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Polymorphic Study of $FecX^G$, $FecG^H$ and Fec^B Mutations in Four Domestic Sheep Breeds in the Lower Yellow River Valley of China

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Abstract: Genetic mutations with major effects on ovulation rate in sheep were recently identified in two genes of the Transforming Growth Factor ($TGF_β$) superfamily and a $TGF_β$ receptor, namely BMP15, GDF9 and BMPRIB. The $FecX^G$, $FecG^H$ and Fec^B were performed to screen four sheep breeds (small tailed Han, Wadi, big tailed Han and Shandi sheep) distributed along the lower Yellow river valley of China by forced PCR-RFLP method if these genes are responsible for their high prolificacies. The Fec^B mutation was found in small tailed Han and Wadi sheep but absent in big tailed Han and Shandi sheep. The 154 small tailed Han sheep included all three genotypes (Fec^B/Fec^B, Fec^B/Fec^+ and Fec^+/Fec^+) at frequencies of 0.54, 0.40 and 0.06, respectively whereas the samples of 30 Wadi sheep were only detected two genotypes, 4 were heterozygous Fec^B/Fec^+ and 26 Fec^+/Fec^+. The frequency of the Fec^B allele in small tailed Han and Wadi sheep were 0.74 and 0.07, respectively. Results indicated that the Fec^B mutation is not fixed in two sheep population. The same $FecX^G$ mutation of the *BMP-15* gene was only found in small tailed Han ewes as in Belclare and Cambridge ewes, frequencies of genotypes $FecX^{G+}$, $FecX^{++}$ and $FecX^{GG}$ were 0.70, 0.27 and 0.03, respectively. There was no evidence of $FecG^H$ in any of the breeds sampled. The discovery of the Fec^B mutation in small tailed Han sheep and Wadi sheep will facilitate the use of Fec^B allele in improving the prolificacy of non-prolific sheep breeds of China.

Key words: $FecX^G$, $FecG^H$, Fec^B, sheep, mutation, prolificacy, China

INTRODUCTION

The lower Yellow river valley is located in East China. Its elevation is between 10 and 100 m above sea level, the mean rainfall and temperature are 600~900 mm and 12~14°C, respectively. In the valley, small tailed Han sheep that has significant characteristics of high prolificacy and non-seasonal ovulatory activity is an excellent local sheep breed (Tu, 1989).

Shandi sheep and large tail Han sheep are known for white fur and draft efficiency,respectively. Wadi sheep is the 2nd prolific sheep of the valley after the small tailed Han sheep (Wang and Yue, 2008; Ren *et al*., 2008). This population is mainly distributed in Yellow river delta including Binzhou, Dongying country.

Previouly studies had investigated the phenotypic variability and DNA polymorphisms of sheep populations. The close genetic relationships among these four sheep breeds have been revealed by RAPD, microsatellite and mtDNA markers (Du and Cao, 2003; Zhang *et al*., 2005). Large genetic variations in the litter size have also been observed among these breeds and within breeds. Recently, genetic mutations with major effects on ovulation rate in sheep were identified including the Fec^B mutation of the BMPR-IB (Wilson *et al*., 2001), locus $FecG^H$ mutation in the *GDF9* gene (Hanrahan *et al*., 2004) and $FecX^G$ mutation in *BMP15* gene (Galloway *et al*., 2000) which causes high prolificacy in sheep, Belclare and Cambridge, respectively. In this valley, Fec^B and $FecX^G$ mutation have been found existed in Small tailed Han sheep with high a frequency (Chu *et al*., 2007). However, a report of Ren *et al*. (2008) revealed that the lambing percentage averaged 250% in Wadi sheep.

On the other hand, Shandi and larger tailed Han sheep usually produce twin's lambs in one lambing but occasionally give triplet. It was thought that the twinning rate in other sheep breed might be linked to known and unknown fecundity genes (Table 1). Therefore, we have investigated the females of four local sheep breeds in the

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Table 1: Mean number of litter records and the mean litter size of various sheep breeds

Breed	Code	No. of individual	Location	Mean no. lambings (±SE)	Mean litter size (±SEM)
Small tailed Han	STH	154	Jiaxiang	3.7 (±0.60)	2.90 (±0.10)
Big tailed Han	BTH	20	Linqing	2.6 (±0.32)	1.90 (±0.10)
Shandi	SD	35	Sishui	3.5 (±0.32)	2.21 (±0.10)
Wadi	WD	30	Binzhou	3.1 (±0.32)	2.49 (±0.10)

Table 2: Conditions of PCR amplification of the Fec^B, $FecX^G$ and $FecG^H$ mutation

Genes	Mutation	Product size (bp)	Primers	Annealing condition	References
BMP-15	$FecX^G$	141	5'-GTCGCTATGGGGAAGTTTGGATG-3' 5'-CAAGATGTTTTCATGCCTCATCAACACGGTC-3'	62.5°C (32 cycles for 30 sec)	Hanrahan *et al.* (2004)
GDF-9	$FecG^H$	139	5'-ATGGATGATGTTCTGCACCATGGTGTGAACCTGA-3' 5'-CTTTAGTCAGCTGAAGTGGGACAAC -3'	62°C (32 cycles for 30 sec)	Hanrahan *et al.* (2004)
BMPRIB	Fec^B	140	5'-CACTGTCTTCTTGTTACTGTATTTCAATGAGAC-3' 5'-GATGCAATACTGCCTGCTTG-3'	60°C (32 cycles for 30 sec)	Wilson *et al.* (2001)

lower Yellow river valley for the presence of Fec^B, $FecX^G$, $FecG^H$ mutations and their relation with twinning if any.

MATERIALS AND METHODS

Sheep breeds and DNA extraction: A total of 239 blood samples were collected from the jugular vein using ACD as anticoagulant by a random sampling from 4 local sheep breeds. The collected samples were transported to the laboratory at 4°C. DNA was extracted using modified salting out procedure (Miller *et al.*, 1988) and stored at -20°C till used in assay.

Detection of the Fec^B, $FecG^H$ and the $FecX^G$ mutations: The Fec^B, $FecG^H$ and the $FecX^G$ genotyping was carried out by forced RFLP-PCR technique using primers as shown in Table 2. The *Fec^B* gene was amplified a 140 bp band. After digestion with Ava II, the Fec^{BB} animals had a 110 bp band, the Fec^{B+} animals had 140 and 110 bp bands and the Fec^{++} animals had a 140 bp band. The *$FecG^H$* gene was amplified a 139 bp band. After digestion with DdeI, the $FecG^{++}$ animals had a 108 bp band, the $FecG^{B+}$ animals had 139 and 108 bp bands and the $FecG^{HH}$ animals had a 139 bp band.

The 141 bp product of $FecX^G$ mutation of the *BMP15* gene was amplified. The PCR product from noncarriers (wild type) had a Hinf I restriction site while carrier individuals lacked the Hinf I site. After digestion, wild type individuals ($FecX^{++}$) should have a 111 and 30 bp fragments, heterozygous individuals ($FecX^{G+}$) 141, 111 and 30 bp fragments and homozygous individuals ($FecX^{GG}$) an uncut 141 bp fragment. PCR reaction was performed in a 25 μL reaction volume containing 2.5 μL of thermophilic DNA polymerase 10×buffer (10 mM Tris-HCl (pH 9.0), 50 m M KCl and 0.1% Triton X-100), 200 uM of dNTPs, 25 mM $MgCl_2$, 10 pmol of each primer and 0.5 units of Taq DNA polymerase (Promega, USA). About 50-100 ng of genomic DNA was used as template. The PCR cycling parameters were optimized separately and the annealing conditions were showed in Table 2.

The PCR products were separated by horizontal submarine agarose gel (3.5%, free from DNAse and RNAse) electrophoresis in 1×TAE buffer at 80 V. The gel was stained with ethidium bromide solution (0.5 ug mL^{-1}) and maintained for 10 min in darkness and photographed using a molecular imager (Gel doc XR, Bio rad). The 5 uL PCR product was digested with enzyme (Table 1) at 37°C for 2 h and resolved on 3% agarose gel.

RESULTS AND DISCUSSION

In the present study, the Fec^B, $FecX^G$ and $FecG^H$ mutations were investigated in four sheep breed distribution in lower Yellow river valley. A total of 154 individuals of small tailed Han sheep were analyzed for the Fec^B mutation, out of which 83 were homozygous (Fec^{BB}), 62 heterozygous (Fec^{B+}) and 9 non-carriers (Fec^{++}). The Fec^B profile of Small tailed Han sheep is shown in gel photograph (Fig. 1). About 94.2% of small tailed Han sheep were found carrier for the Fec^B mutation and frequency of allele was about 0.74.

Thirty Wadi ewes which had at least one record of twin or triplets were genotyped, out of that 4 ewes were carriers (Fec^{B+}) and 26 ewes were non-carriers (Fec^{++}) for the Fec^B mutation. The mean litter size and the mean number of litter records of Wadi ewes were recorded as 2.49±0.10 and 3.1±0.32, respectively. The $FecX^G$ mutation was only found in small tailed Han sheep, frequencies of genotypes $FecX^{G+}$, $FecX^{++}$ and $FecX^{GG}$ were 0.70, 0.27 and 0.03, respectively (Table 3).

The $FecX^G$ profile of small tailed Han sheep is shown in gel photograph (Fig. 2). The $FecG^H$ mutation was analyzed in small tailed Han, Big Tailed Han, Shandi and Wadi sheep. All sheep were found non-carriers for the

Table 3: Gene and genotype frequencies for $FecX^G$ and Fec^B mutations in four domestic sheep breeds

		Fec^B					$FecX^G$				
Population	Sample size	BB	B+	++	B	+	GG	G+	++	G	+
STH	154	83 (0.54)	62 (0.40)	9 (0.06)	0.74	0.26	4 (0.03)	108 (0.70)	42 (0.27)	0.36	0.64
BTH	20	0 (0.00)	0 (0.00)	0 (0.00)	0.00	0.00	0 (0.00)	0 (0.00)	0 (0.00)	0.00	0.00
SD	35	0 (0.00)	0 (0.00)	0 (0.00)	0.00	0.00	0 (0.00)	0 (0.00)	0 (0.00)	0.00	0.00
WD	30	0 (0.00)	4 (0.13)	26 (0.87)	0.07	0.93	0 (0.00)	0 (0.00)	0 (0.00)	0.00	0.00

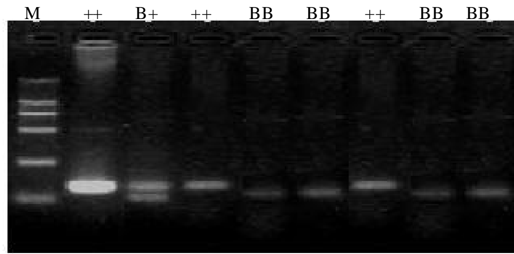

Fig. 1: Forced RFLP-PCR of the *BMPR-1B* gene. M: 100 bp molecular weight marker and lanes 1-9: individuals of small tailed Han and Wadi sheep produced twin or triplet lambs

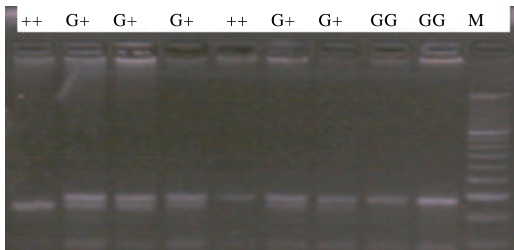

Fig. 2: Forced RFLP-PCR of the *BMP-15* gene. M: 50 bp molecular weight marker and lanes 1-9: individuals of small tailed Han sheep

$FecG^H$ mutation. The results indicated that the twinning/multiple lambing in these breeds is not linked with the $FecG^H$ mutation. The $FecG^H$ profile of small tailed Han sheep is shown in gel photograph.

In the present study, genotyping was carried out in four domestic sheep breeds from the lower Yellow river valley for identification of the Fec^B, $FecX^G$ and $FecG^H$ mutation of which 94.0% small tailed Han sheep and 13% Wadi sheep carried the Fec^B mutation, $FecX^G$ was only found in small tailed Han sheep and no $FecG^H$ mutation was detected. The discovery of Fec^B mutation in small tailed Han sheep which has large effect on litter size. It appears that Fec^B alleles segregating at high frequency in the small tailed Han sheep but the presence of the three genotypes suggests the gene is not fixed in this sheep however, larger samples are required to reliably determine the frequency of the allele. The results showed that the frequency of the Fec^B allele in small tailed Han sheep was about 0.73. Guan *et al*. (2007) reported a similar frequency of the Fec^B allele (0.74) in Hu sheep. Thus, it can be stated that both breeds are similar in their frequency of the Booroola mutation. The Fec^B allele in small tailed Han sheep and Wadi sheep is segregating in a similar way as reported in the Booroola Merino (Piper *et al*., 1985).The result indicated that the majority of small tailed Han sheep with at least one record of twin or triplets was carrier. Their lambing records were also in accordance with the assignment of Fec^B status.

The distribution of Wadi sheep is mainly concentrated in the villages of Binzhou district. The presence of Fec^B mutation in Wadi sheep can be explained by two hypotheses: Firstly, the Fec^B mutation in Wadi and small tailed Han sheep may be a separate event. Secondly, the Fec^B allele of small tailed Han sheep might travel in the local sheep due to out crossing and the farmers could develop this sheep as Wadi. Ren *et al*. (2008) suggested that the presence of some heterozygous individual of small tailed Han sheep might be due to result of out crossing of two sheep breeds. However, some Wadi sheep that produced twins proved to be non-carrier of the Fec^B mutation.

The discovery of $FecX^G$ mutation in the small tailed Han sheep is agreement with Chu *et al*. (2007) which indicated that small tailed Han sheep had the same $FecX^G$ mutation of the *BMP-15* gene as Belclare and Cambridge ewes. Hanrahan *et al*. (2004) reported that $FecX^{GG}$ ewes were sterile. Ewes of this genotype were not detected in 188 small tailed Han sheep ewes (Chu *et al*., 2007) whereas, 12 GG ewes were detected in 129 Cambridge ewes (Hanrahan *et al*., 2004). The current study, 5 $FecX^{GG}$ were detected among the 154 small tailed Han ewes. The results showed that $FecX^{GG}$ were indeed exist in the small tailed Han breed. Chu *et al*. (2002) did not detected the $FecX^{GG}$ maybe the method used to select ewes (only ewes with litter records were used) excluded all infertile $FecX^{GG}$ ewes. These sheep breeds belong to subspecies of Mongolian sheep and mainly distributed the Yellow river valley. Therefore, it was thought that big tailed Han, Shandi and Wadi sheep are prolific and might have a chance of $FecX^G$ and Fec^B mutation because these four sheep and may have some similarities. The present study indicated that the high litter size or twinning rate in big tailed Han and Shandi sheep is not associated with the

$FecX^G$ and Fec^B mutations while multiple births in small tailed Han and Wadi sheep is due to the effect of the Fec^B mutation. Results are in agreement with the findings of Davis *et al.* (2006) that Mountain sheep (brown), Tyrolian Mountain sheep, German white head mutton and Mountain sheep (white) with a litter size of 1.6-1.8, without carrying the $FecX^G$ and Fec^B mutations. None of these breeds were found carrier for $FecG^H$ mutation.

CONCLUSION

In the present study, the Fec^B mutation was identified in the small tailed Han sheep and Wadi sheep of lower Yellow river valley of China. None of sheep breeds carried the $FecG^H$ mutation. The Wadi sheep has been reported as the 2nd prolific sheep of in Shandong province after the small tailed Han sheep. High frequency of the Fec^B and $FecX^G$ mutation in small tailed Han sheep revealed that the mutation has arisen many generations ago but the gene is not fixed in the population. The discovery of the Fec^B mutation in Wadi sheep suggests that either this mutation may be a separate event or it might be introduced by small tailed Han sheep. The discovery of the $FecX^G$ and Fec^B mutation in small tailed Han sheep and the Fec^B in Wadi sheep will allow breeding strategies to be adopted for improving the prolificacy of non-prolific sheep breeds. The small tailed Han sheep would be a better choice since the body weight of this breed is higher than the Wadi sheep. Research efforts should be under taken to conserve and exploit the valuable germplasm of small tailed Han sheep in improving the prolificacy of non-prolific sheep breeds of China in the future. Ongoing investigations into the basis of the prolific phenotype of these four sheep ewes from the Yellow river valley are likely to reveal further insights into the events controlling follicle and oocyte development.

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REFERENCES

Chu, M.X., J.Z. Wang, A.G. Wang, N. Li and J.L. Fu, 2002. Genetic polymorphisms of five microsatellite loci in small tail Han sheep. Acta. Genetic. Sinic., 29: 502-506.

Chu, M.X., Z.H. Liu, C.L. Giao, Y.Q. He and L. Fang *et al.*, 2007. Mutations in BMPR-IB and BMP-15 genes are associated with litter size in small tailed han sheep *Ovis aries*. J. Anim. Sci., 85: 598-603.

Davis, G.H., L. Balakrishnan, I.K. Ross, T. Wilson and S.M. Galloway *et al.*, 2006. Investigation of the Booroola (*FecB*) and Inverdale (*FecXI*) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Anim. Reprod. Sci., 92: 87-96.

Du, L.X. and D.G. Cao, 2003. Studies on microsatellite and RAPD markers in little-tailed Han sheep. Acta Genetica. Sinica., 30: 1041-1044.

Galloway, S.M., K.P. McNatty, L.M. Cambridge, M.P. Laitinen and J.L. Juengel *et al.*, 2000. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nature Genet., 25: 279-283.

Guan, F., L. Shou-Ren, G.Q. Shi and L.G. Yang, 2007. Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. Anim. Reprod. Sci., 99: 44-52.

Hanrahan, J.P., S.M. Gregan, P. Mulsant, M. Mullen, G.H. Davis, R. Powell and S.M. Galloway, 2004. 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., 70: 900-909.

Miller, S.A., D.D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res., 16: 1215-1215.

Piper, L.R., B.M. Bindon and G.H. Davis, 1985. The Single Gene Inheritance of the High Litter Size of the Booroola Merino. In: Genetics of Reproduction in Sheep, Land, R.B. and D.W. Robinson (Eds.). Butterworths, London, pp: 115-125.

Ren, Y.L., J.S. Liu, Y. Guan, J.W. Xie, Z.Q. Shen and J. Wu, 2008. Feeding and management tehnology of meat multiparity Wadi Sheep. J. Jilin Agric. Sci., 33: 47-49.

Tu, Y.R., 1989. The Sheep and Goat Breeds in China. Shanghai Science and Technology Press, Shanghai.

Wang, J. and W. Yue, 2008. Genetic relationships of domestic sheep and goats in the lower reaches of the Yellow River based on microsatellite analysis. Biodiversity Sci., 16: 53-62.

Wilson, T., X.Y. Wu, J.L. Juengel, I.K. Ross and J.M. Lumsden *et al.*, 2001. 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. Biol. Reprod., 64: 1225-1235.

Zhang, C.S., L.Y. Geng, H.W. Wan, J.T. Li and L.X. Du, 2005. Study on sequence variation of mitochondrial cytochrome b gene and phylogenetic relationshi of four native sheep breeds. Acta Vet. Zootechnica Sinica, 36: 313-317.