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Prolificacy in Indian goat breeds is independent of FecB mutation

SONIKA AHLAWAT[1], REKHA SHARMA[2], A MAITRA[3], K N RAJA[4], N K VERMA[5] and M S TANTIA[6]

National Bureau of Animal Genetic Resources, Karnal, Haryana 132 001 India

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ABSTRACT

Genetic studies on sheep prolificacy have indicated that litter size and ovulation rate is determined by the action of single genes, named fecundity (Fec) genes. In the present study, an attempt was made to screen Indian goats for the presence or absence of FecB mutation in BMPRIB gene which has been established as a prolificacy associated marker in sheep. Blood samples were collected and DNA was extracted from 200 goats with history of high twinning rate for three consecutive parities belonging to six prolific breeds (Black Bengal, Beetal, Barbari, Malabari, Sikkim and Jakhrana) of India. These goats were genotyped for the presence of FecB mutation by forced restriction fragment length polymorphism PCR technique. The efficiency and accuracy of the PCR-RFLP assay was confirmed by DNA sequencing of representative samples of each breed. All the goats were found to be homozygous non-carriers indicating absence of FecB mutation. Complete concordance was observed between the results obtained by PCR-RFLP and direct DNA sequencing. These results suggest that fecundity of these prolific goat breeds is not linked to the same locus in BMPRIB gene as in some sheep breeds, and other polymorphism(s) or genes may be involved in governing the fecundity trait in caprines.

Key words: FecB, Indian goats, PCR-RFLP, Prolificacy

India is endowed with a wide diversity of goat genetic resources comprising of 23 well defined breeds of goats which form the backbone of its rural livelihood security systems. India ranks second in the world in goat population with more than 135 million goats which constitute 26.4% of country's total livestock (Livestock census 2012). Goats are amongst the major meat-producing animals in India, whose meat (chevon) is one of the most preferred meat with huge domestic demand. They provide nutritional security to the millions of marginal and small farmers and agricultural laborers. Kidding percentage is the most important factor influencing profitability in goats since a moderate increase in litter size can lead to large profit to the rural poor. Improvement of reproduction by traditional selective breeding methods has proved to be difficult due to low heritability (approximately 0.10) of the trait. Molecular genetics appears promising by supplying tools to analyze genetic variability directly at the DNA level with the possibility of detecting the individual genes influencing the reproductive characteristics.

Genetic studies on sheep prolificacy across continents have indicated that litter size and ovulation rate are determined by the action of single genes, named fecundity (Fec) genes, with major effects (Davis 2004). FecB was the first major gene for prolificacy identified in Australian Merino sheep. The Booroola gene (FecB) is an autosomal mutation identified on the basis of segregation studies on litter size (Piper *et al.* 1982) and ovulation rate (Davis *et al.* 1982). Single point mutation (A to G substitution) at base 746 (A746G) of the coding region of the BMPRIB (Bone morphogenetic protein receptor 1B) gene caused an amino acid change (Q249R) that was associated with the hyper prolific phenotype of Booroola ewes (Souza *et al.* 2001, Wilson *et al.* 2001). This mutation is located in the highly conserved kinase domain of the bone morphogenetic protein receptor IB, and is characterized by 'precocious' differentiation of ovarian follicles leading to the production of large numbers of ovulatory follicles that are smaller in diameter than wild-type follicles (Souza *et al.* 2003).

The FecB mutation has so far been identified in a number of sheep breeds globally, including the Booroola Merino (Australia), Garole (India), Javanese (Indonesia) (Davis *et al.* 2002), DuoLang (China) (Zhong *et al.* 2005), Small Tail Han (China), Hu (China) (Davis *et al.* 2006), Cele black (China) (Shi *et al.* 2010), Kendrapada (India) (Kumar *et al*. 2008), Nilagri (India) (Sudhakar *et al*. 2013) and Kalehkoohi (Iran) (Mahdavia *et al*. 2014) sheep. FecB mutation has been utilized in formulating breeding strategies to maximize the benefits of increased prolificacy in these breeds by introgressing this mutation in non-prolific breeds. Since the tendency of twining and triplets is inherited and possibly common in the two small ruminants (sheep and goat), the objective of this study was to screen prolific Indian

Present address: [1,4]Scientist (sonika.ahlawat@gmail.com, drknraja@yahoo.co.in), [2]Senior Scientist (rekvik@gmail.com), [3]Senior Research Fellow (maitra.avishek@gmail.com), [5,6]Principal Scientist (nkverma.497@gmail.com, tantiams @gmail.com).

goat breeds for FecB mutation so as to validate if the same mutation is responsible for prolificacy of goats also.

MATERIALS AND METHODS

Animal selection, sample collection and DNA isolation: Female goats (200) from 6 breeds viz. Black Bengal (130), Beetal (15), Barbari (15), Malabari (15), Sikkim (20) and Jakhrana (5) reared under farm conditions with history of high twinning rate for 3 consecutive parities were precisely selected. Approximately 5 ml of blood sample was collected aseptically from the jugular vein in EDTA containing vacutainers. Genomic DNA was extracted from white blood cells using standard phenol–chloroform extraction method (Sambrook and Russel 2001) and dissolved in TE buffer before storing at –20ºC for further use.

FecB genotyping: The FecB genotyping was carried by forced PCR-RFLP technique using primers CCAGAGGACAATAGCAAAGCAAA (Forward) and CAAGATGTTTTCATGCCTCATCAACACGGTC (Reverse) as described by Davis *et al.* (2002). The reverse primer has a deliberately introduced point mutation resulting in PCR products with FecB carrier sheep containing an *Ava*II restriction site (G|GACC), whereas products from non-carriers lack this site.

Polymerase chain reaction was carried out in 25 µL reaction volume with about 50–100 ng of genomic DNA using i-cycler (BioRAD, USA). The reaction mixture consisted of 200 µM each of dATP, dCTP, dGTP, dTTP, 1.5 mM $MgCl_2$, 50 pmol primer, 0.5 U *Taq* polymerase and corresponding *Taq* buffer. The amplification reaction conditions were as follows: initial denaturation at 95°C for 90 sec, followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec and final extension at 72°C for 5 min. The 190-base pair (bp) product was then digested using *Ava*II, and the resulting products were separated by electrophoresis on a 3.5% agarose gel and visualized with ethidium bromide. Booroola products digest to yield a 160-bp fragment, whereas non-carrier products remain uncut at 190 bp. The fragments were photographed under gel documentation unit and their sizes were estimated using a 100-bp DNA ladder and then the genotypes were recorded.

Validation of the PCR-RFLP assay: To evaluate the efficiency and accuracy of the PCR-RFLP assay, selected PCR-amplified DNA samples (n =2, respectively, for each breed) were examined by DNA sequencing. Sequence data were edited manually using Chromas Ver.2.33, (http//www.technelysium.com.au/chromas.html) and MegAlign program of LASERGENE software was used to screen Indian goats for FecB mutation. The results obtained by PCR-RFLP were compared with those determined by sequencing.

RESULTS AND DISCUSSION

Genomic DNA of 6 goat breeds was successfully amplified using primers designed by Davis *et al.* (2002) to yield specific amplified products which could be subjected to RFLP using restriction enzyme AvaII. A total of 200 individuals of 6 goat breeds were analyzed for the FecB mutation and all the goats were found to be homozygous non-carriers (Fig. 1).

Validation of the genotype obtained by PCR-RFLP methodology was done by direct sequencing of representative samples from each breed using the primers employed for PCR. We observed complete concordance between the methods. The genotypes scored from the assay were in 100% accordance with direct sequencing (Fig. 2).

Hyperprolificacy in sheep carrying the Booroola gene (FecB) is the result of a mutation in the BMPR-IB gene, which has provided a direct marker for the DNA mutation test for FecB. The FecB was the first major gene associated with prolificacy in Australian Merino sheep (Piper and Bindon, 1982; Davis *et al.* 1982). The effect of FecB gene is additive for ovulation rate and partially dominant for litter size where one copy increases ovulation rate and litter size by 1.3–1.6 and 0.9–1.2 respectively. Ovulation rate is increased by 2.7–3.0 and litter size by 1.1–1.7 in ewes with two copies of FecB (Piper *et al.* 1985). In the FecBB carrier ewes, Q249R substitution impairs the inhibitory effect of BMPR-IB on granulosa cell steroidogenesis resulting in advanced differentiation and maturation of follicles (Mulsant *et al.* 2001). The A746G mutation in the kinase domain of BMPR1B could result in an alteration in the

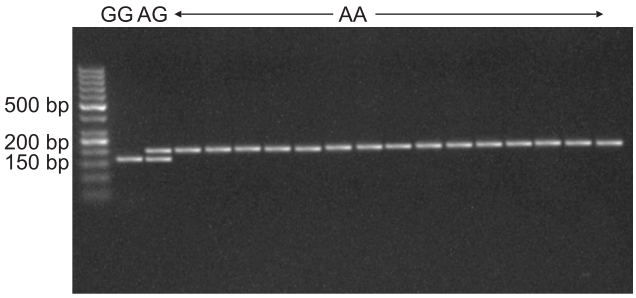

Fig. 1. Forced PCR-RFLP of BMPR-1B gene in Indian goats. Lane 1: 50 bp DNA ladder from GeneRuler; lane 2 and 3: GG and AG genotypes were seen in Kendrapada sheep which were taken as positive control; lanes 4–18: AA genotype (absence of FecB mutation) in prolific Indian goat breeds.

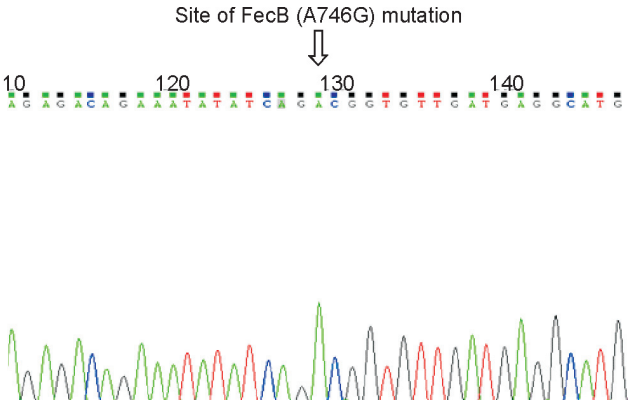

Fig. 2. Sequencing chromatogram showing absence of FecB mutation in investigated goat breeds.

expression and/or phosphorylation of SMADs which are intracellular proteins that transduce extracellular signals from transforming growth factor beta ligands to the nucleus where they activate downstream gene transcription. This eventually leads to phenotype characteristic of the Booroola animals which is the precocious development of a large number of small antral follicles resulting in increased ovulation rate (Souza *et al.* 2001).

The PCR-RFLP based test for FecB has been a useful tool for determining the genetic basis of high prolificacy in distantly related sheep breeds around the world. Sheep breeds across continents that have been found to possess FecB mutation are Booroola Merino (Australia), Javanese (Indonesia), DuoLang, Small Tail Han, Hu, Cele black (China), Garole, Kendrapada, Nilagri (India) and Kalehkoohi (Iran). Knowledge about the presence of FecB mutation in these prolific sheep breeds can pave way for development of breeding strategies that could maximize the benefits in their crosses.

Dr Helen Newton Turner in 1982 concluded that high prolificacy in Merino ewes came from either the Bengal or Cape sheep brought into Australia in the late 18th century from India. Since the tendency of twinning is common and inherited in both sheep and goats and also since the origin of this mutation has been traced to India, we were interested in screening the prolific goat breeds of India for the presence or absence of FecB mutation. However in our study, all the goats from 6 prolific breeds (Black Bengal, Beetal, Barbari, Malabari, Sikkim and Jakhrana) were found to be wild homozygote suggesting absence of FecB mutation. Absence of FecB mutation was reported by Chu *et al.* (2006) in 5 Chinese native goat breeds (Jining Grey, Anhui White, Wendeng Dairy, Liaoning Cashmere and Beijing native goats). These findings are also similar to the results of Hua *et al.* (2008) who found that the point mutation of BMPR1B (FecB) is monomorphic in the prolific goat breeds of China such as Boer, Haimen, Huanghuai, Nubi and Matou. Contrary to all these reports, Polley *et al.* (2009) discovered that the BMPR1B gene was polymorphic in Black Bengal goats with the predominance of heterozygous genotype AG by employing T-ARMS PCR technique to detect the mutation. However, despite screening a large number of Black Bengal goats (130) in our study, the BMPR1B mutation associated with prolificacy could not be detected.

He *et al.* (2010) reported absence of Q249R in prolific Chinese goat breeds which was also confirmed by Chu *et al.* (2010) in high prolificacy as well as low prolificacy goat breeds of China. Thai multi-breed meat goat population was also found to lack the FecB mutation (Supakorn and Pralomkarn 2010). Mohammadi and Alimahmoudi (2011) reported that FecB gene is not cause of prolificacy in Najdi and native goats of khouzestanian which have twinning percentage of 46 and 26% respectively. Gazooei *et al.* (2013) analyzed the FecB mutation by PCR-RFLP method in 110 randomly selected does of Rayini breed of Iran. All the individuals were found to be wild type homozygous for FecB. Another goat breed of Iran, i.e Khouzestan kamouri was also found to lack this mutation (Afzazadeh *et al*. 2013). Palai *et al.* (2013) studied FecB polymorphism in Raighar goat through T-ARMS PCR and reported the monomorphic status of these goats with respect to this gene. Four Egyptian goat breeds (Baladi, Zaraibi, Damascus and Alpine) have also been screened for the FecB mutation and like other goat breeds across the globe, they too are monomorphic for this mutation (Helal *et al*. 2014). Analysis of polymorphism for BMPR1B in Asom hill goat of India indicated that the genetic factor responsible for prolificacy or multiple kidding rates is not related to the reported mutated allele of *BMPR1B* gene (Datta *et al.* 2014). From the studies on Indian, Iranian, Chinese, Egyptian and Malaysian goat breeds, it can be concluded that there is no report of *FecB* gene being responsible for high prolificacy except one report in Black Bengal goats (Polley *et al.* 2009). However, in our study which involved much larger sample size (130) as compared to that investigated by Polley *et al.* (2009), Black Bengal goats were found to be monomorphic. These results suggest that fecundity of these prolific goat breeds is not linked to the same locus in BMPR-IB as in some sheep breeds, and other polymorphism(s) or genes may be involved in governing the fecundity trait in caprines. Thus, there is a dire need for a comprehensive screening of other mutations responsible for fecundity in reasonable sized samples (minimum 40–50 from each breed) in different goat breeds with the aim to identify some new variants and association with prolificacy trait in goats. Searching for other polymorphisms in other candidate genes for fecundity must be resorted to in order to develop marker assisted selection techniques and to study the genetic mechanism of prolificacy in goats.

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