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Screening for S32G mutation of *BMP15* gene in 18 goat breeds

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Abstract: In our early work, one mutation (A898G, which caused Ser32Gly (S32G) change of mature peptide) was identified in the goat *BMP15* gene and the allele G was associated with high litter size in Jining Grey goats (the goat breed with the highest fecundity in China). The aim of this research was to investigate the genetic structure of the prolific *BMP15* gene in 18 goat breeds reared in China, including 12 native breeds, 3 introduced breeds, and 2 cultivated breeds. Genomic DNA of 1011 goats was screened for the S32G mutation. The results showed that this mutation existed in all 3 cultivated and 8 native goat breeds but in none of the 3 introduced goat breeds, which hinted that this mutation may originate from native goat breeds of China. Besides the Jining Grey goats, the *BMP15* gene was also a potential prolificacy gene in Matou goats. Moreover, the structure prediction indicated that the S32G mutation might participate in the binding of *BMP15* with receptors.

Key words: Goat, prolificacy, bone morphogenetic protein 15 gene, functional analysis

1. Introduction

The oocyte-secreted factor, bone morphogenetic protein 15 (BMP15), also known as growth differentiation factor 9B (GDF9B), was proven to be critical for normal fertility in female mammals. It was confirmed that the physiological role of BMP15 was to regulate granulosa cell proliferation and differentiation by promoting granulosa cell mitosis, to stimulate the mRNA expression of granulosa cell kit ligand, to inhibit FSH-dependent progesterone synthesis through the suppression of the mRNA expression of the FSH receptor, and to prevent premature luteinization (1,2). The goat *BMP15* gene contains 2 exons and 1 intron, which showed high homology (99.4%) with that of sheep (3). In goat ovaries, BMP15 mRNA was detected in primordial, primary, and secondary follicles as well as in the oocyte and granulosa cells of antral follicles, while the BMP15 protein was found in oocytes of all types of follicles and granulosa cells of primary, secondary, and antral, but not primordial, follicles (4). BMP15 protein was highly conserved (98.5% homology) between sheep and goats and both lacked the fourth cysteine residue of the 7 cysteines that are typically conserved in the members of the TGFβ superfamily (5). Because of the deletion of the fourth cysteine, goat BMP15

and GDF9 are perhaps linked as homodimeric and/or heterodimeric proteins with noncovalent bonds, rather than disulfide bonds.

To date, 8 mutations have been identified in the sheep *BMP15* gene related to high prolificacy, including FecX^I (6), FecX^H (6), FecX^B (7), FecX^G (7,8), FecX^L (9), FecX^R (10,11), FecX^{Gr} (12), and FecX^O (12) in sheep. The former 6 mutations in the sheep *BMP15* gene were associated with both increased ovulation rate or litter size in heterozygous carriers and sterility in homozygous carriers. The results of our early work showed that the 6 mutations of the *BMP15* gene had no significant effect on the fecundity of Jining Grey goats (JG, the goat breed with the highest prolificacy in China with 2.94 kids per litter) and other goat breeds (13–16). We detected a mutation, A898G (resulted in Ser32Gly (S32G) change of mature peptide), in the goat *BMP15* gene and found that allele G was associated with high litter size in JG goats (3,17); does with genotype GG had 0.71 (P < 0.05) or 1.57 (P < 0.05) kids more than those with genotype GA or AA, and does with genotype GA had 0.86 (P < 0.05) kids more than those with genotype AA (3). The *BMP15* gene may be a potential major gene in goat prolificacy.

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The S32G mutation of the *BMP15* gene in JG goats may have significant effects on fecundity of other goat breeds reared in China. The aim of our research was to investigate the distribution of the S32G mutation in 18 goat breeds by PCR-RFLP and to analyze its functional change.

2. Materials and methods

2.1. Samples and breeds
There are abundant genetic resources of goats in China and they show rich diversity of fecundity (18). The DNA samples of 18 goat breeds with different fecundities were collected (Table 1). Blood samples (10 mL, jugular vein, ACD anticoagulant) were collected from 203 JG goat does that kidded in 2008 (first, second, or third parity) (Jining Grey Goat Conservation Base, Jiaxiang County, Shandong Province, China), 60 Guizhou White goat does (GW, Guizhou White Goat Conservation Farm, Yanhe Tujia Nationality Autonomous County, Guizhou Province, China), 55 Dazu Black goat does (DB, Dazu Black Goat Breeding Farm, Dazu County, Chongqing, China), 30 Shannan White goat does (SW, Jingbian County, Shaanxi Province, China), 54 Nanjiang Brown goat does (NB, Nanjiang Brown Goat Breeding Farm, Nanjiang County, Sichuan Province, China), 40 Gulin Ma goat does (GM, Hujia Farm, Gulin County, Sichuan Province, China), 50 Boer goat does (B, Qinshui Demonstration Farm, Qinshui County, Shanxi Province, China), 32 Chengdu Ma goat does (CM, Chengdu Ma Goat Breeding Farm, Dayi County, Sichuan Province, China), 64 Saanen Dairy goat does (SD, Shaanxi Province, China), 48 Matou goat does (M, Shiyan City, Hubei Province, China), 50 Wendeng Dairy goat does (WD, Wendeng City, Shandong Province, China), 49 Banjiao goat does (BJ, Banjiao Goat Breeding Farm, Wuxi County, Chongqing, China), 45 Jintang Black goat does (JB, Jintang County, Sichuan Province, China), 24 Guanzhong Dairy goat does (GD, Fuping County, Shaanxi Province, China), 60 Chuandong White goat does (CW, Yikouxian Goat Breeding Farm, Yunyang County, Chongqing, China), 41 Angora goat does (A, Qinshui Demonstration Farm, Qinshui County, Shanxi Province, China), 60 Liaoning Cashmere goat does (LC, Liaoning Cashmere Goat Breeding Center, Liaoyang City, Liaoning Province, China), and 46 Inner Mongolia Cashmere goat does (IC, Inner Mongolia White Cashmere Goat Breeding Farm, Etuokeqi, Ordos City, Inner Mongolia Autonomous Region, China). Genomic DNA was extracted from whole blood using the phenol-chloroform method, dissolved in TE buffer (10 mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA (pH 8.0)), and kept at –20 °C.

The 203 Jining Grey goat does were selected at random and represented the progeny of 5 goat bucks (n = 39, 40, 41, 41, 42). Because the 5 bucks had been sold, their blood was not collected for genotyping. No selection on litter

size or other fertility traits had been performed in this flock over previous years. Kidding was partitioned into 4 seasons of 3 months each: March through May (season 1, spring, n = 52), June through August (season 2, summer, n = 46), September through November (season 3, autumn, n = 56), and December through February (season 4, winter, n = 49).

2.2. Genotyping
One pair of primers was designed to amplify part of exon 2 of the goat *BMP15* gene. The primer sequences were as follows: F: 5′-CAAGCAGGCAGTATTGCATC-3′, R: 5′-ATGGGGAGCAATGAT CCAGT-3′. The primers were synthesized by Shanghai Invitrogen Biotechnology Co. Ltd. (Shanghai, China).

Polymerase chain reactions were carried out in a 25-μL volume containing approximately 0.5 μL of 10 μmol/L of each primer, 2.5 μL of 10X PCR buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.0), 0.1% Triton X-100), 1.5 μL of 25 mmol/L MgCl$_2$, 2.0 μL of 2.5 mmol/L of each dNTP, 2.0 μL of 50 ng/μL goat genomic DNA, 1.0 μL of 2.5 U/μL Taq DNA polymerase (Promega, Madison, WI, USA), and ddH$_2$O to complete the volume.

Amplification conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 32 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 20 s, with a final extension at 72 °C for 10 min on the Mastercycler 5333 (Eppendorf AG, Hamburg, Germany).

PCR products were digested by *Alu*I (NEB, China) according to the recommendations. The mixtures were detected at 100 V on a 12% polyacrylamide gel.

2.3. Statistical analysis
The following fixed-effects model was employed for analysis of litter size in JG goat does and least squares means were used for multiple comparisons of litter size among the different genotypes:

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

where y_{ijklm} is the phenotypic value of litter size, μ is the population mean, S_i is the fixed effect of the ith sire (i = 1, 2, 3, 4, 5), KS_j is the fixed effect of the jth kidding 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 the random residual effect of each observation.

Analysis was performed using the general linear model procedure of SAS Version 8.1 (SAS Institute Inc., Cary, NC, USA). Mean separation procedures were conducted using a least significant difference test.

2.4. Structure prediction of goat BMP15
The structures were predicted online with the Phyre program and the deepview/Swiss-pdb Viewer v4.0.1 based on the crystal structure of hBMP7 (pdb ID: c1lx5A).

3. Results and discussion

The mean litter size of the 18 goat breeds ranged from 1.05 (IC) to 2.94 (JG) (18,19). The 18 goat breeds can be divided into 3 categories based on their fecundity with 4 high prolificacy breeds (mean litter size exceeded 2.50 kids, including JG, GW, DB, and SW), 11 moderate prolificacy breeds (mean litter size between 1.50 and 2.49 kids, including NB, GM, B, CM, SD, M, WD, BJ, JB, GD, and CW), and 3 low prolificacy breeds (mean litter size less than 1.49 kids, including A, LC, and IC) (Table 1). The allele G at the 898 locus was detected with a high frequency (0.938) in JG goats; a moderate frequency (0.562) in M goats; a low frequency in WD (0.200), BJ (0.133), and JB (0.133) goats; and an extremely low frequency (less than 0.1) in SW, NB, GM, CM, GD, and CW goats by the PCR-RFLP method (Figure 1). None of the individuals in the GW, DB, B, SD, A, LC, and IC breeds were detected to carry the A898G mutation of *BMP15* (Table 1). The huge difference in genotype distribution of the *BMP15* gene in 18 goat breeds indicated that there could be certain relations between the S32G mutation and litter size in goats.

On the other hand, the 18 goat breeds could be classified into 3 types, with 12 native goat breeds (JG, GW, DB, SW, GM, CM, M, BJ, JB, CW, LC, and IC), 3 introduced goat breeds (B, SD, and A), and 3 cultivated goat breeds (NB, WD, and GD) (19,20). Interestingly, no individuals of the 3 introduced goat breeds (B, SD, and A) carried the A898G mutation, while the mutation was identified in all 3 cultivated breeds (NB, WD, and GD) and some of the native goat breeds (JG, SW, GM, CM, M, BJ, JB, and CW), which was consistent with the hypothesis that this mutation may originate from native goat breeds of China. In the breeding process of the NB goat breed (the first goat breed cultivated for meat production in China in 1998), CM goats were used as one of the paternal breeds and JB goats were used as one of the maternal breeds (21,22). As a result, the A898G mutation was found in the new cultivated breed afterwards and its breeding materials.

In JG goats, frequency of AA, GA, and GG genotypes was 0.015, 0.094, and 0.891, respectively. Genotype distributions of the *BMP15* gene were significantly different (P < 0.001, data not shown) between JG goats

Table 1. Allele and genotype frequencies of *BMP15* in 18 goat breeds.

Breed	N	Genotype frequency[a]			Allele frequency		Mean litter size
		AA	GA	GG	A	G	
JG	203	0.015(3)	0.094(19)	0.891(181)	0.062	0.938	2.94
GW	60	1.000(60)	0.000(0)	0.000(0)	1.000	0.000	2.73
DB	55	1.000(55)	0.000(0)	0.000(0)	1.000	0.000	2.72
SW	30	0.900(27)	0.067(2)	0.033(1)	0.933	0.067	2.59
NB	54	0.944(51)	0.056(3)	0.000(0)	0.972	0.028	2.19
GM	40	0.875(35)	0.125(5)	0.000(0)	0.938	0.062	2.14
B	50	1.000(50)	0.000(0)	0.000(0)	1.000	0.000	2.10
CM	32	0.938(30)	0.031(1)	0.031(1)	0.953	0.047	2.06
SD	64	1.000(64)	0.000(0)	0.000(0)	1.000	0.000	2.00
M	48	0.271(13)	0.333(16)	0.396(19)	0.438	0.562	1.95
WD	50	0.640(32)	0.320(16)	0.040(2)	0.800	0.200	1.85
BJ	49	0.755(37)	0.225(11)	0.020(1)	0.867	0.133	1.84
JB	45	0.733(33)	0.267(12)	0.000(0)	0.867	0.133	1.80
GD	24	0.917(22)	0.083(2)	0.000(0)	0.958	0.042	1.78
CW	60	0.983(59)	0.017(1)	0.000(0)	0.992	0.008	1.66
A	41	1.000(41)	0.000(0)	0.000(0)	1.000	0.000	1.39
LC	60	1.000(60)	0.000(0)	0.000(0)	1.000	0.000	1.18
IC	46	1.000(46)	0.000(0)	0.000(0)	1.000	0.000	1.05

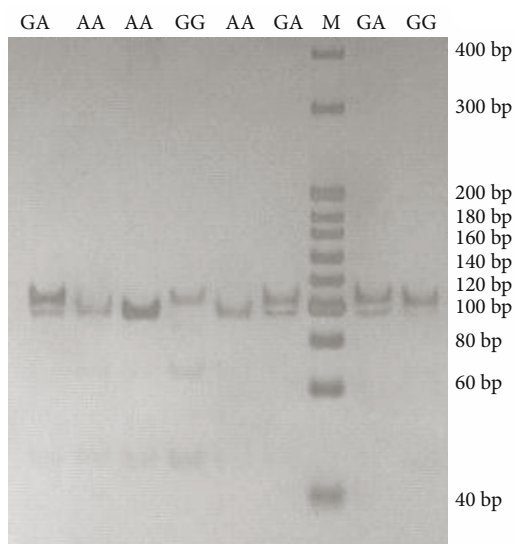[a]The numbers in parentheses are the genotype's individuals.

Figure 1. The PCR-RFLP (*Alu*I) analysis of A898G in goat *BMP15* gene (12% polyacrylamide gel). The amplified fragment had 2 *Alu*I sites located at the 96th and 106th positions of the 138-bp fragment, respectively. Three genotypes: AA, 96/32/10 bp; GA, 106/96/32/10 bp; GG, 106/32 bp. M: 20-bp DNA Ladder Marker (TaKaRa, Dalian, China).

and other high prolificacy goat breeds. The JG does with genotype GG had 0.65 (P < 0.01) or 1.75 (P < 0.01) kids more than those with genotype GA or AA, while the does with genotype GA had 1.10 (P < 0.01) kids more than those with genotype AA (Table 2). The relationship between different genotypes and litter size in the present study was consistent with that of Chu et al. (17) and Feng et al. (3). In moderate prolificacy goat breeds, genotype distributions of the *BMP15* gene were also significantly different (P < 0.001, data not shown) between M goats and other goat breeds. Because the kidding records were not available, we did not estimate the effects of different genotypes on litter size in M goats.

Table 2. Least squares mean and standard errors for litter size of different genotypes of *BMP15* gene in Jining Grey goats.

Locus	Genotype	Number of does	Litter size
	GG	181	$2.83^a \pm 0.08$
898	GA	19	$2.18^b \pm 0.15$
	AA	3	$1.08^c \pm 0.06$

Least squares means with different superscripts differ significantly (P < 0.01).

Mutations of A898G (also named as A963G and resulted in S300G in protein) and C985G (also named as C1050G and resulted in L329V in protein) were reported in exon 2 of the *BMP15* gene in Jining Grey goats (14,17). Another 2 novel SNPs (G735A and C808G) were observed in exon 2 of the *BMP15* gene, which showed no evidence of being related to litter size in Indian goat breeds (23). Six known mutations of exon 2 in the sheep *BMP15* gene, including FecX[I], FecX[H], FecX[B], FecX[G], FecX[L], and FecX[R], were associated with both increased ovulation rate or litter size in heterozygous carriers and sterility in homozygous carriers, which had no significant effect on fecundity of JG goats (13–16). Recently, FecX[Gr] (C950T led to T317I) mutation in exon 2 of the *BMP15* gene in French Grivette sheep and FecX[O] (A1009C led to N337H) mutation in Polish Olkuska sheep were identified to be responsible for an atypical hyperprolificacy phenotype in sheep reproduction (12). It was noteworthy that FecX[Gr] and FecX[O] led to highly prolific ewes of homozygous status (genotype frequency was 92.9% and 65.5% in highly prolific ewes, respectively), in contradiction with other *BMP15* variants described so far. This is the first time that mutations of the sheep *BMP15* gene were associated with a prolific phenotype in homozygous ewes. In our study, homozygous does with the A898G mutation had the highest litter size among 3 genotypes; whether or not this mutation shared the same mechanism as FecX[Gr] and FecX[O] remains to be determined.

Because the structure of BMP15 was not available, the tertiary structure of the BMP15 protein of JG goats was modeled by online prediction method based on the crystal structure of hBMP7 (pdb ID: c1lx5A). The tertiary
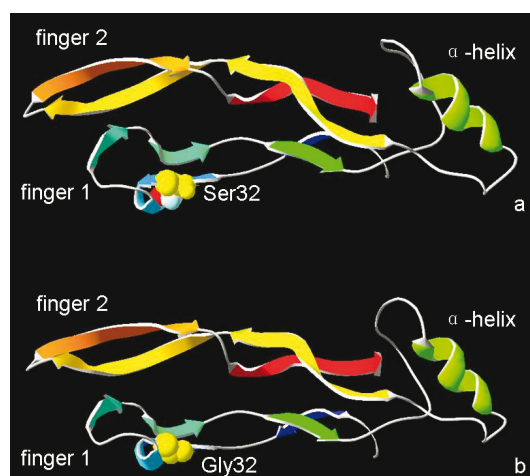

Figure 2. Homology-based molecular model of goat BMP15 and S32G-mutated BMP15 protein molecules. Goat wild-type BMP15 (a) and Gly32 BMP15 (b) monomer models are shown. Residues 22–125 of goat mature BMP15 peptide were modelled on the basis of homology with the tertiary structure of hBMP7.

model of mature BMP15 of JG goats from 22 to 125 was built using the Phyre program online. The results were the same as those of Ran et al. (24) and indicated that the overall structure of BMP15 was curled as left-handed with 1 α-helix and 2 fingers, which was similar to other TGFβ superfamily members (25). Six cysteines of JG goat BMP15 consisted of 3 disulfide bonds including Cys24-90, Cys35-122, and Cys57-124. In the left-hand model, the smaller finger 1 comprised β1, α1, β2, and β3, while the larger finger 2 included β4 to β7 (Figure 2). Mutation of the polar Ser32 to nonpolar Gly located at the surface of finger 1 and palm of BMP15 might participate in the binding of BMP15 with receptors (24). However, Val31Asp mutation (FecX^I, mutation of the hydrophobic Val31 to negatively charged Asp), the closest mutation to Ser32Gly, changed the electrostatic surface potentials in the middle of a noncharged surface area involved in dimer formation (6).

In conclusion, the prolificacy genotype of the *BMP15* gene in JG goats was also found in all 3 cultivated breeds (NB, WD, and GD) and some of the native goat breeds (JG, SW, GM, CM, M, BJ, JB, and CW), but not in the 3 introduced goat breeds (B, SD, and A), which hinted that this mutation may originate from native goat breeds of China. Besides the JG goats, the *BMP15* gene was also a potential prolificacy gene in M goats. Moreover, the structure prediction showed that the S32G mutation might participate in the binding of BMP15 with receptors.

References

1. Otsuka F, Yao Z, Lee T, Yamamoto S, Erickson GF, Shimasaki S. Bone morphogenetic protein-15. Identification of target cells and biological functions. J Biol Chem 2000; 275: 39523–39528.

2. Moore KR, Shimasaki S. Molecular biology and physiological role of the oocyte factor, BMP-15. Mol Cell Endocrinol 2005; 234: 67–73.

3. Feng T, Zhao YZ, Chu MX, Di R, Zhang YJ, Fang L. Bone morphogenetic protein 15 as a candidate gene for prolificacy of Jining Grey goat. Acta Veterinaria et Zootechnica Sinica 2009; 40: 468–475 (article in Chinese with an English abstract).

4. Silva JR, van den Hurk R, van Tol HT, Roelen BA, Figueiredo JR. Expression of growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), and BMP receptors in the ovaries of goats. Mol Reprod Dev 2005; 70: 11–19.

5. 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.

6. Galloway SM, McNatty KP, Cambridge LM, Laitinen MP, Juengel JL, Jokiranta TS, McLaren RJ, Luiro K, Dodds KG, Montgomery GW et al. 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. Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH, Powell R, Galloway SM. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). Biol Reprod 2004; 70: 900–909.

8. Barzegari A, Atashpaz S, Ghabili K, Nemati Z, Rustaei M, Azarbaijani R. Polymorphisms in GDF9 and BMP15 associated with fertility and ovulation rate in Moghani and Ghezel sheep in Iran. Reprod Domest Anim 2010; 45: 666–669.

9. Bodin L, Di Pasquale E, Fabre S, Bontoux M, Monget P, Persani L, Mulsant P. A novel mutation in the BMP15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. Endocrinology 2007; 148: 393–400.

10. Martinez-Royo A, Jurado JJ, Smulders JP, Martí JI, Alabart JL, Roche A, Fantova E, Bodin L, Mulsant P, Serrano M et al. A deletion in the bone morphogenetic protein 15 gene causes sterility and increased prolificacy in Rasa Aragonesa sheep. Anim Genet 2008; 39: 294–297.

11. Monteagudo LV, Ponz R, Tejedor MT, Laviña A, Sierra I. A 17 bp deletion in the Bone Morphogenetic Protein 15 (BMP15) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed. Anim Reprod Sci 2009; 110: 139–146.

12. Demars J, Fabre S, Sarry J, Rossetti R, Gilbert H, Persani L, Tosser-Klopp G, Mulsant P, Nowak Z, Drobik W et al. Genome-wide association studies identify two novel *BMP15* mutations responsible for an atypical hyperprolificacy phenotype in sheep. PLoS Genet 2013; 9: e1003482.

13. He YQ, Chu MX, Wang JY, Fang L, Ye SC. Polymorphism on BMP15 as a candidate gene for prolificacy in six goat breeds. Journal of Anhui Agricultural University 2006; 33: 61–64 (article in Chinese with an English abstract).

14. Jiao CL, Chu MX, Wang JY, Liu ZH, Fang L, Ye SC. RFLP analysis on bone morphogenetic protein 15 as a candidate gene for prolificacy in goats. Journal of Yangzhou University (Agriculture and Life Sciences Edition) 2006; 27: 31–34 (article in Chinese with an English abstract).

15. Xiao JW, Chu MX, Li XW, Fang L. Detection of the FecX^L mutation of BMP15 gene in goats. China Animal Husbandry and Veterinary Medicine 2008; 35: 35–37 (article in Chinese with an English abstract).

16. Di R, Feng T, Chu MX, Zhang YJ, Fang L. Detection of the FecX^R mutation of BMP15 gene in sheep and goats. Acta Veterinaria et Zootechnica Sinica 2009; 40: 1303–1307 (article in Chinese with an English abstract).

17. Chu MX, Jiao CL, He YQ, Wang JY, Liu ZH, Chen GH. Association between PCR-SSCP of bone morphogenetic protein 15 gene and prolificacy in Jining Grey goats. Anim Biotechnol 2007; 18: 263–274.

18. The Editorial Board of China National Commission of Animal Genetic Resources. Animal Genetic Resources in China Sheep and Goats. 1st ed. Beijing, China: China Agriculture Press; 2011.

19. Tu YR. The Sheep and Goat Breeds in China. 1st ed. Shanghai, China: Shanghai Science and Technology Press; 1989.

20. Editor of Report on Domestic Animal Genetic Resources in China. Report on Domestic Animal Genetic Resources in China. 1st ed. Beijing, China: China Agriculture Press; 2004.

21. Wang CX, Tang SF, Liu QH, Xu DG, Liu XJ, Zhong WB, Tang SJ. The reproduction performance of Jitang Black goats. Sichuan Animal and Veterinary Sciences 2002; 29: 20–21 (article in Chinese with an English abstract).

22. Chen Y, Wang WC, Zhang GJ, Xiong ZR, Zhang, HP. Selective breeding of high prolificacy characteristics in Nanjiang Brown goat. Pruataculture and Animal Husbandry 2008; 146: 47–49 (article in Chinese).

23. Ahlawat S, Sharma R, Maitra A. Screening of indigenous goats for prolificacy associated DNA markers of sheep. Gene 2013; 517: 128–131.

24. Ran XQ, Lin JB, Du ZY, Qing C, Wang JF. Diversity of bmp15 and gdf9 genes in white goat of Guizhou province and evolution of the encoded proteins. Zoological Research 2009; 30: 593–602.

25. Griffith DL, Keck PC, Sampath TK, Rueger DC, Carlson WD. Three dimensional structure of recombinant human osteogenic protein 1: structural paradigm for the transforming growth factor beta superfamily. Proc Natl Acad Sci U S A 1996; 93: 878–883.