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Differential expression of mRNAs encoding BMP/Smad pathway molecules in antral follicles of high- and low-fecundity Hu sheep

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ABSTRACT

The Hu sheep is world-famous for its hyper-prolificacy and the bone morphogenetic protein (BMP)/Smad pathway and several other closely related molecules (GDF9, TGF-βRI) have been shown to have a close relationship with reproduction in sheep. In order to investigate the mechanism of high fecundity in Hu sheep and its relationship with the BMP/Smad pathway, 147 Hu sheep were blood sampled for detection of the FecB mutation (A746G) in the *BMPRIB* gene by PCR-SSCP, and sixteen adult Hu ewes classified as either high-fecundity (HF) or low-fecundity (LF) animals were sacrificed for tissue and antral follicle sampling. The tissue distribution patterns of mRNAs encoding BMP/Smad pathway molecules including BMPs (BMP2, BMP4, BMP6, BMP7 and BMP15), BMP receptors (BMPRIA, BMPRIB and BMPRII), intracellular transducers (Smad1, Smad5 and Smad4) and closely related molecules (GDF9 and TGF-βRI) were detected by RT-PCR and the gene expression levels in antral follicles were investigated by real-time PCR. The results showed that all experimental animals were homozygous for the *BMPRIB* (A746G) mutation, and all detected genes related to the BMP/Smad pathway and *GDF9* and *TGF-βRI* were expressed in the ovary. In addition, *BMP4*, *BMPRIB*, *BMPRII*, *Smad4*, *GDF9* and *TGF-βRI* mRNAs were more abundant in the antral follicles of HF animals than those of LF animals ($P < 0.05$), but *BMP15* mRNA was less abundant ($P < 0.01$). This suggests that there could be an unidentified genetic mutation in *BMPRIB*, or other unidentified genes and unknown factors, which controls ovarian number by changing the expression patterns of genes known to regulate ovulation rate via the BMP/Smad pathway and closely related molecules (GDF9 and TGF-βRI).

1. Introduction

Hu sheep are a local breed in South China characterized by high fecundity with an average litter size of 2.29 and a lambing rate of 200–250% (Wang et al., 2000). There has been increasing interest in the identification and utilization of major prolificacy genes in these sheep. It is well known that mutations that increase ovulation rate and affect fecundity have been discovered in the bone morphogenetic protein 15 gene (*BMP15*; breeds showing this mutation include the Inverdale, Hanna, Belclare, Cambridge and Laucune), its receptor gene (*BMPRIB*; the Booroola breed) and in the growth differentiation factor 9 gene (*GDF9*; the Cambridge and Belclare breeds) (Davis, 2005). In Hu sheep, however, point mutations of the *BMP15* gene including $FecX^{I}$, $FecX^{H}$, $FecX^{G}$, $FecX^{B}$, $FecX^{L}$ and mutation of the *GDF9* G8(c-t) gene have not been detected by polymerase chain reaction-single-strand conformation polymorphisms (PCR-SSCP) or by restriction

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Table 1
Oligonucleotide primer sequences used for RT-PCR and real-time PCR in Hu sheep.

Target genes	Primer sequence	Product size (bp)	Annealing temperature (°C)	Reference sequences	Product identity with reference
BMP2	F: 5′-ATCACCTGAACTCCACGAA-3′ R: 5′-TACCACCTTCTCATTCTCATC-3′	140	48	DQ192012	100%
BMP4	F: 5′-GGGGAAGAAAAAAGTCGCCGAGATT-3′ R: 5′-CTCAGGATACTCCAGACCGATGCCC-3′	238	64.5	AF508312	99%
BMP6	F: 5′-GGTGGCAGGACTGGATCATT-3′ R: 5′-CACCGAGATGGCGTTCAGTT-3′	190	58	DQ192014	98%
BMP7	F: 5′-AAAACAGCAGCAGCGACCAGAG-3′ R: 5′-CCTCACAGTAGTAGGCGGCATAGC-3′	123	68	AF508311	100%
BMP15	F: 5′-CAGCAGCCAAGAGGTAGTGAGGT-3′ R: 5′-GTGGAGGGAACACTGGTTACTTT-3′	260	54.6	AY572412	98%
BMPRIA	F: 5′-CGTCGTTGTATCACAGGAG-3′ R: 5′-CTGCTCGTAGACATTCATCAC-3′	163	50.7	NM001076800	98%
BMPRIB	F: 5′-AACATCTTGGGCTTCATTGC-3′ R: 5′-CTGGTTTGCCTTGAGTGCTA-3′	211	59.5	AF312016	100%
BMPRII	F: 5′-GTGAGCCCAACAGTCAATCC-3′ R: 5′-TGCTTGCTGCCGTTCAT-3′	243	64.5	XM617952	98%
Smad1	F: 5′-TGGTTCCAAGACACAGCGAATA-3′ R: 5′-GGTGTATCTGCTGGCATCTGAA-3′	252	58	AY035385	98%
Smad5	F: 5′GCACAGCCTTCTGGTTCA 3′ R: 5′-GGGTAGGGACTATTTGGAG-3′	132	64.5	AF508027	100%
Smad4	F: 5′-CCAAGTAATCGCGCATCAAC-3′ R: 5′-CAGTCCAGGTGGTAGTGCTGTT-3′	252	58	AF508026	100%
GDF9	F: 5′-CTGAGACTTGGTCCTTGCTGAA-3′ R: 5′-CTCCTTGGTAGCGTATGCCTTAT-3′	170	63	AF078545	100%
TGF-βRI	F: 5′-CTGTCGGAAAGCCGTCATCT-3′ R: 5′-TCCTCTTCACTTGGCACTCG-3′	151	56	AY656799	100%
β-actin	F: 5′-AGCCTTCCTTCCTGGGCATGGA-3′ R: 5′-GGACAGCACCGTGTTGGCGTAGA-3′	117	68	U39357	100%

fragment length polymorphisms (RFLP) (Guan et al., 2005a; Chu et al., 2005, 2007; Davis et al., 2006). A homozygous *BMPRIB* gene point mutation (A746G) was found in all Hu sheep (Guan et al., 2005b; Davis et al., 2006), but mean total litter size and mean litter size at the first and the second lambings of a selected population were significantly higher than those in unselected animals (Guan et al., 2005b). These findings indicate that the putative mutations that increase ovulation rate in the *BMPRIB*, *BMP15* and *GDF9* genes might not be responsible for the high prolificacy in Hu sheep. There might be unidentified mutations in some of these genes or in different genes present in Hu sheep that change the gene expression patterns of *BMPRIB*, *BMP15* and *GDF9* and related genes affecting ovulation rate.

Besides BMP15, several other BMPs (BMP2, BMP4, BMP6 and BMP7), belonging to the transforming growth factor β (TGF-β) superfamily, are now known to have effects on reproduction; these act as autocrine/paracrine regulators of ovarian follicular development and ovulation through the BMP/Smad signaling pathway (Galloway et al., 2000; Juengel et al., 2006; Yoshino et al., 2006). The BMP/Smad signaling occurs through the formation of a heteromeric complex of BMP and type II receptor (BMPRII) which then induces the trans-phosphorylation of the type I receptor (BMPRIA or BMPRIB). Phosphorylated BMP type I receptors can induce the phosphorylation of receptor-specific Smads (R-Smad, Smad1, Smad5 or Smad8) and form oligomeric complexes with common partner Smads (Co-smad, Smad4). This R-Smad-co-Smad complex translocates to the nucleus to regulate specific gene expression, causing various biological reactions (Kaivo-oja et al., 2006). BMPRII and TGF-βRI are also downstream of GDF9 (Vitt et al., 2002; Mazerbourg et al., 2004). Furthermore, different modes of cross-talk between the BMP signaling path-

Table 2
Comparison of body weight, number of lambings, average number of lambs born alive and ovulation rate of high- and low-fecundity Hu sheep.

	Animal numbers	Body weight (kg)	Number of lambings	Average number of lambs born alive	Ovarian weight (g)	Ovulation rate
LF	8	37.8 ± 3.96^{a}	2.33 ± 0.21^{a}	1.00 ± 0.00^{B}	1.33 ± 0.16^{b}	2.00 ± 0.00^{B}
HF	8	45.7 ± 3.25^{a}	2.60 ± 0.16^{a}	2.90 ± 0.20^{A}	1.95 ± 0.06^{a}	3.80 ± 0.37^{A}

Note: Number of lambings means how many times the experimental animal had born its lambs. Different letters in the same column indicate significant differences $P < 0.05$ (small letter), $P < 0.01$ (capital letter).