Polymorphisms in GDF9 Gene and Its Relationship with Litter Size in Five Breeds of Black Goats

G.Q. Zhu, Q.I. Wang, Y.G. Kang, Y.Z. Lv and B.Y. Cao

INTRODUCTION

The ovulation rate and litter size are results of well regulated interactions of endocrine and paracrine mediators in mammals. Even though the tendency of twining and triplicate is common in both sheep and goats, how the litter size is precisely controlled remains a critical question in reproductive biology.

Growth differentiation factor 9 (GDF9) was first identified as an oocyte-derived growth factor required for ovarian somatic cell function (Dong et al. 1996), the FecG H mutation leads to the substitution of serine with phenylalanine at position 77 of the mature GDF9 peptide S77F, (Hanrahan et al. 2004). A close homolog of GDF9 was discovered later, namely, GDF9B (BMP15), and it is also expressed in the oocyte from the primary follicle stage continuing through ovulation (Dube et al. 1998). Heterozygous ewe carriers of the FecGHI (high fertility) allele exhibit one to two additional ovulations compared with non-carriers, whereas homozygous mutant ewes are sterile, and have small, flattened streak ovaries containing only follicles that do not develop up to the primary (type 2) stage (Hanrahan et al. 2004; Galloway et al. 2000; Mc Natty et al. 2005; Bodin et al. 2007).

The genetic basis of caprine prolificacy remains to be explored. Recent studies on prolific goat breeds like Boer, Haimen, Huanghuai, Nubi, Matou and Jining Grey suggested that genetic control of higher prolificacy in goats is
different from sheep (Chu et al. 2005; Chu et al. 2007; Hua et al. 2008; Hua and Yang 2009).

The known point mutations of BMPR1B (FecB) and BMP15 (FecXH, FecXJ, FecXG and FecXB) genes are monomorphic in the prolific goat breeds (Hua et al. 2008). Two new point mutations in the BMP15 gene linked with a higher prolificacy in the Jining Grey goat, therefore BMP15 gene was suggested to be the major gene that influences prolifically in this breed (Chu et al. 2007).

The black goats are indigenous species with different fecundities in China. The big foot (BF) black goat in Chongqing city and Jintang (JT) black goat in Sichuan province are of high fecundity, with average litter size of 2.89 and 2.46, respectively. The Hainan (HN) black goat in Hainan province is of middle fecundity, the average litter size is 1.75. The fecundity of Guizhou (GZ) black goat in Guizhou province and Taihang (TH) black goat in Henan province are low, the average litter sizes are 1.52 and 1.43, respectively, which provides a very good natural variation to study the reproductive differences between prolific and non-prolific black goats. The objective of this research is to analyze the polymorphisms of GDF9 gene and to investigate the relationship between different genotypes and litter sizes in these five breeds of black goats.

### MATERIALS AND METHODS

#### Experimental goat breeds and samples

A total of 384 adult female individuals from five breeds of black goats were examined in this study, including big foot black goats (BF, n=96), Jintang black goats (JT, n=81), Hainan black goats (HN, n=59), Guizhou black goats (GZ, n=58) and Taihang black goats (TH, n=90). Approximately 10 mL blood was collected aseptically from the jugular vein into sterile tubes which contained 0.5 mL EDTA (0.5 M) as anticoagulant reagent. All samples were taken back to the laboratory in icebox. The genomic DNA was extracted from white blood cells using standard phenol–chloroform extraction procedure (Sambrook and Russell, 2002).

The DNA samples were dissolved in TE buffer (pH 8.0) to a final concentration of 100 ng/µL, DNA samples were checked by Nano drop 2000 (thermo scientific) for purity and stored at −20 °C for use.

Information of primer sequences and PCR conditions based on the complete GDF9 gene coding sequence (GenBank accession number EF446168.2), four pairs of primers were designed to amplify partial intron, exon 2 and 3'-UTR of the GDF9 gene. The sequences of the four pairs of primers are listed in Table 1. The 12 µL PCR amplification system, containing 1.2 µL reaction buffer (10X), 50 ng genomic DNA, 10 pmol/L each primer, 1.2 µL dNTPs (0.25 mmol/L each), MgCl₂ (4.0 mmol/L or 3.0 mmol/L) and 0.5 U Taq DNA polymerase (MBI), was used. The cycling procedure is as follows: 4 min at 95 °C; 35 cycles of 94 °C 30 s; 61 °C 1 min and 72 °C 1.5 min; with a final extension at 72 °C for 10 min. Polymorphisms were detected by 12% PAGE (82 mm×82 mm×1.0 mm) using constant voltage (200 V) for 2.5 h. The gel was stained with 1% silver nitrate exactly as described by (Bi et al. 2007).

#### Statistical analysis

The average heterozygosity (He) and polymorphism information content (PIC) of GDF9 gene in the five breeds were analyzed by the cluster analysis software (poultry institute of the Chinese academy of agricultural sciences).

The relationship between GDF9 genotypes and litter size in five breeds of black goats were analyzed using GLM (general linear model) method, which was performed by SPSS software (version 13.0). Linear model was:

\[ Y_{ij} = \mu + M_i + e_{ij} \]

Where:

- \( Y_{ij} \): the litter size trait measured on each of the \( ij \)th animal.
- \( \mu \): the overall population means.
- \( M_i \): the fixed effects associated with \( i \)th genotype.
- \( e_{ij} \): the random error.
Zhu et al.

RESULTS AND DISCUSSION

Analysis of GDF9 gene by PCR-SSCP

SSCP analysis showed that only primer pair P1 detected polymorphisms in two out of the five breeds with three genotypes as AA, AB and BB (Figure 1). Other three primer pairs P2, P3 and P4 did not detect polymorphism in all the five breeds of black goats.

![Figure 1](image1.png)

**Figure 1** PCR-SSCP patterns of GDF9 by the primer P1 in 5 breeds black goats

A: big foot black goat; B: Jintang black goat; C: Hainan black goat; D: Guizhou black goat and E: Taihang black goat

Different genotypes (AA, AB and BB) from different individuals are labeled on top of each lane

Confirmation of polymorphisms by sequencing

To confirm the polymorphic results from PCR-SSCP of P1 primer pair and check the specific polymorphisms in DNA sequences, the polymorphic fragments were sequenced in both directions.

Part of the sequencing profiles of the three observed genotypes are shown in Figure 2. A single nucleotide mutation (G792→A) in exon 2 of GDF9 gene was detected, and this mutation resulted in an amino acid change: valine→isoleucine.

![Figure 2](image2.png)

**Figure 2** Partial sequencing maps of AA, AB and BB genotypes in black goats GDF9 gene amplification products by P1

BMP15 and BMP receptors have been detected in goat ovarian follicles at all stages (Silva et al. 2004). Polymorphisms of GDF9 of white goat in Guizhou province had been confirmed in previous investigations (Du et al. 2008; Lin et al. 2007). Studies have found that different genotypes have significant impact on the average litter size (P<0.05) in small tail Han sheep (Li et al. 2003; Chu et al. 2004; Yang et al. 2006). Jining Grey goats have 1.04 and 0.75 more average litter size with genotypes AA and AB, respectively, than that of genotype BB (P<0.01), and the average litter size of genotype AA is 0.29 (Table 3) more than that of AB (P<0.05) (Wu et al. 2005).

Table 3 Genetic structure of the GDF9 gene (P1) for BF and JT breeds

<table>
<thead>
<tr>
<th>Breed</th>
<th>Genotypes</th>
<th>Sample size</th>
<th>Allele frequencies</th>
<th>He</th>
<th>PIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF</td>
<td>AA (33)</td>
<td>33</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AB (17)</td>
<td>17</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BB (46)</td>
<td>46</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JT</td>
<td>AA (44)</td>
<td>44</td>
<td>0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AB (21)</td>
<td>21</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BB (16)</td>
<td>16</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Table 3](image3.png)

The means within the same column with at least one common letter, do not have significant difference (P>0.05). We have investigated the mutations in GDF9 gene of five breeds black goats and confirmed that the mutations of GDF9 were present in two black goat populations. Our re-
search found that mutation of GDF9 gene exists in two high fecundity breeds of five breeds black goats investigated, and this mutation (allele) contributes to the greater litter size of the two polymorphic breeds. GDF9 gene can be therefore considered as a candidate gene of litter size for selecting BF and JT black goats.

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**REFERENCES**


