovary, expression of the BMPR-IB was observed to be widespread throughout the ovary, whereas the expression of BMPR-II was more limited. The converse was observed in the ewe. In addition, in the rat, expression of BMPR-II was not observed in oocytes of antral follicles. The observed differences could be due to technical variations between the studies or to species differences. One obvious difference between the two animal models is that rats are multiple ovulators, whereas sheep normally ovulate one or two follicles per cycle.

In addition to BMPR-IB, BMPR-II can also interact with BMPR-IA to form an active receptor for members of the BMPs. Therefore, it seems likely that expression of BMPR-II in various ovarian cell types that do not appear to contain BMPR-IB would contain BMPR-IA or other type 1 receptors. BMPR-IB can interact with anti-Mullerian hormone (AMH) R-II to transduce a signal for AMH [42]. Whereas AMH has been shown to be produced by the granulosa cells of ovine follicles from the secondary stage onward [43], the pattern of expression of AMHR-II in the ovine ovary is not known. In the rat, mRNA encoding AMHR-II was expressed primarily in granulosa cells of preantral and small antral follicles [44].

Because BMPR-IB and BMPR-II mRNAs are localized in oocytes as well as granulosa cells, and given that a *FecB* mutation exists in the BMPR-IB gene, gene-specific differences may occur during follicular growth in granulosa cells or the oocyte, or indeed, in both cell types. It is known that in BB ewes the granulosa cells undergo only 17 doublings of their population in developing from a type 1 to a preovulatory follicle, whereas in ++ animals, the granulosa cells undergo 18 doublings. Moreover, in terms of differentiative functions, granulosa cells in BB ewes develop a greater sensitivity to FSH with respect to cAMP synthesis in vitro and acquire LH receptors and develop aromatase activity at a smaller follicular diameter compared with these functions in ++ ewes [45]. The present study confirms and expands upon the findings that oocytes in small follicles of BB ewes grow larger than in equivalent sized ++ follicles [46]. When BB and ++ follicles reached mean diameters of 236 and 383 μm, respectively (i.e., the inflection point), the mean oocyte diameters in both genotypes were the same (i.e., 130.5 μm); thereafter, the changes in oocyte diameter

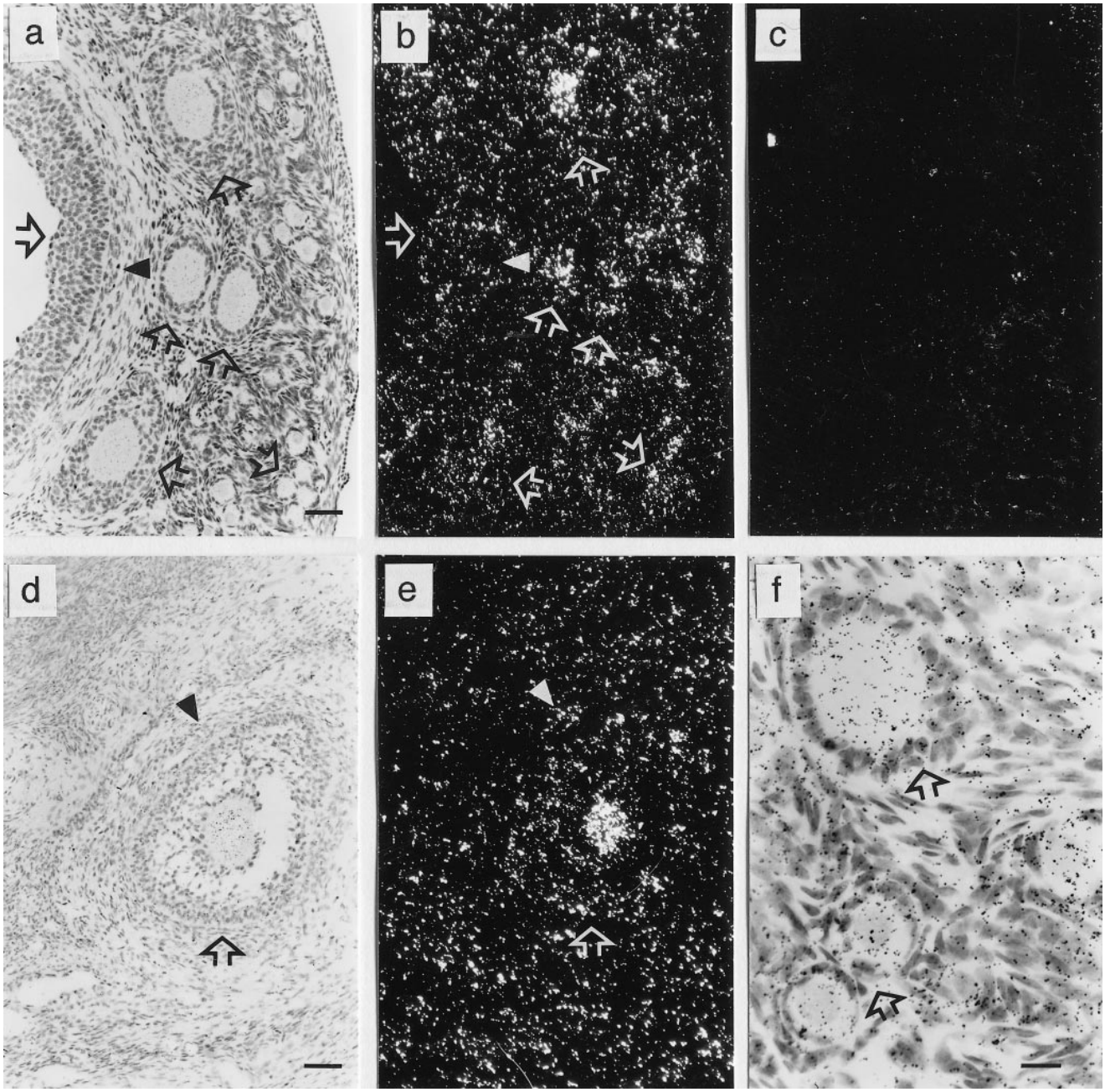

FIG. 8. Panels **a**–**c** represent corresponding brightfield (**a**) and darkfield views of ovaries of a BB sheep following hybridization to the BMPR-II antisense (**b**) and sense (**c**) probe. Corresponding brightfield (**d**) and darkfield (**e**) views of ovaries of ++ sheep following hybridization to the BMPR-II antisense probe. Panel **f** represents a brightfield view of an ovary of a ++ sheep hybridized with the BMPR-II antisense probe. BMPR-II mRNA was evident in oocytes and granulosa cells of types 1–5 follicles in both genotypes (**a**, **b**, **d**–**f**; open arrows). BMPR-II mRNA was also present in theca cells (arrowheads) of type 5 follicles (**a**, **b**, **d**, **e**) and in some but not all stroma cells (**f**). Results for hybridization to the surface epithelium (**a**, **b**) were variable. Bar = 50 μm (**a**–**e**), and 12.5 μm (**f**).

were similar for both genotypes. The differences between the genotypes in oocyte diameters before reaching 130.5 μm precede those observed for granulosa cells [45]. The possibility exists that the changes observed in the oocyte are indirect and due to altered, as yet unknown, granulosa functions, or they occur directly as a consequence of the *FecB* mutation in BMPR-IB within the oocyte. For example, one possibility is that once oocytes reach a diameter of approximately 130 μm, they signal a greater proportion of granulosa cells to enter a differentiative pathway in preference to a proliferative one. Regardless of the mechanism of action, these and earlier studies [46] show that there are gene-specific differences in oocyte growth as well as in granulosa cell characteristics during follicular development.

In addition to AMH, BMP-4 and BMP-7 are expressed within the rat ovary [15]. Furthermore, treatment of granulosa cells with BMP-4 or BMP-7 enhanced FSH-stimulated estradiol production while suppressing FSH-stimulated progesterone secretion [15]. The authors proposed that an intrinsic BMP system within the ovary regulates the granulosa cells responsiveness to FSH during follicular growth and development. In mice lacking active AMH, young animals have increased number of preantral and small antral follicles. In these mice, follicles enter the grow-

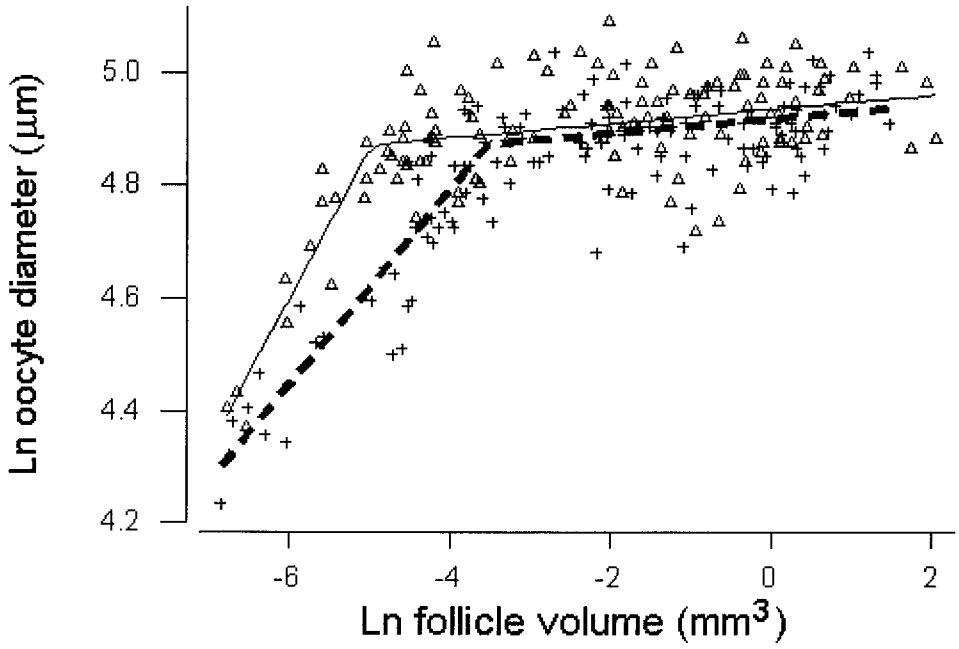

FIG. 9. Relationship between Ln (Logarithm natural) oocyte diameter (μm), and Ln follicle volume (mm^3) with respect to Booroola genotype (BB, △; ++, +). The solid and dotted lines represent the best-fit lines for the BB and ++ genotypes, respectively.

ing pool in greater numbers, which would lead to an earlier depletion of the primordial pool of follicles [47]. It has been shown that AMH inhibits mitosis of human granulosa-luteal cells [48] and ovarian cancer cells [49].

Oocytes express various members of the TGF-β superfamily, including GDF9, BMP15/GDF9b, and BMP6 [6, 11, 13, 14, 50–52]. Whereas mice that lack active BMP6 are fertile [53], inactivating mutations of GDF9 in mice [54] or BMP15/GDF9b in sheep [6] cause follicular development to be arrested just after the growing phase is entered. In vitro, GDF9 stimulates the growth of rat preantral follicles [55] and granulosa cell mitosis [56]. Culture of rat granulosa cells with BMP15/GDF9b also stimulates thymidine incorporation [57]. The receptors that transduce the GDF9 and BMP15/GDF9b signal have not been identified.

It is interesting that whereas ewes that are homozygous for inactivating mutations of BMP15/GDF9b (Inverdale) are infertile due to a block in follicular growth, those that are heterozygous for the mutation have increased ovulation rates [6]. Furthermore, many of the physiological characteristics observed in granulosa cells collected from BB ewes are also observed in granulosa cells collected from ewes with a single copy of the BMP15/GDF9b mutation [58]. Finally, the interaction between the Booroola and Inverdale mutations appear to be multiplicative in that animals that are heterozygous for both the Booroola mutation and the Inverdale mutation have ovulation rates greater than the increase expected for an additive effect alone [59]. This interaction between the Inverdale and Booroola mutation is consistent with the hypothesis that the BMPR-IB may be involved with BMP15/GDF9b signaling. However, as discussed above, many other members of the BMP family and AMH have also been shown to regulate granulosa cell mitosis and responsiveness to FSH, which are characteristic of changes observed in the Booroola granulosa cells. Thus, identification of the ligand or ligands that interact with BMPR-IB within the sheep ovary as well as the changes in signaling mechanisms that initiate the characteristic physiological changes of Booroola granulosa cells leading to increased ovulation rate remain to be elucidated.

In summary, we have identified a mutation in the highly conserved intracellular kinase signaling domain in the BMP-IB receptor in carriers of the *FecB* locus. BMPR-IB maps within the critical region for the locus and segregates with the *FecB* phenotype in all the flocks tested. BMPR-IB was expressed in oocytes and granulosa cells in the sheep ovary and the pattern of expression was consistent with an important role for this receptor in oocyte and granulosa cell development within the ovary.

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REFERENCES

1. Davis GH, Montgomery GW, Allison AJ, Kelly RW, Bray AR. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. N Z J Agric Res 1982; 25:525–529.
2. Piper LR, Bindon BM. The Booroola Merino and the performance of medium non-Peppin crosses at Armidale. In: Piper LR, Bindon BM, Nethery RD (eds.), The Booroola Merino; Melbourne, Australia: CSIRO; 1982: 9–20.
3. Montgomery GW, Lord EA, Penty JM, Dodds KG, Broad TE, Cambridge L, Sunden SL, Stone RT, Crawford AM. The Booroola fecundity (*FecB*) gene maps to sheep chromosome 6. Genomics 1994; 22: 148–153.
4. Montgomery GW, Crawford AM, Penty JM, Dodds KG, Ede AJ, Henry HM, Pierson CA, Lord EA, Galloway SM, Schmack AE, Sise JA, Swarbrick PA, Hanrahan V, Buchanan FC, Hill DF. The ovine Booroola fecundity gene (*FecB*) is linked to markers from a region of human chromosome 4q. Nat Genet 1993; 4:410–414.
5. Davis GH, McEwan JC, Fennessy PF, Dodds KG, Farquhar PA. Ev-

idence for the presence of a major gene influencing ovulation rate on the X chromosome of sheep. Biol Reprod 1991; 44:620–624.
6. Galloway SM, McNatty KP, Cambridge LM, Laitinen MPE, Juengel JL, Jokiranta TS, McLaren RJ, Luiro K, Dodds KG, Montgomery GW, Beattie AE, Davis GH, Ritvos O. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat Genet 2000; 25:279–283.
7. Massague J. TGF-beta signal transduction. Annu Rev Biochem 1998; 67:753–791.
8. Hogan BL. Bone morphogenetic proteins: multifunctional regulators of vertebrate development. Genes Dev 1996; 10:1580–1594.
9. Winnier G, Blessing M, Labosky PA, Hogan BL. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. Genes Dev 1995; 9:2105–2116.
10. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. Annu Rev Immunol 1998; 16:137–161.
11. Dube JL, Wang P, Elvin J, Lyons KM, Celeste AJ, Matzuk MM. The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. Mol Endocrinol 1998; 12:1809–1817.
12. Elvin JA, Yan C, Matzuk MM. Oocyte-expressed TGF-beta superfamily members in female fertility. Mol Cell Endocrinol 2000; 159:1–5.
13. Aaltonen J, Laitinen MP, Vuojolainen K, Jaatinen R, Horelli-Kuitunen N, Seppa L, Louhio H, Tuuri T, Sjoberg J, Butzow R, Hovatta O, Dale L, Ritvos O. Human growth differentiation factor 9 (GDF-9) and its novel homolog GDF-9B are expressed in oocytes during early folliculogenesis. J Clin Endocrinol Metab 1999; 84:2744–2750.
14. Laitinen M, Vuojolainen K, Jaatinen R, Ketola I, Aaltonen J, Lehtonen E, Heikinheimo M, Ritvos O. A novel growth differentiation factor-9 (GDF-9) related factor is co-expressed with GDF-9 in mouse oocytes during folliculogenesis. Mech Dev 1998; 78:135–140.
15. Shimasaki S, Zachow RJ, Li D, Kim H, Iemura S, Ueno N, Sampath K, Chang RJ, Erickson GF. A functional bone morphogenetic protein system in the ovary. Proc Natl Acad Sci U S A 1999; 96:7282–7287.
16. Massague J, Weis-Garcia F. Serine/threonine kinase receptors: mediators of transforming growth factor beta family signals. Cancer Surv 1996; 27:41–64.
17. ten Dijke P, Miyazono K, Heldin C. Signaling via hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors. Curr Opin Cell Biol 1996; 8:139–145.
18. Carcamo J, Weis FM, Ventura F, Wieser R, Wrana JL, Attisano L, Massague J. Type I receptors specify growth-inhibitory and transcriptional responses to transforming growth factor beta and activin. Mol Cell Biol 1994; 14:3810–3821.
19. Kretzschmar M, Liu F, Hata A, Doody J, Massague J. The TGF-beta family mediator Smad1 is phosphorylated directly and activated functionally by the BMP receptor kinase. Genes Dev 1997; 11:984–995.
20. Miyazono K. Signal transduction by bone morphogenetic protein receptors: functional roles of Smad proteins. Bone 1999; 25:91–93.
21. Crawford AM, Dodds KG, Ede AJ, Pierson CA, Montgomery GW, Garmonsway HG, Beattie AE, Davies K, Maddox JF, Kappes SW, Stone RT, Nguyen TC, Penty JM, Lord EA, Broom JE, Buitkamp J, Schwaiger W, Epplen JT, Matthew P, Matthews ME, Hulme DJ, Beh KJ, McCraw RA, Beattie CW. An autosomal genetic linkage map of the sheep genome. Genetics 1995; 140:703–724.
22. Haley CS, Knott SA, Elsen JM. Mapping quantitative trait loci in crosses between outbred lines using least squares. Genetics 1994; 136: 1195–1207.
23. Dodds KG. Haley-Knott regression mapping of quantitative trait loci in extended pedigrees. Proc Assoc Adv Anim Breeding Genet 1999; 13:313–316.
24. Broom MF, Hill DF. Construction of a large-insert yeast artificial chromosome library from sheep DNA. Mamm Genome 1994; 5:817–819.
25. Lumsden JM, Lord EA, Cato SA, Richardson TE, van Stijn TC, Broom MF, Patel K, Montgomery GW. The application of AFLP fingerprinting to construct a YAC contig containing ADH2 and MTP on sheep chromosome 6. Cytogenet Cell Genet 1999; 84:225–229.
26. Smith P, O W-S, Hudson NL, Shaw L, Heath DA, Condell L, Phillips DJ, McNatty KP. Effects of the Booroola gene (FecB) on body weight, ovarian development and hormone concentrations during fetal life. J Reprod Fertil 1993; 98:41–54.
27. Rosenzweig BL, Imamura T, Okadome T, Cox GN, Yamashita H, Dijke PT, Heldin C-H, Miyazono K. Cloning and characterization of a human type II receptor for bone morphogenetic proteins. Proc Natl Acad Sci U S A 1995; 92:7632–7636.
28. Juengel JL, Nett TM, Anthony RV, Niswender GD. Effects of luteotrophic and luteolytic hormones on expression of mRNA encoding insulin-like growth factor I and growth hormone receptor in the ovine corpus luteum. J Reprod Fertil 1997; 110:291–298.
29. Tisdall DJ, Fidler AE, Smith P, Quirke LD, Stent VC, Heath DA, McNatty KP. Stem cell factor and c-kit gene expression and protein localization in the sheep ovary during fetal development. J Reprod Fertil 1999; 116:277–291.
30. Jolly PD, Smith PR, Heath DA, Hudson NL, Lun S, Still LA, Watts CH, McNatty KP. Morphological evidence of apoptosis and the prevalence of apoptotic versus mitotic cells in the membrana granulosa of ovarian follicles during spontaneous and induced atresia in ewes. Biol Reprod 1997; 56:837–846.
31. Smith P, Braw-Tal R, Corrigan K, Hudson NL, Heath DA, McNatty KP. Ontogeny of ovarian follicle development in Booroola sheep fetuses that are homozygous carriers or non-carriers of the $FecB^B$ gene. J Reprod Fertil 1994; 100:485–490.
32. Lundy T, Smith P, O'Connell A, Hudson NL, McNatty KP. Populations of granulosa cells in small follicles of the sheep ovary. J Reprod Fertil 1999; 115:251–262.
33. Chen D, Ji X, Harris MA, Feng JQ, Karsenty G, Celeste AJ, Rosen V, Mundy GR, Harris SE. Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. J Cell Biol 1998; 142:295–305.
34. Ide H, Katoh M, Sasaki H, Yoshida T, Aoki K, Nawa Y, Osada Y, Sugimura T, Terada M. Cloning of human bone morphogenetic protein type IB receptor (BMPR-IB) and its expression in prostate cancer in comparison with other BMPRs. Oncogene 1997; 14:1377–1382.
35. Beppu H, Minowa O, Miyazono K, Kawabata M. cDNA cloning and genomic organization of the mouse BMP type II receptor. Biochem Biophys Res Comm 1997; 235:499–504.
36. Weis-Garcia F, Massague J. Complementation between kinase-defective and activation-defective TGF-beta receptors reveals a novel form of receptor cooperativity essential for signaling. EMBO J 1996; 15: 276–289.
37. Nishimura R, Kato Y, Chen D, Harris SE, Mundy GR, Yoneda T. Smad5 and DPC4 are key molecules in mediating BMP-2-induced osteoblastic differentiation of the pluripotent mesenchymal precursor cell line C2C12. J Biol Chem 1998; 273:1872–1879.
38. Yi SE, Daluiski A, Pederson R, Rosen V, Lyons KM. The type I BMP receptor BMPRIB is required for chondrogenesis in the mouse limb. Development 2000; 127:621–630.
39. Mishina Y, Suzuki A, Ueno N, Behringer RR. Bmpr encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. Genes Dev 1995; 9:3027–3037.
40. Beppu H, Kawabata M, Hamamoto T, Chytil A, Minowa O, Noda T, Miyazono K. BMP type II receptor is required for gastrulation and early development of mouse embryos. Dev Biol 2000; 221:249–258.
41. Gilboa L, Nohe A, Geissendorfer T, Sebald W, Henis YI, Knaus P. Bone morphogenetic protein receptor complexes on the surface of live cells: a new oligomerization mode for serine/threonine kinase receptors. Mol Biol Cell 2000; 11:1023–1035.
42. Gouedard L, Chen YG, Thevenet L, Racine C, Borie S, Lamarre I, Josso N, Massague J, di Clemente N. Engagement of bone morphogenetic protein type IB receptor and Smad1 signaling by anti-Mullerian hormone and its type II receptor. J Biol Chem 2000; 275:27973–27978.
43. Bezard J, Vigier B, Tran D, Mauleon P, Josso N. Immunocytochemical study of anti-Mullerian hormone in sheep ovarian follicles during fetal and post-natal development. J Reprod Fertil 1987; 80:509–516.
44. Baarends WM, Uilenbroek JTJ, Kramer P, Hoogerbrugge JW, van Leeuwen ECM, Themmen APN, Grootegoed JA. Anti-Mullerian hormone and anti-Mullerian hormone type II receptor messenger ribonucleic acid expression in rat ovaries during postnatal development, the estrous cycle, and gonadotrophin-induced follicle growth. Endocrinology 1995; 136:4951–4962.
45. Montgomery GW, McNatty KP, Davis GH. Physiology and molecular genetics of mutations that increase ovulation rate in sheep. Endocr Rev 1992; 13:309–328.
46. Cognie Y, Benoit F, Poulin N, Driancourt MA. Effect of follicle size and of the FecB Booroola gene on oocyte function in sheep. J Reprod Fertil 1998; 112:379–386.
47. Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, Themmen AP. Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. Endocrinology 1999; 140:5789–5796.
48. Kim JH, Seibel MM, MacLaughlin DT, Donahoe PK, Ransil BJ, Hametz PA, Richards CJ. The inhibitory effects of mullerian-inhibiting

substance on epidermal growth factor induced proliferation and progesterone production of human granulosa-luteal cells. J Clin Endocrinol Metab 1992; 75:911–917.
49. Masiakos PT, MacLaughlin DT, Maheswaran S, Teixeira J, Fuller AF Jr, Shah PC, Kehas DJ, Kenneally MK, Dombkowski DM, Ha TU, Preffer FI, Donahoe PK. Human ovarian cancer, cell lines, and primary ascites cells express the human Mullerian inhibiting substance (MIS) type II receptor, bind, and are responsive to MIS. Clin Cancer Res 1999; 5:3488–3499.
50. McGrath SA, Esquela AF, Lee S-J. Oocyte-specific expression of growth/differentiation factor-9. Mol Endocrinol 1995; 9:131–136.
51. Lyons KM, Pelton RW, Hogan BLM. Patterns of expression of murine Vgr-1 and BMP-2a RNA suggest that transforming growth factor-β-like genes coordinately regulate aspects of embryonic development. Genes Dev 1989; 3:1657–1668.
52. Bodensteiner KJ, Clay CM, Moeller CL, Sawyer HR. Molecular cloning of the ovine growth/differentiation factor-9 gene and expression of growth/differentiation factor-9 in ovine and bovine ovaries. Biol Reprod 1999; 60:381–386.
53. Solloway MJ, Dudley AT, Bikoff EK, Lyons KM, Hogan BL, Robertson EJ. Mice lacking Bmp6 function. Dev Genet 1998; 22:321–339.
54. Dong J, Albertini DF, Nishimori K, Rajendra Kumar T, Lu N, Matzuk MM. Growth differentiation factor-9 is required during early ovarian folliculogenesis. Nature 1996; 383:531–535.
55. Hayashi M, McGee EA, Min G, Klein C, Rose UM, van Duin M, Hsueh AJW. Recombinant growth differentiation factor-9 (GDF-9) enhances growth and differentiation of cultured early ovarian follicles. Endocrinology 1999; 140:1236–1244.
56. Vitt UA, Hayashi M, Klein C, Hsueh AJW. Growth differentiation factor-9 stimulates proliferation but suppresses the follicle-stimulating hormone-induced differentiation of cultured granulosa cells from small antral and preovulatory rat follicles. Biol Reprod 2000; 62:370–377.
57. Otsuka F, Yao Z, Lee Th, Yamamoto S, Erickson GF, Shimasaki S. Bone morphogenetic protein-15: identification of target cells and biological functions. J Biol Chem 2000; 275:39523–39528.
58. Shackell GH, Hudson NL, Heath DA, Lun S, Shaw L, Condell L, Blay LR, McNatty KP. Plasma gonadotropin concentrations and ovarian characteristics in Inverdale ewes that are heterozygous for a major gene (FecXI) on the X chromosome that influences ovulation rate. Biol Reprod 1993; 48:1150–1156.
59. Davis GH, Dodds KG, Bruce GD. Combined effect of the Inverdale and Booroola prolificacy genes on ovulation rate in sheep. Proc Assoc Adv Anim Breeding Genet 1999; 13:74–77.