RESEARCH NOTE

Increasing litter size in a sheep breed by marker-assisted selection of *BMPR1B* A746G mutation

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Introduction

The substitution A746G in the bone morphogenetic protein receptor-1B (*BMPR1B*) gene has been shown to be a causative mutation for ovine prolificacy, which enhances ovulation rate and litter size. To develop a new breed with improved prolificacy and meat production, 110 ewes from Small-tailed Han sheep (Han sheep) were selected and crossed with 18 Dorper sires, and the prolific B allele of *BMPR1B* A746G mutation was selected in the breeding programme using created restriction-site PCR method. B allele frequency was promoted remarkably in the close-breed population (1.000) compared to that in F_1 (0.432) and backcross (BC) population (0.265). The genotype effect on litter size presented significant difference ($BB > B+ > ++$) ($P < 0.01$). For different flocks, the litter size increased by selection of B alleles, and the average litter size of the close-breed population (1.76±0.03) was significantly greater than Dorper (1.16±0.06), F_1 (1.65±0.03) and backcross populations (1.37±0.04) ($P < 0.01$). The selective effect of the *BMPR1B* gene slightly acted on the growth trait of the 12-month weight, and the ++ genotype presented faster growth gain than *BB* or *B+* individuals ($P < 0.05$). These results indicated that the prolific B allele transmitted to hybrids by crossing with Han sheep, and the litter size was promoted in the developing sheep breed by marker-assisted selection (MAS).

The prolificacy and growth are equally important economic traits in sheep breeding and meat production, which breeders desire to integrated into a breed with high efficiency. The *BMPR1B* gene, mapped to ovine chromosome 6, is reported as a causative gene for high prolificacy (Mulsant *et al*. 2001), and the B allele with G substitution (the opposite allele is + with A substitution) for the A746G mutation is shown to be strongly associated with the prolific phenotype of Booroola ewes (Fogarty 2009). The effect of *BMPR1B* gene on the litter size has been reported in widespread breeds and flocks, including Bonpala sheep (Roy *et al*. 2011), Han (Chu *et al.* 2003, 2007, 2011) and Hu sheep (Chu *et al.* 2011). MAS of the *BMPR1B* gene allows the selection pressure on traits and leads to the promotion of genetic gain, which can be used for incorporation of a major gene for prolificacy into a flock (Davis 2005).

In recent decades, a number of sheep breeds that have the merit of fast growth have been introduced into China. However, most of these breeds, such as Dorper and Suffolk, are not prolific, and most individuals carry ++ genotype of *BMPR1B* gene (Guan *et al.* 2007). Davis *et al.* (2006) reported that a majority of individuals of Han breed possessed the B allele, and litter sizes of *BB* ewes were greater 0.97 ($P < 0.05$) and 1.5 ($P < 0.01$) lambs than those of ++ individuals in the first and later parities, respectively (Liu *et al.* 2003). Han sheep is thus deemed as the desirable maternal parent in cross breeding utilizations.

Materials and methods

In the present study, the *BMPR1B* gene was used as the selection marker for promoting the litter size in a developing breed. The breeding programme was conducted by the combination of growth and prolificacy merits by crossbreeding method, in which Dorper was used as the paternal parent for the growth character, and Han sheep as the maternal

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parent for the prolificacy merit. The breeding programme was performed by cross-breeding method with MAS of the B allele of *BMPR1B* A746G mutation in Hebei Liansheng Mutton Sheep Breeding Farm (Zhuozhou, Hebei, China) from September 2006 to May 2013. In the beginning of the breeding programme, 228 Han sheep (114 ewes and 114 rams) from a large number flock and 40 Dorper sheep (22 ewes and 18 rams) were selected to construct the experimental populations. Among the experimental populations, 110 Han ewes identified as carriers of B allele of the *BMPR1B* A746G mutation (genotypes with *BB* or *B*+) were selected as maternal subjects to be crossed with 18 Dorper rams who had ++ genotypes. In F_1 hybrids, 373 lambs (including 147 lambing ewes) were born from 2007 to 2008, and 87 ewes identified with the B allele (*B*+ genotype) were selected to be backcrossed with 18 Dorper rams. In BC hybrids, 206 lambs (Dorper × F_1) were born in two lambing cycles from May 2008 to December 2009, and 109 lambs were determined with B allele, which were selected after adult to mate with selected F_1 sheep (genotype *B*+) to produce the F_2 flock. The F_2 population included 309 lambs, in which only *BB* individuals were selected to enroll in the close-breed population. Till now, the close-breed population (the first to the third generation) reached 618 sheep (including 147 lambing ewes). The breeding programme is illustrated in figure 1.

During the experimental process, all animals were kept on dietary-based grain and name of one kind pasture under intensive management system. The litter size and 12-month weight were measured when sheep grew to adulthood. In this study, litter size is defined as the number of lambs produced at one birth by a single ewe. For breeding experiment all sheep were ear tagged and maintained under standard farm management practices, which is according to the guidelines of the experimental animal management of China Agricultural University. The current study was approved by the Experimental Animal Care and Use Committee of CAU.

Blood samples were collected from each experimental sheep in EDTA vacutainers and preserved at −20°C until DNA extraction. Genomic DNA was isolated using the standard phenol – chloroform – isoamyl alcohol extraction method from frozen blood samples (Sambrook and Russell 2001).

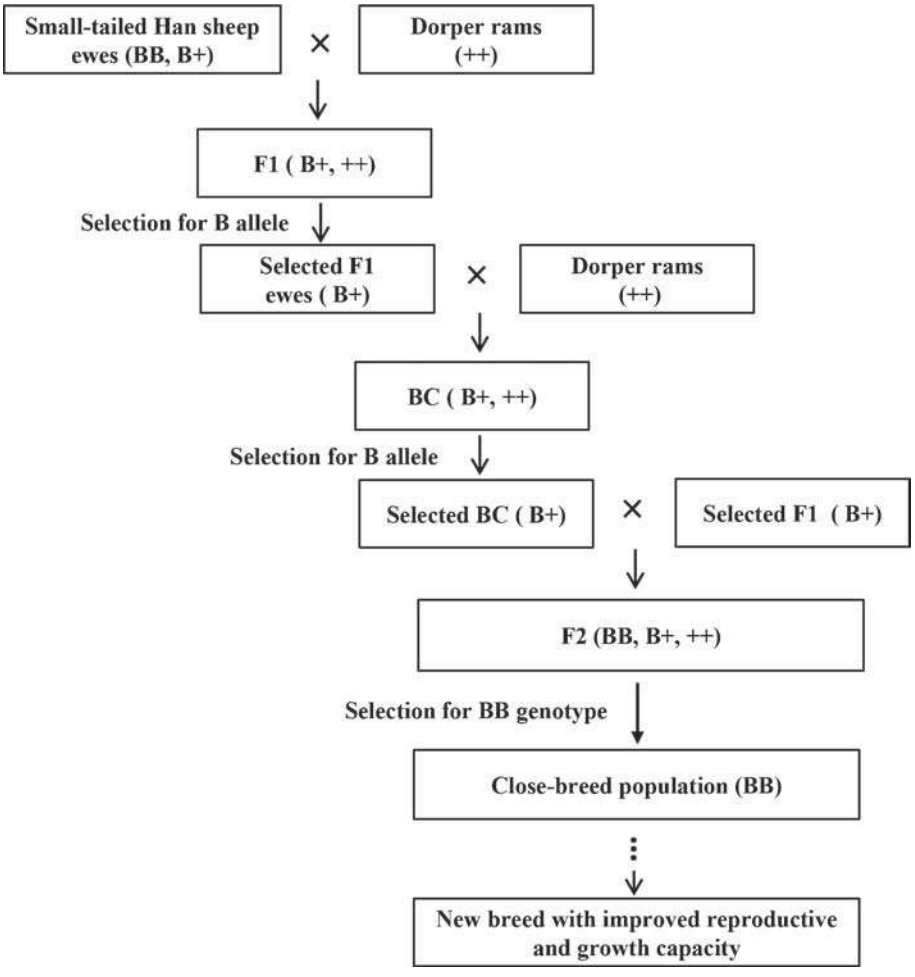

Figure 1. The breeding programme for MAS by *BMPR1B* A746G mutation in the developing breed of Dorper × –Han sheep. *BB*, *B*+ and ++ are genotypes of *BMPR1B* A746G in sheep.

Primers were designed based on the sequences described previously (Wilson *et al.* 2001). Genotyping of *BMPR1B* A746G mutation was carried out using created restriction-site PCR (CRS-PCR) method as described by Davis *et al.* (2006). The effect of genotype on mean litter size and 12-month weight in different flocks were analysed using fixed model: $y_{ijknm} = \mu + G_i + S_j + F_k + P_n + e_{ijknm}$. When this model is employed for analysis of mean litter size, S_j is excluded, y_{iknm} is the litter size, and when this model is employed for analysis of 12-month weight, P_n is not included, where y_{ijkm} is the 12-month weight, μ is the population mean. G_i is the genotype effect ($i = 1, 2, 3$); S_j is gender effect ($j = 1, 2$); F_k is the flock effect ($k = 1, 2, 3, 4, 5$); P_n is the parity effect; e_{ijknm} is the random residual effect of each observation. Analysis was performed using the general linear model procedure of SAS ver. 8.1 (SAS Institute, Cary, USA).

Results and discussion

Han sheep is prolific breed, and the allelic frequency of B allele reached 0.728, and the genotypic frequencies of *BB* and *B*+ added to 0.965. Dorper sheep is poor on reproductive performance, and all individuals presented ++ genotypes. In F_1 and BC hybrids, due to backcrossing with Dorper rams (++ genotype), the *BB* genotype disappeared, and the B allele frequency decreased from 0.432 in F_1 to 0.256 in BC. The *BB* genotype appeared in F_2, and the B allele frequency reached 0.508 (table 1). Our results indicated that the B allele was capable of being introduced into offspring by crossbreeds using *BB*/*B*+ Han sheep as maternal parent, and the litter size was rapidly promoted. This result is consistent with the previous studies (Kumar *et al.* 2006), the B allele frequency increased in hybrid sheep with the promotion of Han sheep bloodline proportion.

Through the selection of B allele, the litter size increased apparently in F_1 and BC flocks ($P < 0.01$) compared with the paternal parent Dorper, while for the maternal parent, Han sheep reached 2.24±0.03 in litter size, which was significantly greater than that of other flocks ($P < 0.01$). However, in spite of the allele frequency consistency, the litter size decreased in BC compared to that of F_1 ($P < 0.01$). In the close-breed population, all ewes were selected by *BB* genotype of *BMPR1B* gene, which eventually led to a high level of the litter size (1.76±0.03), which presented significantly greater than F_1, BC and the paternal parent Dorper flocks ($P < 0.01$). The results indicated that the litter size of offspring increased with the promotion of the bloodline proportion of Han sheep (table 2). For the genotype effect on litter size (all experimental ewes from different flocks were sorted by genotypes), the *BB* individuals reproduced the greatest numbers of lambs (2.13±0.03) amongst *B*+ and ++ewes ($P < 0.01$), and the *B*+ ewes (1.71±0.02) significantly outnumbered the ++ individuals (1.28±0.06) ($P < 0.01$). Our results demonstrated similar gene effects of *BMPR1B* gene on litter size in Han sheep and its crossbreeds, which demonstrated significant improvement with B allele. The litter size of the close-breed population was significantly greater than that of F_1 and BC. The reason was due to the genotype effect, were *BB* (the close-breed population) ewes produced more lambs than *B*+ or ++ individuals (in F_1 or BC populations). On the other hand, the B allele frequency of BC and F_1 was equal to 0.500, but the litter size of F_1 was significantly greater than BC. This contradiction may be explained assuming that *BMPR1B* gene is not the only factor which regulates the litter size, it involves several other factors such as correlative functional genes, maternal contributions and environmental effects (Davis 2004). In the present case, the Dorper bloodline proportion in BC (67.5%) is greater than F_1 (50%), and some Dorper specific genes may influence the litter size, which reduces the capacity of prolificacy.

For the growth trait of 12-month weight, Dorper sheep evidently exceeded all experimental flocks ($P < 0.01$), and Han sheep had the lowest value ($P < 0.01$). Sheep of the close-breed population and BC grew significantly faster than Han sheep and F_1 ($P < 0.01$), and there was no significant difference between close-breed sheep and BC ($P > 0.05$). For the genotype effect on growth trait of 12-month weight, there was no significant difference between *BB* (61.99 kg) and *B*+ (61.67 kg) individuals ($P > 0.05$), however, the ++ sheep (62.60 kg) were slightly greater than *BB* or *B*+ individuals ($P < 0.05$). The 12-month weight presented significant difference on *BMPR1B* genotypes, and the ++ sheep weighed significantly more than *BB* or *B*+ individuals ($P < 0.05$). Thus, the Dorper × Han hybrids weighed significantly than

Table 1. Genotypic and allelic frequencies of *BMPR1B* A746G in different sheep flocks.

Flock	Number	Genotypic frequency			Allelic frequency	
		BB	*B*+	++	*B*	+
Han sheep	228	0.491 (112/228)	0.474 (108/228)	0.035 (8/228)	0.728 (332/456)	0.272 (124/456)
Dorper sheep	40	0	0	1	0	1
F_1	373	0	0.863 (322/373)	0.137 (51/373)	0.432 (322/746)	0.568 (424/746)
BC	206	0	0.529 (109/206)	0.471 (97/206)	0.265 (109/412)	0.735 (303/412)
F_2	309	0.252 (78/309)	0.511 (158/309)	0. 236 (73/309)	0.508 (314/618)	0.492 (304/618)
The close-breed population	618	1.000 (618/618)	0	0	1.000 (1236/1236)	0

F_1, offspring of Han sheep crossed with Dorper rams; BC, backcrosses of F_1 with Dorper rams; F_2, hybrids of selected BC×F_1.

Table 2. Litter size and B allele frequency of *BMPR1B* in different flocks.

Flock	Number of lambing ewes	B allele frequency	Litter size ± SE*
Han sheep	110	0.755 (166/220)	2.24 ± 0.03^{A}
Dorper	22	0	1.16 ± 0.06^{B}
F_1	147	0.500 (147/294)	1.65 ± 0.03^{C}
BC	55	0.500 (55/110)	1.37 ± 0.04^{D}
The close-breed population	147	1.000 (294/294)	1.76 ± 0.03^{E}

*Within a column, means without a common superscript letter differ at $P < 0.01$; SE, standard error; F_1, offspring of Han sheep crossed with Dorper rams; BC, backcrosses of F_1 with Dorper rams

Han sheep ($P < 0.01$), and eventually the body weight of close-breed population increased when crossed with Dorper sheep.

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