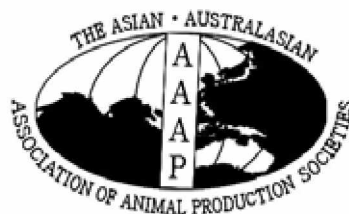

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PCR-SSCP Polymorphism of Inhibin β_A Gene in Some Sheep Breeds

M. X. Chu*, C. T. Xiao[1], Y. Fu[1], L. Fang and S. C. Ye

Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100094, P. R. China

ABSTRACT : Inhibins participate in the regulation of pituitary follicle-stimulating hormone synthesis and secretion, follicular maturation and steroidogenesis in the female. Inhibin β_A gene (*INHBA*) was studied as a candidate gene for the prolificacy of sheep. Single nucleotide polymorphisms of the entire coding region and partial 3' untranslated region of *INHBA* were detected by PCR-SSCP in two high fecundity breeds (Small Tail Han and Hu sheep) and six low fecundity breeds (Dorset, Texel, German Mutton Merino, South African Mutton Merino, Chinese Merino and Corriedale sheep). Only the PCR products amplified by primers 3, 4 and 5 displayed polymorphisms. For primer 3, genotype CC was only detected in Chinese Merino sheep, genotype AA was detected in the other seven sheep breeds. Genotype BB was only detected in Hu sheep. Only Hu sheep displayed polymorphism. Eight or four nucleotide mutations were revealed between BB or CC and AA, respectively, and these mutations did not result in any amino acid change. For primer 4, genotypes EE, EG and GG were detected in Dorset and German Mutton Merino sheep, genotypes EE, EF and FF were detected in Chinese Merino sheep, only genotype EE was detected in the other five sheep breeds. Only Dorset, German Mutton Merino and Chinese Merino sheep displayed polymorphism. Sequencing revealed one nucleotide mutation (114G→A) of exon 2 of *INHBA* gene between genotype FF and genotype EE, and this mutation did not cause any amino acid change. Another nucleotide change (143C→T) was identified between genotype GG and genotype EE, and this mutation resulted in an amino acid change of serine→leucine. For primer 5, genotypes KK and KL were detected in German Mutton Merino and Corriedale sheep, genotypes KK, LL and KL were detected in the other six sheep breeds. Genotype MM was only detected in Hu sheep. All of these eight sheep breeds displayed polymorphism. Sequencing revealed one nucleotide mutation (218A→G) of exon 2 of the *INHBA* gene between genotype LL and genotype KK, and nine nucleotide mutations between genotype MM and genotype KK. These mutations did not alter amino acid sequence. The partial sequence (395 bp for exon 1 and 933 bp for exon 2) of the *INHBA* gene in Small Tail Han sheep (with genotype KK for primer 5) was submitted into GenBank (accession number EF192431). Small Tail Han sheep displayed polymorphisms only in the fragment amplified by primer 5. The Small Tail Han ewes with genotype LL had 0.53 ($p<0.05$) or 0.63 ($p<0.05$) more lambs than those with genotype KL or KK, respectively. The Small Tail Han ewes with genotype KL had 0.10 ($p>0.05$) more lambs than those with genotype KK. (**Key Words :** Sheep Breeds, Inhibin β_A Gene, PCR-SSCP)

INTRODUCTION

Inhibin is glycoprotein hormone belonging to the transforming growth factor-β superfamily that suppresses follicle-stimulating hormone (FSH) synthesis and secretion (Ling et al., 1985; Rivier et al., 1985; Robertson et al., 1985; Woodruff et al., 1996). It consists of two subunits, α and β, linked by disulphide bonds. Two inhibins, sharing a common α-subunit but different β-subunits (β_A or β_B) had been identified (Mason et al., 1985). Inhibin expressed in the organs of ovary, testis, uterus of human, mouse, pig, sheep (Phillips, 2005). In sheep, follicles are a major source of inhibin (Rodgers et al., 1989) but there is evidence for extraovarian sources (McNatty et al., 1992). Inhibin β_A subunit could be used as a marker gene in detecting human pancreatic cancer (Kleeff et al., 1998). Inhibin β_A gene (*INHBA*) had been mapped to chromosome 4q26 in sheep (Brunner et al., 1995). Fleming et al. (1992) had found significantly higher β_A-inhibin mRNA levels in follicles of homozygous Fec^B gene carriers compared with controls. Inhibin β_A gene had significant effect on litter size in some sheep breeds (Leyhe et al., 1994; Hiendleder et al., 1996a, b).

The Small Tail Han sheep breed that has significant characteristics of high prolificacy and year-round estrus is an excellent local sheep breed in China (Tu, 1989; Liu et al., 2006). Mean litter size of Small Tail Han, Hu, Chinese

* Corresponding Author: M. X. Chu. Tel: +86-10-62819850, Fax: +86-10-62895351, E-mail: mxchu@263.net

[1] College of Animal Sciences, Zhejiang University, Hangzhou 310029, P. R. China.

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Table 1. Primer sequence, amplified region and product size of PCR amplification for entire coding region and partial 3'UTR of *INHBA* gene in sheep

Primer	Primer sequence (5'→3')	Product size	Amplified region
Primer 1	F: CAGGATGCCCTTGCTTT	224 bp	Exon 1 (1351-1574)
	R: CATCGGGTCTCTTCTTCAA		(U16238)
Primer 2	F: CACTTGAAGAAGAGACCCG	193 bp	Exon 1 (1553-1745)
	R: CACCTGATTCCGCGAAC		(U16238)
Primer 3	F: GGCACAGCCAGGAAGACG	335 bp	Exon 2 (398-732)
	R: CGTATGTCCAGGGAGCTCTTG		(U16239)
Primer 4	F: ATACGGATTGCCTGTG	333 bp	Exon 2 (728-1060)
	R: CTCACAGTAGTTGGCGT		(U16239)
Primer 5	F: GCTACCACGCCAACTACTGT	293 bp	Exon 2 (1038-1330)
	R: TCTCTGGACCATCTCGCTC		(U16239)

F stands for forward primer; R stands for reverse primer.

Merino and Corriedale sheep was 2.61, 2.29, 1.23 and 1.25, respectively (Tu, 1989). Mean litter size of Dorset, Texel, German Mutton Merino and South African Mutton Merino sheep was 1.45 (Casas et al., 2004), 1.41 (Casas et al., 2004), 2.00 (Chu et al., 2005) and 1.71 (Brand and Franck, 2000), respectively. Based on the important role of *INHBA* gene in reproduction, *INHBA* gene was considered as a possible candidate gene for the prolificacy of Small Tail Han sheep. The objectives of the present study were firstly to detect single nucleotide polymorphisms (SNPs) in coding region of *INHBA* gene in both two high fecundity breeds (Small Tail Han and Hu sheep) and six low fecundity breeds (Dorset, Texel, German Mutton Merino, South African Merino, Chinese Merino and Corriedale sheep) by polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP), and secondly to investigate the association between *INHBA* gene and high prolificacy of Small Tail Han sheep.

MATERIALS AND METHODS

Animals

All procedures involving animals were approved by the animal care and use committee at the respective institution where the experiment was conducted. All procedures involving animals were approved and authorized by the Chinese Ministry of Agriculture.

Venous jugular blood samples (10 ml per ewe) were collected from 136 Small Tail Han ewes lambed in 2004, along with data on litter size in the first, second, or third parity (Jiaxiang Sheep Breeding Farm located in Jiaxiang County, Shandong province, China), 36 Dorset, 40 Texel and 21 German Mutton Merino ewes (HITEK Ranch (Beijing) Ltd. Co. located in Dasungezhuang Town, Shunyi District, Beijing, China), 37 South African Mutton Merino and 31 Corriedale ewes (Qinshui Demonstration Farm located in Zhengzhuang Town, Qinshui County, Shanxi province, China), 39 Chinese Merino ewes (Ziniquan Breeding Sheep Farm located in Shihezi City, Xinjiang Uygur Autonomous Region, China), and 48 Hu ewes (Yuhang Hu Sheep Breeding Farm located in Yuhang District, Hangzhou City, Zhejiang province, China) using acid citrate dextrose as an anticoagulant. These ewes were chosen at random. Genomic DNA was extracted from whole blood by phenol-chloroform method, and then dissolved in TE buffer (10 mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA (pH 8.0)) and kept at -20°C.

However, among these sheep breeds, the 136 Small Tail Han ewes were the progeny of 6 rams. Because the 6 rams were sold, their blood was not collected and they were not genotyped. No selection on litter size or other fertility traits was performed in the flock over previous years. Lambing seasons consisted of 3-mo groups starting with March through May as season 1 (spring), June through August as season 2 (summer), September through November as season 3 (autumn), and December through February as season 4 (winter).

Primers and PCR amplification

Five pairs of primers were designed according to exon 1 (GenBank accession number U16238) and exon 2 (GenBank accession number U16239) of bovine inhibin β_A precursor gene (Thompson et al., 1994), and mRNA (GenBank accession number L19218) of ovine inhibin β_A subunit (Fleming et al., 1995). Entire coding region (1,278 bp) and 42 bp of 3' untranslated region (UTR) were amplified. These primers were synthesized by Shanghai Invitrogen Biotechnology Limited Corporation (Shanghai, China). Primer sequence, amplified region and PCR product size were listed in Table 1.

Polymerase chain reaction was carried out in 25 μl volume containing approximately 2.5 μl of 10×PCR buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.0), 0.1% Triton X-100), 1.5 mmol/L $MgCl_2$, 200 μmol/L each dNTP, 1 μmol/L each primer, 50 ng genomic DNA, and 1 U *Taq* DNA polymerase (Promega, Madison, WI, USA). Amplification conditions were as follows: denaturation at

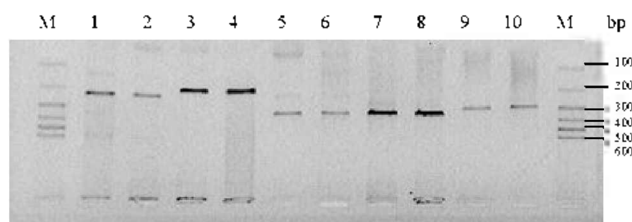

Figure 1. PCR products of five pairs of primers of ovine *INHBA* gene. 1, 2: primer 1; 3, 4: primer 2; 5, 6: primer 3; 7, 8: primer 4; 9, 10: primer 5.

94°C for 6 min; followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 55-62°C for 30 s, extension at 72°C for 30 s; with a final extension at 72°C for 10 min on Mastercycler® 5333 (Eppendorf AG, Hamburg, Germany).

SSCP detection

A volume of 1.5 μl PCR product was transferred in an Eppendorf tube, mixed with 6 μl gel loading solution containing 98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol, 20 mmol/L EDTA (pH8.0), 10% glycerol. The mixture was centrifugalized and denatured at 98°C for 10 min, then chilled on ice for 5 min and loaded on 8% to 10% neutral polyacrylamide gels (acrylamide: bisacrylamide = 29:1). Electrophoresis was performed in 1×Tris borate (pH 8.3)-EDTA buffer at 9 to 15 V/cm at 4°C overnight. After electrophoresis, the DNA fragments in the gels were visualized by silver staining, photographed and analyzed using an AlphaImager™ 2200 and 1220 Documentation and Analysis Systems (Alpha Innotech Corporation, San Leandro, CA, USA).

Cloning and sequencing

After SSCP analysis, PCR products of different homozygous genotypes were separated on 1.0% agarose gels and recovered using Geneclean II kit (Promega). Each DNA fragment was ligated into the pGEM-T Easy vector (Promega) according to the manufacturer's instructions. The ligation reactions were carried out in 10 μl volume containing PCR product 1 μl, pGEM-T Easy vector (50 ng/μl) 1 μl, T_4 ligase (3 U/μl) 1 μl, 2×ligation buffer 5 μl, ddH_2O 2 μl. Each DNA fragment was then transformed into *Escherichia coli* DH5α competence cell. Positive clones of transformed cells were identified by restriction enzyme digestion. Two clones of each homozygous genotype were selected and sequenced. Each clone was sequenced for twice. The target DNA fragments in recombinant plasmids were sequenced from both directions using an automatic ABI 377 sequencer (Perkin Elmer Applied Biosystems, Foster City, CA, USA) by Shanghai Invitrogen Biotechnology Ltd. Co. (Shanghai, China).

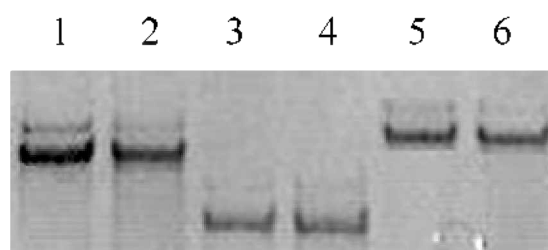

Figure 2. SSCP analysis of PCR amplification using primer 3 in different sheep breeds. 1, 2: AA genotype; 3, 4: BB genotype; 5, 6: CC genotype.

Figure 3. SSCP analysis of PCR amplification using primer 4 in different sheep breeds. 1, 2, 11, 12: EE genotype; 3, 4: EF genotype; 5: FF genotype; 6, 7: GG genotype; 8, 9, 10: EG genotype.

Statistical analysis

The following fixed effects model was employed for analysis of litter size in Small Tail Han ewes and least squares mean was used for multiple comparison in litter size among different genotypes.

$$y_{ijklm} = \mu+S_i+LS_j+P_k+G_l+e_{ijklm}$$

Where y_{ijklm} is phenotypic value of litter size; μ is population mean; S_i is the fixed effect of the ith sire (i = 1, 2, 3, 4, 5, 6); LS_j is the fixed effect of the jth lambing season (j = 1, 2, 3, 4); P_k is the fixed effect of the kth parity (k = 1, 2, 3); G_l is the fixed effect of the lth genotype (l = 1, 2, 3); and e_{ijklm} is random error effect of each observation. Analysis was performed using the general linear model (GLM) procedure of SAS (Ver 8.1) (SAS Institute Inc., Cary, NC, USA). Mean separation procedures were performed using a least significant difference test.

RESULTS

PCR amplification

Genomic DNA of eight sheep breeds was amplified using five pairs of primers for *INHBA* gene. PCR products were detected by running a 2% agarose gel electrophoresis (see Figure 1). Fragments amplified by primer 5 were not obtained in Chinese Merino sheep. The amplified products were consistent with the target fragments and had good specificity, which could be directly analyzed by SSCP.

SSCP analysis

Only the PCR products amplified by primers 3, 4 and 5

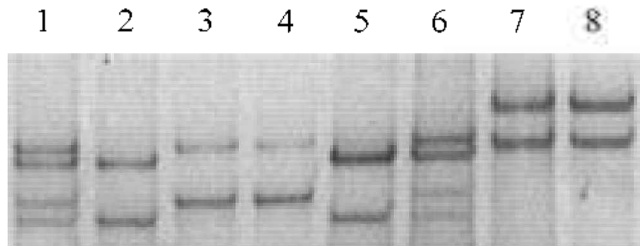

Figure 4. SSCP analysis of PCR amplification using primer 5 in different sheep breeds. 2, 5: KK genotype; 3, 4: LL genotype; 1, 6: KL genotype; 7, 8: MM genotype.

displayed polymorphisms. Three genotypes (AA, BB and CC) were detected by primer 3 (Figure 2), five genotypes (EE, FF, EF, GG and EG) were detected by primer 4 (Figure 3), and four genotypes (KK, LL, KL and MM) were detected by primer 5 (Figure 4).

Sequencing of different homozygous genotypes and nucleotide mutations

In the current study, the partial sequence (395 bp for exon 1 and 933 bp for exon 2) of *INHBA* gene in Small Tail Han sheep (with genotype KK for primer 5) was submitted into GenBank (accession number EF192431).

For primer 3, sequencing revealed eight nucleotide mutations of exon 2 of *INHBA* gene between genotype BB and genotype AA, and four nucleotide mutations between genotype CC and genotype AA (see Table 2). The deduced amino acids of the nucleotide sequences of genotypes AA, BB and CC were identical, so these mutations were silent. While comparing the deduced amino acid sequences with the sequence of ovine inhibin β_A subunit published on GenBank (accession number NP_001009458), an amino acid change (Gln142Lys) was detected, that is, glutamine in GenBank sequences changed into lysine in the present sequences.

For primer 4, sequencing revealed one nucleotide mutation (114G→A, GGG→GGA) (see Figure 5a) of exon 2 of *INHBA* gene between genotype FF and genotype EE, and this mutation did not cause any amino acid change. Another nucleotide change (143C→T, TCG→TTG) was identified between genotype GG and genotype EE, this mutation resulted in an amino acid change of serine → leucine (see Figure 5b), corresponding to serine residue at 287 of amino acid sequence of ovine inhibin β_A subunit

Table 2. The nucleotide mutations between three genotypes amplified by primer 3

Genotype	Nucleotide sequences (5'→3')
	1 24 66 69 93 150 213 255 279 282 300 335
AA	GGC…CATTT…AGCGTG…AGG…AG…AGA…AA…CGTTT…GTT…ACG
BB	GGC…CACTT…AACGTG…AAG…AG…AAA…GA…TGTCT…CTT…ACG
CC	GGC…CACTT…AGCGCG…AGG…AA…AGA…AA…CGTCT…GTT…ACG

The number indicates nucleotide site corresponding to amplified fragments in Table 1.

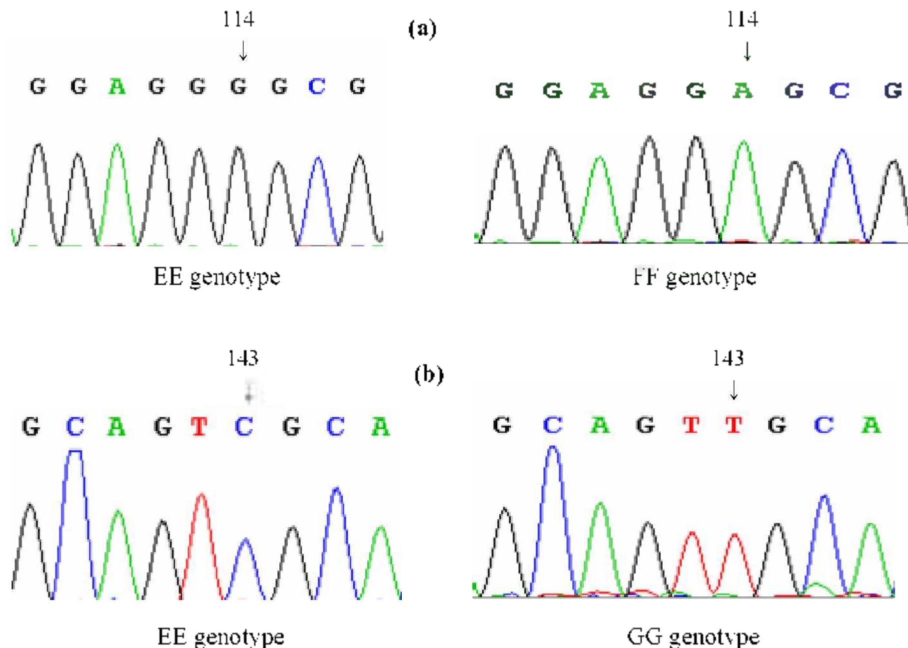

Figure 5. Nucleotide mutations between genotypes FF, GG and EE in sheep.

Table 3. The nucleotide mutations between three genotypes amplified by primer 5

Genotype	Nucleotide sequences (5'→3')
	1 53 83 110 185 218 242 263 264 272 293
	↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓↓ ↓ ↓
KK	GCT…CGT…CAG…GTC…ATG…AAA…GCT…CCAG…ACG…AGA
LL	GCT…CGT…CAG…GTC…ATG…AGA…GCT…CCAG…ACG…AGA
MM	GCT…CAT…CGG…GCC…ACG…AGA…GTT…CTGG…ATG…AGA

The number indicates nucleotide site corresponding to amplified fragments in Table 1.

Table 4. Allele and genotype frequencies of *INHBA* gene in eight sheep breeds

Breed			Small Tail Han sheep	Hu sheep	Texel	Dorset	German Mutton Merino	South African Mutton Merino	Corriedale	Chinese Merino
No. of samples			136	48	40	36	21	37	31	39
Primer 3	Genotype	AA	1.000(136)	0.708(34)	1.000(40)	1.000(36)	1.000(21)	1.000(37)	1.000(31)	0.000(0)
	frequency	BB	0.000(0)	0.292(14)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)
		CC	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	1.000(39)
	Allele	A	1.000	0.708	1.000	1.000	1.000	1.000	1.000	0.000
	frequency	B	0.000	0.292	0.000	0.000	0.000	0.000	0.000	0.000
		C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Primer 4	Genotype	EE	1.000(136)	1.000(48)	1.000(40)	0.417(15)	0.619 (13)	1.000(37)	1.000(31)	0.641(25)
	frequency	FF	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.026(1)
		EF	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.333 (13)
		GG	0.000(0)	0.000(0)	0.000(0)	0.055(2)	0.095(2)	0.000(0)	0.000(0)	0.000(0)
		EG	0.000(0)	0.000(0)	0.000(0)	0.528(19)	0.286(6)	0.000(0)	0.000(0)	0.000(0)
	Allele	E	1.000	1.000	1.000	0.681	0.762	1.000	1.000	0.808
	frequency	F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.192
		G	0.000	0.000	0.000	0.319	0.238	0.000	0.000	0.000
No. of samples			136	46	40	35	21	37	31	
Primer 5	Genotype	KK	0.471(64)	0.304(14)	0.450(18)	0.942(33)	0.905(19)	0.324(12)	0.452(14)	
	frequency	LL	0.220(30)	0.239(11)	0.025(1)	0.029(1)	0.000(0)	0.189(7)	0.000(0)	
		KL	0.309(42)	0.044(2)	0.525(21)	0.029(1)	0.095(2)	0.487(18)	0.548(17)	
		MM	0.000(0)	0.413(19)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	0.000(0)	
	Allele	K	0.625	0.326	0.713	0.957	0.952	0.568	0.726	
	frequency	L	0.375	0.261	0.287	0.043	0.048	0.432	0.274	
		M	0.000	0.413	0.000	0.000	0.000	0.000	0.000	

The numbers in the parentheses are the individuals that belong to the respective genotypes.

published in GenBank (accession number NP_001009458).

For primer 5, sequencing revealed one nucleotide mutation (218A→G) of exon 2 of *INHBA* gene between genotype LL and genotype KK, and nine nucleotide mutations between genotype MM and genotype KK (see Table 3). However, these amino acid sequences of the nucleotide sequences of the three genotypes were the same, and which were identical to amino acid sequences of ovine inhibin β_A subunit published in GenBank (accession number NP_001009458).

Allele and genotype frequencies of *INHBA* gene in eight sheep breeds

The frequencies of 12 genotypes and 9 alleles in eight sheep breeds were presented in Table 4.

For primer 3, genotype CC was only detected in Chinese Merino sheep, genotype AA was detected in other seven sheep breeds. Genotype BB was only detected in Hu sheep. Only Hu sheep displayed polymorphism.

For primer 4, genotypes EE, EG and GG were detected in Dorset and German Mutton Merino sheep, genotypes EE, EF and FF were detected in Chinese Merino sheep, only genotype EE was detected in other five sheep breeds. Only Dorset, German Mutton Merino and Chinese Merino sheep displayed polymorphism.

For primer 5, genotypes KK and KL were detected in German Mutton Merino and Corriedale sheep, genotypes KK, LL and KL were detected in other six sheep breeds. Genotype MM was only detected in Hu sheep. All of these eight sheep breeds displayed polymorphism.

Based on sequencing results, primer 3 had 10 DNA variations, primer 4 had two DNA variations, and primer 5 had 9 DNA variations. In total of the 21 mutations, 17 DNA variations were originated from the Hu breed, five DNA variations (two mutations 24T→C and 282T→C of them were the same as those in the Hu breed) were found in the Chinese Merino breed, and one SNP was detected in the Dorset and German Mutton Merino breeds.

These results indicated that the polymorphisms in coding region of *INHBA* gene were rather abundant among

Table 5. Least squares means and standard errors for litter size of different genotypes of *INHBA* gene in Small Tail Han sheep

Primer	Genotype	No. of samples	Litter size
Primer 5	KK	64	$1.90^{b}\pm0.15$
	KL	42	$2.00^{b}\pm0.17$
	LL	30	$2.53^{a}\pm0.20$

Least squares means with the different superscripts for the same pair of primer differ significantly ($p<0.05$).

these sheep breeds.

Influence of fixed effects on litter size in Small Tail Han sheep

Sire significantly influenced litter size in Small Tail Han sheep ($p<0.01$). Both lambing season and parity significantly influenced litter size in Small Tail Han sheep ($p<0.05$). The *INHBA* genotype significantly influenced litter size in Small Tail Han sheep ($p<0.05$).

The least squares means and standard errors for litter size of different genotypes of *INHBA* gene in Small Tail Han sheep were given in Table 5. The Small Tail Han ewes with genotype LL had 0.53 ($p<0.05$) or 0.63 ($p<0.05$) lambs more than those with genotype KL or KK, respectively. The Small Tail Han ewes with genotype KL had 0.10 ($p>0.05$) lambs more than those with genotype KK.

DISCUSSION

Polymorphisms of *INHBA* gene in sheep

A *Taq*I polymorphism at the ovine *INHBA* locus had been identified (Hiendleder et al., 1992). Jaeger and Hiendleder (1994) identified polymorphisms in the coding regions and regulatory elements of ovine *INHBA* gene. Leyhe et al. (1994) revealed two *Taq*I alleles (1.9 kb and 1.5 kb) of ovine *INHBA* gene, and found that the four sheep breeds (Rhoenschaf, Merinolandschaf, East Friesian Milksheep and Romanov) with different reproductive performance differed significantly ($p<0.001$) in *Taq*I allele frequencies. Hiendleder et al. (1996a) reported two *Taq*I alleles at the ovine *INHBA* locus.

The present study identified 21 new nucleotide polymorphisms in the entire coding region and partial 3' UTR of ovine *INHBA* gene, only one of which disrupted the protein product among eight sheep breeds, and another amino acid change was detected between the investigated sheep and ovine amino acid sequence from GenBank. These polymorphisms determined 12 genotypes and 9 alleles in eight sheep breeds. What is cause that leads to such a high rate of mutation in *INHBA* gene without changing the amino acid sequence in these sheep breeds deserves further study.

In the current study, 17 of 21 SNPs were from the Hu breed. The Hu sheep breed is a special local breed in China. Chinese Hu sheep are used mainly for lambskin production and may be found throughout the Taihu Lake area that covers Jiangsu province, Zhejiang province and the vicinity of Shanghai. This sheep breed is famous for its beautiful lambskin, early sexual maturity, year-round estrus, and high prolificacy (Tu, 1989; Yue, 1996; Chu and Wang, 2001; Chu et al., 2006). Why so many variations in the Hu breed deserve further study.

Relationship of *INHBA* gene with prolificacy of sheep

As inhibin plays an important role in FSH regulation and acts as a growing factor in ovary, it was proposed as a candidate gene for reproductive performance (Xue et al., 2004). Leyhe et al. (1994) and Hiendleder et al. (1996a) reported that the frequency of the *Taq*I allele A of *INHBA* gene coincided with the average litter size in each sheep breed examined. Average litter size was 1.10, 1.44, 1.48, 1.76, 3.00, whereas the frequency of the *Taq*I allele A was 0.0, 0.20, 0.29, 0.35, 0.65, respectively, in wild sheep, Merinolandschaf, Rhoenschaf, East Friesian Milksheep and Romanov. Jaeger and Hiendleder (1994) analyzed 1,000 lambing records and found that *INHBA* gene had obvious genetic effect on litter size of sheep. Hiendleder et al. (1996b) reported that *INHBA* gene had significant influence on ovine litter size. Animal model analysis of 1,562 litters of 389 Merinolandschafe ewes showed that the substitution effect of *INHBA* gene reached 0.04 lambs ($0.25\sigma_A$), and the analysis of 620 litters of 155 East Friesian Milksheep ewes indicated that the substitution effect of *INHBA* gene reached 0.09 lambs.

Combined with previous studies, the present study preliminarily showed that *INHBA* gene is a strong candidate gene for the ovine litter size. Further extensive sampling and DNA analysis would be required to verify these results. Ongoing investigations into the basis of the prolific phenotype of Small Tail Han and Hu sheep are likely to reveal further insights into the events controlling follicle and oocyte development.

ACKNOWLEDGMENTS

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