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FECUNDITY GENE LINKED MICROSATELLITE MARKERS IN MALABARI GOATS

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Abstract : The DNA samples from 120 Malabari goats were utilized to study the polymorphism of ovine fecundity genes related microsatellite markers in goats. A total of 15 alleles with 167-195 bp (base pairs) and 8 alleles with 98-114 bp were observed for loci BM1329 and TGLA68 with polymorphic information content (PIC) of 0.8526 and 0.7823 respectively. The genotypes 175/185 of BM1329 and 104/106 of TGLA68 were found to be related to a higher litter size. For the allele 104 bp of TGLA68 highest frequency (0.4545) was observed in triplets followed by twins (0.2177) and singles (0.1847). The study indicates the possibilities of using these markers for selection for high prolificacy in Malabari goats.

Key words: fecundity gene, Booroola, Inverdale, microsatellite markers, Malabari goats,

Introduction

The goat occupies a special niche in the Indian agricultural production system as it utilises poor quality grass and crop residues. The Malabari goat is one of the most prolific breeds of goats in India. The majority of animals are white or a combination of white with other colours. Fast growth rate, high prolificacy, short kidding interval and early maturity are the important features of this breed. Genetic improvement of prolificacy, expressed as increased litter size has been difficult with traditional selection methods due to the low heritability of this trait. So identification of genetic markers that are linked to quantitative trait loci would be useful in selection. Because of the genome similarity in sheep and goat, the microsatellite markers associated with fecundity genes *viz*., Booroola and Inverdale in sheep were used to study the polymorphism and relationship of these markers with prolificacy in Malabari goats.

Materials and Methods

Isolation of genomic DNA: A total of 120 Malabari does maintained in the field area of All India Coordinated Research Project (AICRP) on Malabari goat improvement were utilized. The Malabari does with litter size of singles, twins, triplets and quadruplets in their second and subsequent parities were selected. DNA was extracted from five ml whole blood using the standard phenol chloroform extraction procedure with modifications. The purity of DNA was assessed by estimating the ratio between the optical densities at 260 nm and 280nm wavelengths.

Polymerase Chain Reaction (PCR): Working solutions of DNA samples were prepared to get a final concentration of 50 ng/µl. One µl of this working solution was used in every 10 µl PCR reaction. A total of five microsatellite markers were selected, in which three microsatellite markers *viz*., OarAE101, BMS2508 and BM1329 were linked to the Booroola (Fec^B) fecundity gene in sheep (*Montgomery et al., 1994; Lord et al., 1998*) and two microsatellite markers TGLA54 and TGLA68 were linked to the Inverdale ($FecX^I$) fecundity gene in sheep *(Davis et al., 1992)*. The primers for these markers were custom synthesised and typed for their polymorphism. The PCR conditions for each microsatellite locus were standardized separately.

The primers obtained in lyophilized form were reconstituted in sterile ultra pure distilled water to make a stock solution of 100 pM/µl concentration. The forward primer for each marker was radio-labeled at the 5′ end with γ^{32} P-ATP for visualizing PCR products by autoradiography. The reaction was carried out with the DNA End-labeling Kit1 (Bangalore Genei).

Agarose gel electrophoresis: The presence of PCR products were checked in 1.5 per cent agarose in 1X TAE buffer in horizontal electrophoresis unit. *HaeIII* digested pBR322 DNA was used as molecular size marker.

Sequencing M13 bacteriophage DNA: Single stranded M13 phage DNA was sequenced using the DNA Sequencing Kit Version 2.0 (Amersham Biosciences Corporation, USA) for determining the allele size of microsatellite markers by comparison.

Denaturing polyacrylamide gel electrophoresis and Autoradiography: Denaturing polyacrylamide gel electrophoresis was performed on a Vertical Sequencer (Consort, Belgium) using 6 per cent denaturing polacrylamide gel. The gel was cast between two glass plates and allowed to set for 30 minutes before electrophoresis. The PCR products were mixed with 3.5 µl formamide loading buffer, denatured at 95^{o}C for 5 minutes and cooled immediately on ice. About 3 µl each of this mixture was loaded into each well. Sequenced products of M13 DNA

which were also denatured at 94°C for 5 minutes were loaded simultaneously in the middle or side wells.

The gels were electrophoresed at 40 W for three hours maintaining a temperature of around 50° C. The bromophenol dye and Xylene cyanol dyes in the loading buffer acted as indicators of the mobility of DNA fragments and had the mobility equivalent to a 25 and 100 base fragments respectively. The gel was covered with klin film and dried in a gel drier at 80° C for one and a half hours. The klim film was removed after drying and the gel was set for autoradiography with X-ray film (Kodak, 35.6 x 43.2 cm) in a cassette (Kiran Hypercassette) fitted with an intensifying screen. The X-ray film was developed after 24 to 48 hours depending on the intensity of radioactive signal, by transferring the film serially into IX developer solution (Kodak) for three to five minutes, 1 per cent acetic acid for a minute followed by washing in distilled water and finally into fixer solution (Kodak) for six to ten minutes in dark room. The developed film was washed thoroughly in running water and air dried.

Microsatellite typing: The genotypes of animals were determined for each microsatellite loci by comparing the sizes of alleles with M13 sequencing ladder. The G, A, T and C sequences were read from the bottom to the top in order. The allele sizes were assigned corresponding to the G, A, T, C bands. The frequency at each locus was determined by direct counting.

Statistical Analyses: Chi- square test for proportions was conducted to test the significance of different allele frequencies and genotypic frequencies of microsatellite markers BM1329 and TGLA68 between different types of birth. Student't-test was used to compare the genotype mean of litter size with that of population mean. Heterozygosity was calculated by the method of *Ott (1992)* and the polymorphic information content (*Botstein et al., 1980*).

Results and Discussion

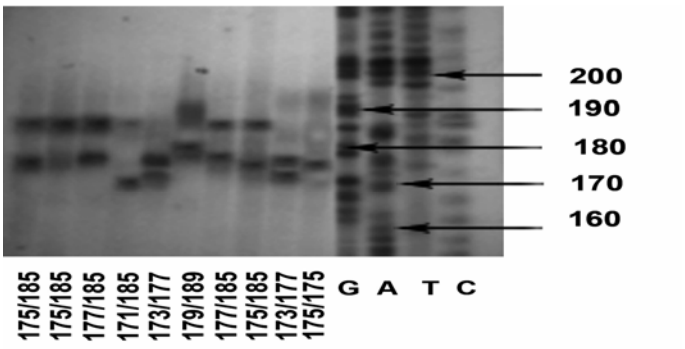
Incidence of multiple births: The overall incidence of multiple births in Malabari goats were 38.33, 51.67, 9.17 and 0.83 per cent for singles, twins, triplets and quadruplets respectively.

Yield and purity of DNA: The mean value of yield of DNA from Malabari goats in the study was found to be 357 ± 23.057 µg per five ml of blood. The average value of the ratio of optical density at 260 and 280 nm was 1.652 ± 0.017. This indicated the purity of DNA isolated.

Polymorphism of microsatellite markers linked to Booroola gene: Among the three microsatellite markers OarAE101, BMS2508 and BM1329 linked

to Booroola gene, the markers OarAE101 and BMS2508 were found to be monomorphic in Malabari goats.

The BM1329 locus was found to be highly polymorphic with 15 alleles ranging in size from 167-195 bp (Plate 1). The allele 177 bp was found in highest frequency and the alleles 167 bp and 193 bp were present in lowest frequency in the population. A significant difference in the alleles 181 bp and 191 bp ($P \leq 0.01$) and the alleles 179 bp and 185 bp ($P \leq 0.05$) for the locus BM1329 were found in different types of births. The highest frequency (0.3182) for the allele 177 bp was observed in triplets followed by twins (0.2419) and singles (0.2065). The frequency of the allele 179 bp was highest in singles followed by twins and triplets. A highest frequency of 0.1818 was recorded in triplets followed by singles and twins for 181 bp allele. For the 185 bp allele, the highest frequency of 0.1048 was recorded in twins followed by triplets and singles and for 191 bp allele, highest frequency was observed in singles followed by triplets and twins.



G A T C represents M13 sequence used as marker

Plate1. Autoradiograph showing polymorphism at BM1329 locus in Malabari goat

A total of 34 genotypes were detected at the BM1329 loci. The genotype 173/177 was present in highest frequency. The genotype 177/191 was found to be different in different types of births ($P \leq 0.01$) with the highest frequency (0.1957) was observed in singles followed by twins (0.0323) and absent in triplets.

The mean litter size of different BM1329 genotypes in Malabari goats are given in Table1. The litter sizes in genotypes 177/191 and 175/185 were significantly different when compared with average litter size. The genotype 175/185 was found to be significantly different with a higher litter size (2.091) when compared with the population average (1.733) in Malabari goats ($P \leq 0.01$).

The results indicated that the microsatellite locus BM1329 has a significant effect on litter size in Malabari goats.

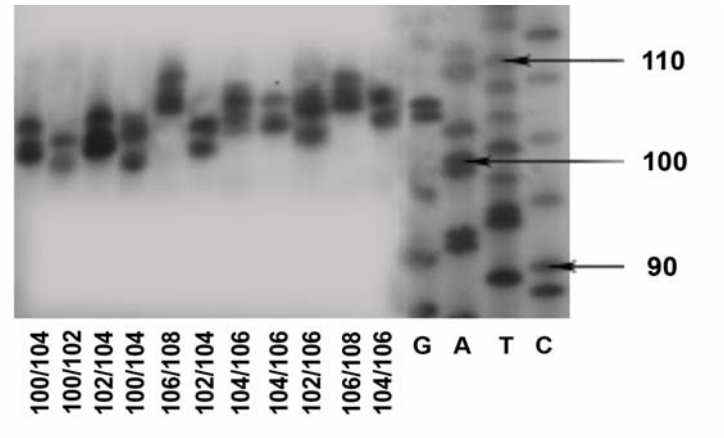
Table 1.The mean litter size of different BM1329 genotypes in Malabari goat

Sl.No.	Genotype	Litter size (mean ±SE)	Sl.No.	Genotype	Litter size (mean ±SE)
1	171/173	1.000	18	173/181	1.000
2	177/191	1.182± 0.1163**	19	189/195	1.000
3	177/185	1.333 ± 0.2722	20	181/191	1.670± 0.5443
4	173/177	1.880± 0.1210	21	177/189	2.200 ± 0.1789
5	173/179	1.460 ± 0.1759	22	175/185	2.091 ±0.087**
6	169/171	1.005 ± 0.3530	23	185/193	2.000
7	173/187	1.330± 0.2722	24	171/175	2.000
8	171/181	1.500 ± 0.3540	25	177/183	2.000
9	177/179	1.250± 0.2165	26	171/177	2.000
10	173/183	1.500 ± 0.3535	27	171/195	2.000
11	175/177	2.143 ± 0.2415	28	173/195	2.000
12	177/187	1.500	29	175/175	2.000
13	179/189	1.500	30	177/181	3.000
14	175/189	1.500	31	167/173	3.000
15	173/175	1.500	32	171/189	2.000
16	175/179	1.333 ± 0.1925	33	171/185	2.000
17	175/181	1.000	34	173/173	4.000

**P≤0.01 SE – Standard Error

Polymorphism of microsatellite markers linked to Inverdale gene: Among the two microsatellite markers TGLA54 and TGLA68 linked to Inverdale gene, the marker TGLA54 was found to be monomorphic in Malabari goats.

At the locus TGLA68 eight different alleles with a size range of 98-114 bp and 12 different genotypes could be detected (Plate 2). The frequency of allele 104 bp is significantly different in different types of birth with triplets having a frequency of 0.4545, followed by twins (0.2177) and singles (0.1848) (P≤0.01).



G A T C represents M13 sequence used as marker

Plate 2. Autoradiograph showing polymorphism at TGLA68 locus in Malabari goat

Genotypes and frequencies at the microsatellite locus TGLA68 in different types of kidding in Malabari goats are given in Table 2. The genotype 104/106 was found to be significantly different in different types of births ($P \leq 0.01$). The highest frequency (0.4545) was observed in triplets followed by singles (0.1087) and twins (0.0333).

No significant difference between litter sizes of different genotypes of the microsatellite marker TGLA68 could be detected when compared with the population average of 1.733 in Malabari goats ($P \geq 0.05$). The highest average litter size of 2 was observed for the genotypes 98/100, 104/106, 102/104, 106/114 and 104/114 and the lowest litter size average of 1.38 was observed for the genotype 102/106. The average litter size of the population under study was 1.733. *Chu et al. (2003)* reported an average litter size of 2.57 for the genotype 98/98. So there is chance that the animals with the allele 104 bp will produce high litter size.

Heterozygosity and polymorphic information content (PIC): The heterozygosity values for each locus were found out by the method suggested by *Ott (1992)* and the value indicates the usefulness of a marker. The values obtained were 0.8660 for BM1329 and 0.8024 for microsatellite marker TGLA68.

Polymorphic information content was calculated using the method of *Botstein et al. (1980)* and it indicates the level of information about a marker. The PIC values obtained were 0.8526 for BM1329 and 0.7823 for microsatellite marker TGLA68.

Table 2. Genotypes and frequencies at the microsatellite locus TGLA68 in different types of kidding in Malabari goats.

Sl. No	Genotype	Genotypic frequency in Malabari goats given birth to							
		Singles		Twins		Triplets		Quadruplets	
		Frequency	No.	Frequency	No.	Frequency	No.	Frequency	No.
1	102/106	0.108696	5	0.048387	3	0.00000	0	0.0000	0
2	100/104	0.130435	6	0.225806	14	0.272727	3	0.0000	0
3	106/108	0.326087	15	0.225806	14	0.090909	1	1.0000	1
4	104/108	0.086957	4	0.033333	4	0.00000	0	0.0000	0
5	104/106**	0.108696	5	0.033333	4	0.454545	5	0.0000	0
6	102/104	0.043478	2	0.033333	4	0.181818	2	0.0000	0
7	106/110	0.130435	6	0.129032	8	0.00000	0	0.0000	0
8	108/110	0.065217	3	0.080645	5	0.00000	0	0.0000	0
9	100/102	0.000000	0	0.032258	2	0.00000	0	0.0000	0
10	106/114	0.00000	0	0.032258	2	0.00000	0	0.0000	0
11	104/114	0.00000	0	0.016129	1	0.00000	0	0.0000	0
12	98/100	0.00000	0	0.016129	1	0.00000	0	0.0000	0
	Total	1.00000	46	1.000000	62	1.00000	11	1.0000	1

**P≤0.01 No. is the number of genotypes

Conclusion

Malabari goats are highly prolific breed of goat of northern Kerala. Selection for high prolificacy is not effective as it is very lowly heritable triat. Since there is high degree of homogeneity between ovine and caprine genome, an attempt is made to find out the relationship between microsatellite markers linked to ovine fecundity genes and prolificacy in Malabari goats. Microsatellite markers linked to Booroola gene *viz*., OarAE101, BMS2508 and BM1329 and those linked to the Inverdale gene *viz*., TGLA54 and TGLA68 in sheep were selected for the study. Among these only two markers BM1329 and TGLA68 were polymorphic in Malabari goats. The study has revealed that the genotype 175/185 of the microsatellite marker BM1329 and the allele 104 bp and genotype 104/106 of the marker TGLA68 were associated with significantly higher litter size. The animals with these genotypes can be selected for future breeding program for increased prolificacy in Malabari goats. The highest heterozygosity (He) and PIC values was observed for the marker BM1329 followed by TGLA68, indicates their suitability of theses markers for the future studies. The speed and efficiency of selection is expected to increase by use of these molecular markers in selection. Since prolificacy related molecular studies are meager in goats, the observations made in this study will provide a base for more research on this line, in the future.

Gen plodnosti povezan sa mikrosatelit markerima kod malabari koza

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Rezime

Uzorci DNK od 120 koza rase malabari su korišćeni u ispitivanju polimorfizma gena plodnosti kod ovaca i povezanosti sa mikrostelit markerima kod koza. Ukupno 15 alela sa 167-195 bp (baznih parova) i 8 alela sa 98-114 bp su utvrđeni na lokusu BM1329 i TGLA68 sa sadržajem polimorfnih informacija (PIC) od 0.8526 i 0.7823 respektivno. Utvrđeno je da genotipovi 175/185 od BM1329 i 104/106 od TGLA68 su povezani sa povećanjem veličine legla. Za alel 104 bp od TGLA68 najviša frekvencija(0.4545) je utvrđena kod trojki i dvojki (0.2177) i jedinaca (0.1847). Ovo istraživanje ukazuje na mogućnost korišćenja markera u selekciji na plodnost kod koza malabari rase.

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