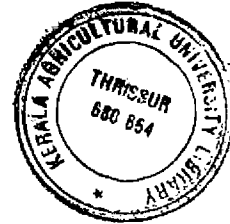# POLYMORPHISM OF OVINE FECUNDITY GENE LINKED MICROSATELLITE MARKERS IN MALABARI GOATS

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Thesis submitted in partial fulfillment of the
requirement for the degree of

Master of Veterinary Science

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2006

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DECLARATION

I hereby declare that this thesis, entitled "**POLYMORPHISM OF OVINE FECUNDITY GENE LINKED MICROSATELLITE MARKERS IN MALABARI GOATS**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.
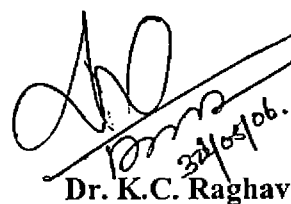
Mannuthy

31.08.2006

SEENA. T. X.

CERTIFICATE

Certified that this thesis, entitled **"POLYMORPHISM OF OVINE FECUNDITY GENE LINKED MICROSATELLITE MARKERS IN MALABARI GOATS"** is a record of research work done independently by **Dr. Seena. T. X,** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Mannuthy

31.08.2006

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We, the undersigned members of the Advisory Committee of **Dr. Seena. T. X,** a candidate for the degree of Master of Veterinary Science in Animal Breeding and Genetics, agree that this thesis entitled **"POLYMORPHISM OF OVINE FECUNDITY GENE LINKED MICROSATELLITE MARKERS IN MALABARI GOATS"** may be submitted by **Dr. Seena. T. X,** in partial fulfilment of the requirement for the degree.

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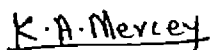College of Veterinary and Animal sciences,
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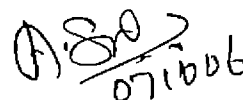Department of Animal Breeding
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Dedicated to

My Beloved Parents

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Introduction

1. INTRODUCTION

The goat (*Capra hircus*) was one of the earliest ruminants domesticated by humans. Today India ranks first in genetic resources and numerical superiority of goats. Goats contribute greatly to the subsistence of small holders and landless poor in our country. Goats play an important role in employment generation and in improving household nutrition of the rural poor. The goat occupies a special niche in the Indian agricultural production system and it is an efficient converter of otherwise unutilized poor quality grass and crop residues into meat and milk. Hence, profitable and sustainable goat production is a high developmental priority for India.

The Malabari goat is one of the most prolific breeds of goats in India and it was originated by crossing of Jamnapari, Surti and Arab goats with local goats. This is a dual-purpose breed found in Kozhikode, Kannur and Malappuram districts of northern Kerala. The majority of animals are black, white, brown or grey in colour. High prolificacy, short kidding interval and early maturity are the important features of the breed.

Prolificacy is a critical factor affecting the profitability of the goat industry. Though there has been improvement in growth and production performance due to modern breeding and management practices, enhancement in reproductive parameters was not considered as a priority. The prolificacy, expressed as litter size in goats is one of the most important economic traits. So the concept of breeding for increased fecundity has received a great deal of attention recently.

Genetic improvement for increased litter size has been difficult with traditional selection methods due to the low heritability of the traits involved. So

identification of quantitative trait loci (QTL) and genetic markers that are linked to these loci would be useful in choosing animals for selective breeding programmes.

Genetic markers can be used to identify specific regions of chromosomes where genes affecting quantitative traits are located. Finding molecular markers linked to QTL is the first step in marker assisted selection (MAS). MAS uses information about QTL in livestock selection programmes to identify individuals with favorable combination of QTLs. The detection of QTLs and their use in MAS could therefore enhance the selection responses.

A large number of genetic markers like RFLP, RAPD, and microsatellites are now available for molecular genetic studies. Among these, microsatellite markers are found to be more effective because of their abundance, even distribution, high polymorphism and high degree of heterozygosity with variable allele combinations. Microsatellites are the loci where short specific sequences of DNA or nucleotides are repeated in tandem arrays. They are the markers of choice in the search for linkage. These molecular markers can be used to identify specific regions of chromosomes where the genes affecting quantitative traits like high prolificacy, milk production *etc.* are located. This information can be used in breeding programmes to identify the individuals with favorable combination of QTL.

Different genes associated with high prolificacy have been discovered in sheep. Among these, Booroola and Inverdale are the fecundity genes with major effects. Booroola sheep is a highly prolific strain of Merino, initially developed in Australia by two commercial sheep breeders. But there are evidences to show that the Booroola gene was first discovered in Garole sheep, the only known prolific sheep breed in India. It is a native of Sunderban, the swampy delta region of the Ganga river in West Bengal. Later it was introgressed into the Australian Merino (Nimbkar *et al.,* 1998; Davis *et al.,* 2002). Booroola gene has been mapped on sheep chromosome 6 (Montgomery *et al.,* 1994). Inverdale prolificacy gene was first

discovered in a prolific family of Romney sheep that had descended from a ewe in Invermay Agricultural Centre in New Zealand. The gene in homozygous condition increases ovulation rate and litter size, whereas in heterozygotes, it produces "streak" gonads. The gene is located in sheep chromosome X and is maternally imprinted (Davis *et al.*, 1991).

It· is reported that the sequence similarity in microsatellite flanking regions between ovine and caprine loci was 88 per cent (Weimann *et al.*, 2000) and the homology between sheep and goat genomes is more than 80 per cent. Because of the genome similarity in sheep and goat, the microsatellite markers associated with fecundity in sheep were used for analysing the association between these markers and litter size in goats. Knowledge regarding a positive association will help farmers in improving the prolificacy of goats and increase the net return from the farm. Hence the present study was conducted with the following objectives:

- To study polymorphism of microsatellite markers linked to Booroola (Fec^B) and Inverdale ($FecX^1$) genes in Malabari goats.

- To examine the association of these microsatellite loci with the prolificacy in Malabari goats.

Review of Literature

2. REVIEW OF LITERATURE

The highly prolific breed of Malabari is reported to have originated centuries ago by crossing of Jamnapari, Surti and Arab goats with local goats (Kaura, 1952). Detailed account of origin and distribution of this breed was given by Pattabiraman (1955). Mason (1969) in his classical work "A World dictionary of livestock breeds, types and varieties" described them as goats belonging to a mixed population of Arab Indian goats including Kutch cross and Tellechery. He used the name "West Coast" as a synonym for this breed.

The breed is generally white, white and black or white and brown in colour, other colours like black, brown or a combination of all these colours are also found. Majority of theses animals have short hairs at forequarters and on hindquarters. Curly hair is rare phenomenon in Malabari goats. Generally animals are long eared and horned with convex forehead. Only 12 per cent of animals have tassels and 6.24 per cent are bearded. The udder is generally round with funnel shaped teats (Anon., 2005).

According to Devendra and Burns (1970), prolificacy expressed as number of kids per birth, is one of the most important economic traits in goats. The high prolificacy of Malabari goats was reported as early as 1957 (Shanmugasundaram, 1957). Though a lot of literature is available about litter size in different Indian and exotic breeds of goats, the details on the effect of genetic markers on prolificacy, especially DNA markers are scanty. The review is presented under the following main heads.

1.   Prolificacy in different breeds of goats.

2.   Microsatellite markers

3.   Microsatellite markers linked to prolificacy genes.

2.1 PROLIFICACY IN DIFFERENT BREEDS OF GOATS

Indian breeds of goats are generally highly prolific. The prolificacy of different Indian breeds of goats is presented in Table 2.1. From the table it can be inferred that Malabari breed is one of the highly prolific breeds of India.

Singh (1973) reported that the frequency of twins increased with the increase in age of the doe.

Singh and Singh (1974) observed that the frequency of twinning increased with the advancement of age. The percentage of single births was higher in does below 36 months and percentage of twins births were higher in does above 54 months in Jamnapari goats.

Mittal (1981) reported a high incidence of twin births than single and triplet births in Barbari goat breed.

Singh (1985) observed an incidence of singles, twins, and triplets kidding were 54.9 per cent, 39.2 per cent and 5.9 per cent respectively in Saanen goats. The kids born as triplets had significantly lower birth weight than those born single or twins. There was a negative correlation of birth weight with litter size in Saanen kids.

Table 2.1 The prolificacy of different Indian breeds of goats

Sl. No.	Breed	Origin and distribution	Type of Birth in per cent			
			single	Twin	Triplet	Quadruplet
1	Barbari	Etawah, Agra, Mathura and Aligarh districts of U. P	25	65	10	0
2	Bengal	Eastern and North eastern India	22	54	20	2.7
3	Beetal	Punjab and Hariana	40.6	52.6	6.52	0.22
4	Chigu	Himachal Pradesh	99	1	0	0
5	Gaddi	Himachal Pradesh	85	15	0	0
6	Ganjam	Southern districts of Orissa	98.4	1.6	0	0
7	Gohilwadi	Bhavananagar, Amreli districts of Gujarat	84.2	15.8	0	0
8	Jamnapari	Agra , Mathura and Etawa districts of U.P	56.2	43	0.7	0
9	Jhakrana	Alwar districts of Rajasthan	57	41	2	0
10	Kutchi	Kutch district of Gujarat	84.1	15.2	0.7	0

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Table 2.1 (continued...) The prolificacy of different Indian breeds of goats

Sl. No.	Breed	Origin and distribution	Type of Birth in per cent			
			single	Twin	Triplet	Quadruplet
11	Kannaiadu	Ramanadhapuram and Thirunelveli districts of Tamil Nadu	90	10	0	0
12	Malabari	Calicut, Cannanore and Malapuram districts of Kerala	30	60	10	0
13	Marwari	Marwar region of Rajasthan	100	0	0	0
14	Mehsana	Mehsana, Gandhinagar and Ahamedabad districts of Gujarat	89.7	10.3	0	0
15	Osmanabadi	Osmanabad district of Maharashtra	70.5	29	0.5	0
16	Sangamneri	Poona and Ahmednagar districts of Maharashtra	69.5	30	0.5	0
17	Sirohi	Sirohi district of Rajasthan	91.5	8.5	0	0
18	Zalawadi	Surendranagar and Rajkot districts of Gujarat	82.1	17.9		0

(Source: Acharya, 1982)

Crepaldi *et al.* (1999) found that the prolificacy was affected by parity and herd-year factor. Kidding that took place in the beginning of the year had shown higher prolificacy and maximum prolificacy was attained late in fourth and fifth lactation in Alpine goats.

Faruque *et al.* (2002) reported that Black Bengal goats were highly prolific and reached maturity at an early age. The litter size for Black Bengal was expected to increase from second parity and in subsequent parities. They studied the effect of selection and crossbreeding in Black Bengal goats on various reproductive characteristics like litter size, age at first kidding *etc.* and found that the litter size was not affected significantly by genetic groups.

Raghavan *et al.* (2004) reported that the percentage of singlet was highest in the first parity (80 per cent) in Malabari goats. Percentage of twins was lowest in first parity which increased as parity advanced with a value of 70 per cent in fourth parity.

2.2 MICROSATELLITE MARKERS

Microsatellites were one of the best classes of molecular markers for studies of genetic processes in natural population. They can be used as genetic markers, based on the difference in the length of short tandem repeat sequences.

Microsatellites are one to six nucleotide repeats, interspersed throughout the genome. The mutation rate of microsatellites was thought to be high and there are often large numbers of alleles that vary in size at a single locus. A simple repeat family in human genome has been shown to reveal numerous polymorphisms (Ali *et al.*, 1986)

Weber and May (1989) reported that the copy or allele inherited from each parent needs to be identified in order to follow the inheritance or segregation of a locus or chromosome segment. DNA based methods provide genetic markers with sufficient variability in population to track the inheritance of all chromosome segments. Microsatellite markers remain the markers of choice for the construction of linkage maps because they are highly polymorphic and require small amount of DNA for each test.

Shiue *et al.* (1999) found that microsatellite based markers are more useful when compared to other DNA markers.

Microsatellites are found to be excellent genetic markers because of their high polymorphism and abundant distribution through out the genome (Chu *et al.*, 2003; Ihara *et al.*, 2004).

The microsatellite based genetic map is reported to be an excellent tool for linkage mapping of monogenic as well as polygenic traits of interest (Ihara *et al.*, 2004)

Williams (2005) reported that microsatellite loci typically contained five to twenty copies of a short sequence motif that is between 2 bp and 4 bp in length and is repeated in tandem. The number of repeat units varied between individuals resulting in a large number of alleles for a given locus.

2.2.1 Polymorphism of microsatellite loci

Schlotterer and Tautz (1992) showed the possibility of synthesizing all types of repetitious di- and tri- nucleotide motifs starting from short primers and a

polymerase *in vitro* and the rate of synthesis depended as a sequence specific slippage rate.

Bishop *et al.* (1994) found that the highly variable DNA segments or variable number of tandem repeats microsatellites (di-, tri-, tetra- nucleotide repeats) were extremely polymorphic and generally specific to closely related species.

Forbes *et al.* (1995) compared the allele sizes at eight $(AC)_n$ microsatellite loci in domestic sheep (*Ovis aries*) and wild rocky Mountain bighorn sheep (*Ovis canadensis canadensis*). Genetic variation, mean allele sizes and allele-size variances were higher in domestic sheep than in bighorn sheep.

Collins *et al.* (1998) reported that about 90 per cent of the sequence variants in human were due to single nucleotide polymorphisms (SNPs). They assembled a DNA polymorphism discovery resource, which was valuable for the discovery of human genetic variation.

Tomasco *et al.* (2002) observed that the allele frequencies differed from those reported in literature for most microsatellite loci analysed and opined that independent estimates were essential for different regions and races from the study conducted in Uruguayan sheep using microsatellite markers.

Tantia *et al.* (2004) found out population bottle necks in two goat populations using the data on microsatellite allele frequency by means of several statistical tests and stated that population bottle necks in both the populations were about 200 generations back.

Hanotte and Jianlin (2005) reported that DNA based polymorphism was the markers of choice for molecular- based surveys of genetic variation. Polymorphic DNA markers like autosomal microsatellites showing different patterns of Mendelian inheritances can be studied in nearly all-major livestock species.

2.2.2 Causes of polymorphism at microsatellite loci

Schlotterer and Tautz (1992) found that slippage during replication was the cause of the observed length polymorphism of simple sequence stretches between individuals of the population.

Forbes *et al.* (1995) opined that if microsatellite polymorphism was associated with repeat size and repeat size varied among taxa, a serious implication was that mutation rates at homologous loci might differ among taxa.

Pandey *et al.* (2003) suggested that the use of a mixture of highly variable microsatellites reduced the risk of over estimating genetic variability which occurs if only highly variable loci were used for genetic diversity analysis.

Hanotte and Jianlin (2005) assumed that the polymorphisms observed at the molecular markers are neutral and the variation in allele frequencies between populations will reflect the distribution of genetic diversity amongst populations.

2.2.3 Applications of microsatellite markers

Microsatellites markers were used for construction of genetic linkage maps in different species of animals *viz.*, in cattle (Bishop *et al.*, 1994; Ihara *et al.*, 2004), sheep (Crawford *et al.*, 1995; De Gortari *et al.*, 1998; Maddox *et al.*, 2001) goat (Schibler *et al.*, 1998) and deer (Slate *et al.*, 2002).

The first scientific journal report of detection of Quantitative Trait Loci (QTL) effects in dairy cattle with microsatellites was published by Ron *et al.* (1994).

Schibler *et al.* (1998) used microsatellite markers for constructing the comparative ruminant map to predict the number of rearrangements between ruminant and human genomes.

Shiue *et al.* (1999) found the application of microsatellite markers in constructing the synteny map of the horse genome.

Cocket *et al.* (2001) analysed the sheep genome by means of microsatellite markers and opined that analysis of traits unique to sheep such as Booroola fecundity had extended our understanding of the functional role and regulation of the genes beyond what was in mice and humans.

Chu *et al.* (2003) conducted the association analysis between microsatellite loci linked to the Fec^B gene in Small Tail Han sheep and found that there was significant association between the microsatellite loci located in 10cM region covering the fecundity gene and litter size in second parity.

Kotze *et al.* (2004) characterized the indigenous Kalahari Red goat breeds of South Africa by using four microsatellite multiplexes.

Mukesh *et al.* (2004) reported high polymorphism at 25 microsatellite loci typed in Sahiwal cattle and opined that the substantial amount of genetic variability within this breed could prove useful in planning future breeding strategies.

Christina *et al.* (2005) used thirty-one microsatellite markers covering 22 chromosomes for differentiation of southern and western European sheep breeds.

Takahashi *et al.* (2005) developed chicken linkage map based on microsatellite markers genotyped on Japanese Large Game and White Leghorn cross. They opined that the resulting linkage map would contribute efficient selection of Quantitative Trait Loci (QTL) in chicken resource populations.

2.2.4 Conservation of microsatellite loci among different species

Hamada *et al.* (1982) examined a huge number of stretches of dT- dG alternating sequence of variable length that has been shown to adopt the Z- DNA conformation in all eukaryotes genomes, indicating extraordinary evolutionary conservation by southern blotting and hybridization analysis.

Davis *et al.* (1991) reported that chromosomal locations for effects on ovulation rate in cattle were different from the location of genes influencing ovulation rate in sheep.

Bishop *et al.* (1994) found that the number of informative markers linked to the present bovine genetic map provided an initial frame work from which informative templates of markers would be selected in a concerted effect to identify economic trait loci, in any breed or breed cross.

Forbes *et al.* (1995) observed longer microsatellite repeats in the focal species than in related among such divergent taxa as sheep.

Ellegren *et al.* (1997) conducted a reciprocal study of repeat length at homologous loci in cattle and sheep using markers derived from the bovine as well as the ovine genome. In both cases the amplification products were longer in focal species and loci were also more polymorphic in the species from which they were originally clones. Thus the authors confirmed that microsatellites could evolve directionally and at different rates in closely related species.

De Gortari *et al.* (1998) reported that about 50 per cent of the bovine microsatellite primer pairs amplify informative markers in sheep and 80 per cent of

the linked microsatellites in the second- generation sheep map were bovine primer pairs.

Pang *et al.* (1999) reported that about 48 primer pairs for chicken microsatellite loci were tested in PCR amplification of Japanese quail genomic DNA and found that 23 per cent of primer sets designed for chicken could be useful in the analysis of the quail genome.

Van Hooft *et al.* (1999) used bovine autosomal microsatellite markers for population genetic studies on African buffalo and about 83 per cent of the markers were found polymorphic. They concluded that the most bovine microsatellite markers were applicable in buffalo populations.

Noor *et al.* (2001) suggested that extreme caution should be taken in using microsatellite sizes for phylogenetic analyses or to infer divergences between populations due to the extensive variation in flanking region length when alleles were sequenced in distantly related species and also due to the existence of different repeat arrays between the same primer binding sites in some species.

Slate *et al.* (2002) found that any region that was suspected to have poor marker coverage could be mapped rapidly by typing microsatellites of other markers from the homologous cattle or sheep regions. They also reported that a striking degree of concerned synteny between deer and human and cattle. The marker order was found to be highly conserved between cattle and deer.

Arora *et al.* (2003) evaluated heterologous microsatellite markers for genetic polymorphism and genetic distancing in indigenous buffalo populations and suggested that the heterologous markers could be used for population structure and phylogenic studies.

Pandey *et al.* (2003) used heterologous microsatellite markers for genetic diversity analysis in three Indian breeds of poultry.

Kim *et al.* (2004) tested cross-species amplification of 34 bovine microsatellite loci in Korean Goral and domestic goats and found that among the amplified products, 55.2 per cent were polymorphic in Korean Goral and 81.5 per cent in domestic goats.

Yang *et al.* (2004) observed that the existence of conservation of microsatellite markers between sheep and goat in five different microsatellite loci.

2.2.5 Microsatellite analysis

2.2.5.1 Isolation of genomic DNA

Common methods for the extraction of DNA from blood involve several phenol extraction steps (Blin and Stafford., 1976).

Jeanpeirre (1987) designed a rapid method for the purification of DNA from human blood, which involved the use of proteinase K for protein digestion, which works efficiently in guanidium hydrochloride solution, and then the DNA was precipitated with ethanol. These methods for the extraction of DNA have eliminated the phenol extraction steps.

Miller *et al.* (1988) developed a rapid, safe and inexpensive method for extraction of DNA from buffy coats of nucleated cells obtained from anticoagulated blood. It involved salting out of the cellular proteins by denaturation and precipitation with a saturated NaCl solution, after deproteinization with proteinase K. This method avoids the use of hazardous organic solvents like phenol and

isochloroform. The quantity of DNA obtained was comparable to those obtained from phenol chloroform extraction method.

John *et al.* (1990) developed a rapid procedure to isolate DNA from leucocytes by using the phenol-chloroform-isoamyl alcohol extraction and the DNA was recovered by ethanol precipitation. The DNA produced was of good quality and was suitable for PCR amplification.

Senthil *et al.* (1996) utilized a simple procedure called high salt method for isolation of high molecular weight genomic DNA from cattle blood for RFLP studies.

Aravindakshan *et al.* (1997) described the procedure of extraction of DNA from white blood cells using phenol: chloroform: isoamyl alcohol (25:24:1) extraction or by a single salting out procedure. The DNA was recovered by ethanol precipitation and was resuspended in 400 µl of the TE buffer. They also described a modified procedure for extraction of DNA from semen samples by phenol-chloroform- isoamyl alcohol extraction procedure.

Aravindakshan *et al.* (1998) compared the three methods like guanidine hydrochloride method, phenol- chloroform extraction method and high salt method for the extraction of DNA from cattle white blood cells and found that the phenol chloroform and high salt methods produced good yields of high molecular weight DNA and suitable for both conventional and PCR-RFLP studies, whereas the guanidine hydrochloride method failed to yield clean DNA and not suitable for PCR-RFLP studies.

Ali (2003) extracted DNA from venous blood samples mixed with EDTA as the anticoagulant using the phenol- chloroform extraction procedure. DNA was

precipitated with chilled ethanol in the presence of high content of salts. The pellets were washed with 70 per cent ethanol and dissolved in TE buffer.

Arora *et al.* (2003) isolated genomic DNA using standard laboratory protocol using proteinase K digestion followed by phenol/ chloroform/ isoamyl alcohol extraction. The DNA was precipitated with absolute ethanol and dissolved in TE buffer.

The DNA isolation method described by Pandey *et al.* (2003) from whole blood involved lysis of RBC and digestion of protein using proteinase K and precipitation of protein using phenol chloroform isoamyl alcohol. DNA was precipitated by gentle addition of 2.5 volumes ethanol and 250 μl of sodium acetate pH 5.2. The resulting DNA strands were spooled out and washed twice with ice cold 70 per cent ethanol to remove excess salts. The DNA was re- dissolved in 500-750 μl of TAE buffer pH 8.0.

Phenol-chloroform extraction protocol was used in the isolation of genomic DNA from whole blood samples by Kim *et al.* (2003), Mukesh *et al.* (2004), Tantia *et al.* (2004) and Jia *et al.* (2005).

Bhattacharya *et al.* (2004) used 0.5 ml of 0.5M EDTA as an anticoagulant for collection of blood from jugular vein.

Yang *et al.* (2004) used 0.5 per cent of EDTA as an anticoagulant to extract DNA.

Kolte *et al.* (2005) isolated DNA by standard proteinase K digestion followed by phenol chloroform extraction and ethanol precipitation.

2.2.5.2 Yield and purity of DNA

Apparao *et al.* (1994) reported that 250- 350 µg DNA per 15 ml of cattle blood by using conventional phenol- chloroform extraction method.

Senthil *et al.* (1996) reported that the average yield of DNA from 15 ml of blood was 615.55 ± 0.72 µg for high salt method and 444.58 ± 21.54 µg for phenol-chloroform method.

Aravindakshan *et al.* (1998) reported the mean yield of DNA from 10 ml of blood isolated by phenol chloroform and high salt methods as 394.50 µg and 344.25 µg respectively. The ratios of optical density at 260 nm and 280 nm were consistently between 1.75 and 1.90 indicating good deproteinisation.

Chitra (2002) and Mathew (2004) reported DNA yields of 231.097 ±11.65 µg and 350.056 ±10.048 µg respectively, from 5 ml of blood from Malabari goats.

Tantia *et al.* (2004) checked the purity of the DNA on 0.8 per cent agarose gel prepared in TAE buffer.

2.2.5.3 Polymerase chain reaction (PCR)

Mullis *et al.* (1986) found that Polymerase Chain Reaction (PCR) was a powerful DNA amplification technique which is currently used in a wide variety of molecular biological studies. They described the term PCR as the reciprocal interaction of two oligonucleotides and the DNA polymerase extension products whose synthesis they prime, when they are hybridized to different strands of DNA template in a relative orientation such that their extension products overlap. The

method consisted of repetitive cycles of denaturation, hybridization and polymerase extension.

Gyllensten and Erlich (1988) used unequal molar amounts of two amplification primers, in a single step to amplify a single copy gene by PCR and produced an excess of single stranded DNA of chosen strand.

Innis *et al.* (1988) modified the PCR condition for direct sequencing of asymmetric PCR products without intermediate purification by using Taq DNA polymerase.

Jayaraman *et al.* (1989) developed an alternate method for gene synthesis which involved a combination of a single step ligation of oligonucleotides and PCR amplification of this crude ligation mixture.

Lowe *et al.* (1990) designed a computer based algorithm which rapidly scans nucleic acid sequences to select all possible pairs of oligonucleiotides suitable for use as primers to direct efficient DNA amplification by PCR. This program facilitates the rapid selection of effective and specific primers from long gene sequences. This program also displayed information such as length and melting temperature of the amplified product, which may be of additional help in choosing primers.

Don *et al.* (1991) interpreted that the appearance of spurious small bands in the PCR product spectrum is due to mispriming of one or both of the oigonucleotide amplimers internal or external to the target template. The occurrence of spurious bands can be reduced by adjusting the $[Mg^{2+}]$ concentration or increasing the annealing temperature of the PCR.

Rose (1991) reported that from both conceptual and practical perspectives, the PCR represents a fundamental technology for genome mapping and sequencing.

Moreover, applications of PCR have provided efficient approaches for identifying, isolating, mapping and sequencing DNA.

Housman (1995) used polymerase chain reaction to measure variation in very short tandemly repeated sequences, only two, three, four base pairs long in human genome. In PCR, the binding sites of oligonucleotide primers were the fixed points that determined the ends of the DNA fragment.

Bradley and Hillis (1997) successfully employed PCR to examine polymorphisms within populations and the technique was not error free. *Invitro* recombination can also occur when amplifying alleles of single–gene loci in heterozygous individuals, and that recombination frequency can vary as a function of different polymerases in the extension step of PCR.

Ellegren *et al.* (1997) carried out PCR in 10 µl volume containing 100 ng genomic DNA, 25 nM of both forward and reverse primer, 1 unit of Taq DNA polymerase, 200 µM of each dNTP, 100 mM Tris-Cl (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, 0.1 per cent Triton X-100 and 0.01 per cent gelatin.

According to Schnell and Mendoza (1997) in PCR, the product formed after 'n' successive cycles, Tn, should ideally result in the geometric accumulation.

$$Tn = 2^n\ To$$

where To was the initial amount of target DNA.

Segev *et al.* (1998) carried out PCR reaction in a total volume of 10 µl containing 5-20 ng genomic DNA, 4 ng labeled primer and 20 ng unlabeled primer in 35 cycles consisting of denaturing 94^0C for 60 seconds, annealing at $55-60^0C$ for 60 seconds, and extension at 72^0C for 120 seconds.

Xu *et al.* (1999) performed PCR with the reaction mixture (25 µl) containing 100 ng DNA, 7.5 ng of reverse primer, 50ng of forward primer, 2 mM $MgCl_2$, 0.2 mM dNTPs and 2.5 units of Taq polymerase.

Polymerase chain reaction conditions for microsatellite analysis were standardized as 10 µl final volume of PCR mix containing 50 ng of template DNA, 1 µl of 10X PCR buffer (100 mM Tris- pH 8.3, 500 mM KCl), 1.25 -1.5 mM MgCl2, 200 mM dNTP, 5 pM of end labeled forward primer, 5 pM of reverse primer and 0.3 units of Taq DNA polymerase. (Anilkumar, 2003; Preethy, 2004; Jacob, 2005; Thomas, 2005; Uthaman, 2005).

Mukesh *et al.* (2004) carried out PCR in 25 µl reaction volume containing 1.5 mM $MgCl_2$, 200 mM dNTPs, 50ng of each primer approximately 100 ng of template DNA and 0.5 units of Taq DNA polymerase using TC 100 thermocycler. PCR products were analysed on ethidium bromide (1.5 µg/ml) stained 2 per cent agarose gel for amplification.

Mainguy *et al.* (2005) performed PCR in 10 µl containing 10 ng genomic DNA, 0.16 µM of each primer, 0.2 mM of each dNTP, 2.0 mM mgCL2 and 0.05 µl BioTaq DNA Polymerase in the manufactures buffer (final concentrations 16 mM $(NH_4)So_4$, 67 mM Tris- HCl, pH 8.8 , 0.01 per cent Tween-20).

2.2.5.4 M13 sequencing ladders

Sanger *et al.* (1977) developed a method for DNA sequencing called didoxy nucleotide method. In this method, nucleotide sequences in DNA are determined by making use of the 2', 3'- dideoxy and arabinonucleoside analogues of the normal deoxy nucleoside triphosphates, which act as a specific chain- terminating inhibitors

of DNA polymerase and they applied this technique to the DNA of bacteriophage ΦX174.

The sequencing of M13 mp10 DNA reaction were stopped by the addition of 2 μl of 95 per cent deionized formamide with 0.1 per cent bromophenol blue, 0.1 per cent xylene cyanol and 10 mM EDTA (pH 7.0) (Biggin *et al.*, 1983).

Tabor and Richardson (1987) described a chemically modified derivative of the DNA polymerase induced by phage T7 with properties ideal for DNA sequencing by the chain termination method. They used template DNA of vector M13 Mp 8 that contain a 2707 bp fragment of phage T7 DNA. This method increased electrophoretic band resolution and ensured uniform radioactive intensity of the bands in the gels.

Gyllensten and Erlich (1988) reported that the individual alleles of the single copy gene amplified by PCR in a heterozygote can be sequenced directly by using allele-specific oligonucleotides either in amplification reaction or as sequencing primers.

Innis *et al.* (1988) conducted DNA sequencing with *Thermus aquaticus* DNA polymerase by using the stranded M13 mp10 DNA as the template. The PCR reaction was stopped by adding 4 μl of 91 per cent formamide with 20 mM EDTA (pH 8.0) and 0.05 per cent each of xylene cyanol and bromophenol blue.

Rose (1991) used PCR based sequencing methods which allowed rapid large scale sequencing of DNA. By this most of the time spent for obtaining the original DNA clones containing gene of interest, sub-cloning and handling the DNA before the actual sequencing reaction could be saved.

Bishop *et al.* (1994) found that the allele size was approximate by comparison to M13 and Mp18 ssDNA sequencing ladders.

Hulme *et al.* (1994) reported that the allele sizes were determined by comparison with the amplification product of the sequenced ladders.

Ellegren *et al.* (1997) scored and sized the microsatellite alleles with reference to lanes of M13 / pUC 19 sequences and DNA standard.

2.2.5.5 End labeling of primers

Hamada *et al.* (1982) examined a huge number of stretches of dT- dG alternating sequence of variable length that has been shown to adopt the Z- DNA conformation in all eukaryotes genomes, indicating extraordinary evolutionary conservation by southern blotting and hybridization analysis. This was done using $[\gamma^{32}P]$ –labeled (dT-dG) poly (dC-dA) as a probe.

Tabor and Richardson (1987) carried out termination DNA sequencing by the chain termination method using 5'- ^{32}P labeled 17-mer primers annealed to the M13 DNA template.

Gyllensten and Erlich (1988) labeled oligonucleotides with $[\gamma^{32}P]$ dATP by using polynucleotide kinase in sequencing reactions.

Innis *et al.* (1988) 5'- end –labeled oligonucleotide primers (3×10^6cpm/ pmol) with $[\gamma^{32}P]$ ATP and T4 polynucleotide kinase.

Kaluz and Reid (1991) designed a novel rapid method for detection of PCR products. In this method, the internal oligonucleotide was labeled with $[\gamma^{32}P]$ dATP by polynucleotide kinase. This procedure eliminated the problem of generation of truncated products, which occurs if the labeled internal probe is added before the last few steps of the PCR.

Rose (1991) described an approach aiming at identifying polymorphism involving the incorporation of radioactivity into amplified PCR products, followed by denaturation and segregation.

Bishop *et al.* (1994) labeled PCR products radioisotopically by including 0.1μci [α-^{32}P] dATP and 3.0 μM dATP. End labeled primers were used when direct incorporation of the ^{32}P in to amplified products increased sub banding and hindered scoring.

Hulme *et al.* (1994) conducted PCR by direct incorporation of 0.7 μci of ^{32}P dCTP in to each 15 μl reaction mix.

End labeling of 5' end of the microsatellite primer with γ^{32}P-ATP and separation of PCR amplified products microsatellites on 6 per cent denaturing polyacrylamide gel for autoradiography was used by several workers (Usha, 1995; Anilkumar, 2003; Preethy, 2004).

PCR based microsatellite analysis in which one of the primers was end labeled with [γ^{32}P] dATP was employed by Ellegren *et al.* (1997).

Esposito *et al.* (1998) proposed two common methods for PCR labeling. They are either end labeling of primers before the reaction or incorporation of labeled nucleotide during PCR amplification. Microsatellite typing obtained using [α^{35}S] dATP as the label (3 μCi) yielded results comparable with those obtained in an overnight exposure using a lower amount (0.5 μCi) of [α ^{32}P] dATP.

Xu *et al.* (1999) reported that the reverse primer was labeled with γ^{32}P in 25 μl polymerase chain reaction mixture.

Slate *et al.* (2002) amplified microsatellites in a 96 well thermocycler and labeled by direct incorporation of $[\alpha^{32}P]$ dCTP were run out on a 6 per cent polycrylamide gel for approximately 2 hrs.

2.2.5.6 Polyacrylamide gel electrophoresis

Biggin *et al.* (1983) used gels containing 6 per cent acrylamide and 460 g of urea/liter, polymerized by addition of 1.5 µl of 25 per cent ammonium persulphate stock solution and 1.5 µl of N,N,N',N'- tetramethyl ethylene diamine per ml of gel mix. The gels were dried and saran wrap removed for subsequent autoradiography of S35- labeled products.

Tabor and Richardson (1987) carried out electrophoresis of the sequenced M13 DNA in a 8 per cent acrylamide gel containing 7 M urea and a buffer of 100 nM Tris borate (pH 8.3) and 1 mM EDTA.

Bishop *et al.* (1994) labeled PCR products radioisotopically by including 0.1 µci $[\alpha^{-32}P]$ dATP and 3.0 µM dATP. The amplified PCR products were diluted with equal volume of diluting buffer and resolved in 6 per cent polyacrylamide gels between 3 and 6hr (based on product size) at 40 V/cm. Gels were vacuum dried for 15 min in to 3 mm chromatography paper and exposed to X-ray film overnight.

Housman (1995) reported that the number of copies of the tandem repeated sequences determine the total length of the fragment that can be visualized by separating the products of the PCR on a polyacrylamide gel.

Ellegren *et al.* (1997) end labeled one of the primers with $[\gamma^{32}P]$ dATP using T4 polynucleotide kinase in microsatellite analysis. PCR products were

electrophoresed through 6 per cent denaturing polyacrylamide-sequencing gels and visualized by autoradiography.

Esposito *et al.* (1998) mixed PCR products with 1 volume of formaldehyde buffer and subjected to denaturing electrophoresis in 5 per cent polyacrylamide gels containing 8 mol/L urea. Gels were dried and autoradiographied for 1 to 4 days at room temperature.

Segev *et al.* (1998) conducted PCR based microsatellite analysis in which one primer from each pair was end labeled with T4 kinase and γ [^{32}P] ATP. PCR reaction was carried out in a total volume of 10 μl containing 5-20 ng genomic DNA, 4 ng labeled primer and 20 ng unlabeled primer in 35 cycles. After that the PCR products were resolved in 40 per cent formamide and 8.3 mol/L urea, 6 per cent polyacrylamide gels and the gels were dried and autoradiographied for 4-48 hrs at room temperature or at -70°c

Slate *et al.* (2002) amplified microsatellites and labeled by direct incorporation of [α^{32}P] dCTP and were run out on a 6 per cent polycrylamide gels for approximately 2 hr.

Chu *et al.* (2003) loaded PCR products to non- denatured polyacrylamide gel in 1X TBE at 170 volt for 15 to 20 hr.

The PCR products were resolved in 6 per cent urea polyacrylamide gels by Kim *et al.* (2003).

The PCR products or DNA bands were resolved in 6 per cent urea- PAGE and the gel was stained with silver nitrate (Pandey *et al.*, 2003; Mukesh *et al.*, 2004).

The DNA bands resolved in 12 per cent polyacrylamide gels were visualized with silver staining technique (Guo *et al.*, 2004; Jia *et al.*, 2005).

2.3 MICROSATELLITE MARKERS LINKED TO PROLIFICACY GENES

2.3.1 Microsatellite markers linked to Booroola gene

The Booroola sheep is a highly prolific strain of Merino, initially developed in Australia by two commercial sheep breeders, the Seears brothers of 'Booroola', New South Wales and later by the divisions of Animal Genetics and Animal Production of the Common Wealth Scientific and Industrial Research Organisation. Researchers in New Zealand and Australia confirmed the single gene inheritance of high prolificacy in Booroola sheep. The locus was named as Fec^B (Fec = fecundity, B = Booroola) by the Committee on Genetic Nomenclature of Sheep and Goats. The effect of the Fec^B gene is additive to ovulation rate and partially dominant to litter size (Davis *et al.*, 1982).

On an average the gene increases litter size by one or two extra lambs with one copy of the Fec^B mutation (Montgomery *et al.*, 1992) and located in sheep chromosome 6.

Association of microsatellite markers with fecundity gene Booroola has been documented by Chu *et al.* (2003).

Fabre *et al.* (2003) reported that the hyper prolific phenotype of Booroola ewes was due to the presence of Booroola gene (Fec^B).

Davis (2004) reported that Booroola and Inverdale are the major genes affecting ovulation rate in sheep.

2.3.1.1 OarAE101

Montgomery *et al.* (1993) found that microsatellite marker OarAE101 was linked to the fecundity gene with a maximum lod (likelihood of the odds) score of 17.33 at a distance of 13 cM.

Gootwine *et al.* (1998) described the use of OarAE101 in a marker assisted selection program for high prolificacy of the Fec^B carriers in the Booroola- Awassi crosses.

Nowak and Charon (2001) identified a total of six alleles from 97 bp to 119 bp in microsatellite OarAE101 and the marker of the Fec^B gene seemed to be an allele of 97 bp.

Chu *et al.* (2003) observed a total of nine alleles for the microsatellite marker OarAE101 with a size range of 97 to 135 bp. The heterozygosity observed was 0.363.

Sun *et al.* (2004) reported the allele sizes of the microsatellite marker OarAE101 ranged from 75 to 131 bp. The PIC value and heterozygosity obtained were 0.9138 and 0.9195 respectively, in goats.

Yang *et al.* (2004) reported the allele size of the microsatellite marker OarAE101 ranged from 93 to113 bp in Hu breed of sheep, 85-119 bp in Tag sheep breed and 75-131bp in Yantse River Delta White goat (YRD). The annealing temperature used was 57^0C and 1 μl of 25 mM/ml $MgCl_2$ was used for each 20 μl PCR reaction for the primer OarAE101.

2.3.1.2 BMS2508

Mulsant *et al.* (1998) reported that the closest flanking markers of the Fec^B gene where bovine microsatellite BMS2508 and caprine microsatellite LSCV043, which were situated about 2 cM on either side of the gene.

Mulsant *et al.* (2001) reported that BMS2508 was linked to the Fec^B gene at a distance of 2 cM.

Tomasco *et al.* (2002) evaluated the polymorphism of ten microsatellite marker loci in Uruguayan sheep flocks and reported an allele size of 88-116 bp for BMS2508.

Chu *et al.* (2003) observed a total of six alleles for the microsatellite marker BMS2508 with a size range of 93 to 115 bp. The heterozygosity observed was 0.642.

2.3.1.3 BM1329

Bishop *et al.* (1994) used the microsatellite marker BM1329 for constructing the genetic linkage map of cattle. They reported an annealing temperature of 58^0C and allele size ranged from 145 to 161 bp. The number of alleles detected was eight.

Gootwine *et al.* (1998) reported the use of microsatellite marker BM1329 for a marker assisted selection program for high prolificacy of the Fec^B carriers in the Booroola- Awassi crosses.

Lord *et al.* (1998) mapped the fecundity gene to a 10cM region between microsatellite markers BM1329 and OarAE101. Booroola Awassi cross-bred ewes and rams were identified as B-allele carriers using the BM1329 (Lord *et al.*, 1998;

Gootwine *et* al., 1998) and these markers were found to be informative in some of the Booroola Awassi families.

Nowak and Charon (2001) identified a total of five alleles from 162 to 174 bp at BM1329 locus and the marker of the Fec^B gene seemed to be an allele of 162bp.

Chu *et al.* (2003) observed a total of six alleles for the microsatellite marker BM1329 with a size range of 160 to 180 bp. The heterozygosity observed was 0.516.

Kim *et al.* (2003) used microsatellite marker BM1329 for the quantitative traits loci linkage mapping of Han Woo (Korean cattle) chromosome 6 for daily gain marbling score.

Martinez *et al.* (2004) used the microsatellite marker BM1329 for genetic characterization of the Blanca Andaluza goat and reported that the number of alleles was nine and heterozygosity of 0.8864 and PIC value of 0.80.

2.3.2 Microsatellite markers linked to Inverdale gene

Davis *et al.* (1991) identified Inverdale fecundity gene ($FecX^l$) in a prolific New Zealand sheep family descended from a Romney female that produced 33 lambs in 11 lambing and progeny testing suggested that this prolific gene was located on the X chromosome.

Davis *et al.* (1992a) found that one copy of the Inverdale gene increased litter size by 0.58, mean litter size of carrier ewes was 2.17 and 32 per cent of litters had three or more lambs.

Davis *et al.* (1992b) reported that the Inverdale gene increased ovulation rate in animals heterozygous for the gene but produced 'streak' ovaries in homozygous condition.

Mc Natty (1994) narrated the history of the discovery of Inverdale gene and stated that George Davis is the discoverer of Inverdale X-linked fecundity gene.

Davis *et al.* (2001) reported the existence of a novel gene for prolificacy located on the 'X' chromosome.

2.3.2.1 TGLA54

Galloway *et al.* (2000) used microsatellite marker TGLA54 which is in the 10 cM region covering the Inverdale gene.

Chu *et al.* (2003) observed a total of five alleles for the microsatellite marker TGLA54 with a size range of 116 to 136 bp. The heterozygosity observed was 0.267.

2.3.2.2 TGLA68

Chu *et al.* (2003) observed two alleles for the microsatellite marker TGLA68 with a size range of 98 and 100 bp. The heterozygosity observed was 0.497.

2.3.3 Heterozygosity and Polymorphic Information Content (PIC)

Ott (1992) calculated the heterozygosity as

$$H_e = 1 - \sum_{i=1}^{n} P_i^2$$

Where 'P$_i$' is frequency of allele 'i' in the locus, 'n' is the No. of alleles in the locus. The above formula for calculating heterozygosity of microsatellite markers was used by (Chu *et al.*, 2003).

Pandey *et al.* (2003) estimated genetic variation by calculating the observed/ direct amount and unbiased heterozygosity for microsatellite loci in three breeds of poultry.

The formula for calculating the polymorphic information content is given by Botstein *et al.* (1980) was as follows:

$$PIC = 1 - \left(\sum_{i=1}^{n} P_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 P_i^2 P_j^2$$

Here 'Pi' is the frequency of the ith allele and 'Pj' is the frequency of the jth allele. This formula for finding out PIC was used by several workers (Jacob, 2005; Thomas, 2005; Uthaman, 2005).

2.3.4 Prolificacy linked microsatellite markers in selection

Gootwine (1995) used DNA markers linked to the Booroola gene in selection of lambs carrying the gene.

Gootwine *et al.* (1998) described the use of microsatellite markers OarAE101 and BM1329 in a marker assisted selection program for high prolificacy of the Fec^B carriers in the Booroola- Awassi crosses and found that the speed and efficiency of selection was increased by use of molecular markers associated with the Fec^B gene in selecting the desired genotypes in the breeding program.

Microsatellite markers were shown to be suitable for identifying major genes for genetic improvement of livestock (Montaldo and Meza- Herrera, 1998).

Williams (2005) reported that the genetic markers for QTL that are linked to the trait gene could be used to choose animals for selective breeding programs. The most effective markers are functional mutations within the trait genes.

2.3.5 Association between seasonality and type of birth

Singh and Singh (1974) inferred that the month of kidding could not influence the frequency of single and twin birth based on the distribution of kidding.

Sharma (1985) reported that the oestrus activity and the conception rate during June-July in Jamnapari does under tropical conditions was 69 per cent and 57.62 per cent respectively, whereas 68.93 per cent and 56.33 per cent in October to November period. The multiple births were 31.8 per cent and 30 per cent in the two seasons respectively. He suggested that a slightly higher conception rate and multiple births were observed during June- July seasons than the October- November seasons.

Singh *et al.* (1987) reported an average litter size of 1.34, 1.33 and 1.35 in summer, monsoon and winter season respectively in Black Bengal goats.

Mishra (1991) reported that kidding was spread through out the year with the highest incidence of 20 per cent in the month of April in local goats of Sikkim.

Nandy *et al.* (2001) observed that the month of kidding could not influence the frequency of single and twin birth. Age of doe showed a marked effect on the type of birth.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

The Malabari goats are the prolific goat breed of southern India and they were selected as the subjects for the study. A total of 120 Malabari does maintained in the field area of All India Coordinated Research Project (AICRP) on Malabari goat improvement *viz.*, Tanur, Tellechery and Badagara regions of Kerala, were utilised. The number of animals from Tanur, Tellechery and Badagara regions were 30, 36 and 54 respectively. The Malabari does with litter size of singles, twins, triplets and quadruplets in their second and subsequent parities were used for the study of polymorphism of ovine fecundity gene linked microsatellite markers.

3.2. MICROSATELLITE ANALYSIS

3.2.1 Isolation of genomic DNA

3.2.1.1 Source of genomic DNA

Whole blood collected from Malabari goats were used as the source of genomic DNA.

3.2.1.2 Collection of blood samples

Five ml of blood sample was collected from the jugular vein aseptically using EDTA (1mg/ml of blood) as anticoagulant in 15 ml sterile disposable centrifuge tubes. The samples were brought in ice to the laboratory and stored at 4^0C until processing.

3.2.1.3. Isolation of DNA from whole blood

DNA was extracted from whole blood using the standard phenol chloroform extraction procedure with modifications. The procedure followed was

1. To the 5 ml blood, double the volume of ice-cold RBC lysis buffer (150 mM NH₄Cl, 10 mM KCl, 0.1 mM EDTA) was added, mixed well and kept in ice with occasional mixing for 10 minutes for complete lysis of red blood cells.

2. The leucocytes were pelleted by centrifuging at 3500 rpm for 15 min. and the supernatant containing lysed RBC's was discarded.

3. The pellets were resuspended in ice-cold RBC lysis buffer and the above two steps were repeated till a clear pellet without any unlysed erythrocytes were obtained.

4. The pellet was then washed twice with 10 ml of Tris buffered saline (TBS-140 mM NaCl₂, 0.5 mM KCl, 0.25 mM Tris) by vigorous vortexing followed by centrifugation at 3000 rpm for 10 minutes.

5. Resuspended the washed white cell pellet in 5 ml saline EDTA buffer (SE-75 mM NaCl, 35 mM EDTA) completely by vortexing so that no cell clumps remain. To this mixture 0.25 ml of 20 per cent SDS and 25 µl of Proteinse-K (20 mg/ml)and were added, mixed well and incubated at 50°C in water bath with occasional mixing for a minimum of three hours.

6. Cooled the digested samples to room temperature 300 µl of 5 M NaCl was added and mixed by vortexing. An equal volume of phenol (pH 7.8) saturated with Tris-HCl, was added, mixed thoroughly by inversion of the tubes for 10 minutes and centrifuged at 3500 rpm for 15 minutes.

7. The aqueous phase containing DNA was collected in fresh tubes, to which an equal volume of saturated phenol:chloroform:isoamyl alcohol (25:24:1) was added. The contents were mixed thoroughly by inversion for 10 minutes and centrifuged at 3500 rpm for 15 minutes.

8. Collected the aqueous phase in fresh tubes, equal volume of chloroform: isoamyl alcohol (24:1) was added, mixed for 10 minutes and centrifuged at 3500 rpm for 15 minutes.

9. The supernatant was transferred to a sterile 50 ml beaker and one tenth volume of 3 M Sodium acetate (pH 5.5) was added and mixed.

10. To this mixture, equal volume of chilled isopropyl alcohol was added and the precipitated DNA was spooled out using a fresh micropipette tip, washed in 70 per cent ethanol and air-dried.

11. Dried DNA was resuspended in 0.5 ml of Tris buffer (TE-10 mM Tris base, 0.1 mM EDTA) and stored at −20°C.

3.2.2 Estimation of yield and purity of DNA

Yield and purity of isolated DNA samples were estimated by using spectrophotometer (Jenway, UK). Twenty μl of the DNA stock solution was diluted to 2 ml with sterile distilled water. Optical densities (OD) were measured at 260 nm and 280 nm wavelengths for each diluted DNA sample. Yield and purity of DNA samples were estimated as follows.

3.2.2.1 Yield of DNA samples

An OD of 1 at 260 nm wavelength corresponds to approximately 50 μg/ml of double stranded DNA. Concentration of DNA stock solution was calculated as follows.

Concentration of DNA stock solution (μg/ml) = OD_{260} x Dilution factor x 50

The total yield of DNA obtained from blood was calculated by multiplying the concentration with the volume of DNA stock solution.

3.2.2.2 Purity of DNA samples

Purity of DNA was assessed by estimating the ratio between the readings at 260nm and 280nm wavelengths. The quality of the DNA sample was ensured electrophoretically on 0.8 per cent agarose gel in 1X Tris Acetate EDTA (TAE) buffer in horizontal submarine electrophoresis.

3.2.3.2 Selection of Primers

A total of five microsatellite markers were selected, in which three microsatellite markers viz., OarAE101, BMS2508 and BM1329 were linked to the Booroola fecundity gene in sheep and two microsatellite markers TGLA54 and TGLA68 were linked to the Inverdale fecundity gene in sheep. The primers for these markers were custom synthesised and typed for their polymorphism. The sequences of the forward and reverse primers for each locus were as follows.

Locus   Primer sequence (5'-3')

OarAE101

     F 5'-TTCTTATAGATGCACTCAAGCTAGG-3'
     R 5'-TAAGAAATATATTTGAAAAAACTGTATCTCCC-3'

BMS2508

     F 5'- TTTCTGGGATTACAAAATGCTC-3'
     R 5'-TTTCTTAGGGGAGTGTTGATTC-3'

BM1329

     F 5'-TTGTTTAGGCAAGTCCAAAGTC-3'
     R 5'- AACACCGCAGCTTCATCC-3'

TGLA54

     F 5'-CTCAATATTTTGCAATAACATATAAGG-3'
     R 5'-ACGATATCATGTTAGTTTCAGGTG-3'

TGLA68

     F 5'- ATCTTACTTACCTTCTCAGAGCT -3'
     R 5'- GGGACAAAATTTTACATATACACTT-3'

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 solutions were incubated at room temperature for one hour and then stored at -20°C. Working solutions of the primers were prepared by diluting the stock solution.

3.2.3.3 Incorporation of Radioactivity: End-Labeling of Primers

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). The procedure followed for end-labeling was as follows:

The following components were taken in a 0.2 ml microcentrifuge tube

10X Polynucleotide kinase (PNK) buffer	- 1 µl
Forward Primer (100 pM/µl)	- 2 µl
T$_4$ Polynucleotide kinase (10 U/µl)	- 0.5µl
γ^{32} P-ATP (10 mCi/ml)	- 1 µl
Nuclease free water	- 5.5µl

The mixture was spun and incubated at 37°C for 30 minutes and the final volume was made up to 40 µl with sterile ultra pure water. For every 10 µl reaction 1 µl of end labeled primer was used.

3.2.3.4 PCR conditions

The PCR conditions for each microsatellite loci were standardized separately. Each reaction was carried out in a final volume of 10 µl. PCR reaction was carried out in a PTC 200 thermal cycler (MJ Research, USA). Each reaction

consisted of 1μl of 10 X PCR buffer (15 mM $MgCl_2$, 100 mM Tris-p^H 8.3, 500 mM KCl), 200 μM dNTP, 1 μl of end-labeled and diluted forward primer, 5 pM of reverse primer and 0.3 U of Taq DNA polymerase. Concentration of $MgCl_2$ used was 1.5 mM for all the primers selected. The reaction mixture was mixed well and subjected to PCR. The samples were then cooled down to 4°C and stored at -20°C till further analysis. The PCR conditions for different primers are given in Table 4.3.

3.2.3.5 Agarose gel electrophoresis

The presence of PCR products were checked in 1.5 per cent agarose in 1X TAE buffer in horizontal electrophoresis unit. 2 μl of PCR product was mixed with 6 X gel loading buffer (Bromophenol blue 0.25 per cent, Xylene cyanol 0.25 per cent, sucrose 40 per cent) were loaded on the agarose gel. *HaeIII* digested pBR322 DNA was used as molecular size marker.

3.2.4 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. The procedure followed was

1.   Preparation of annealing mixture

The composition of the mixture was as follows:

M13 phage DNA (0.2 μg/μl)	-	5μl
5 X Sequenase reaction buffer	-	2μl
Forward primer (5pM/μl)	-	1μl
Sterile ultra pure water	-	2μl

The mixture was mixed well. Spun and incubated at 65°C for 2 minutes. Then cooled to room temperature over 15-30 min, and kept in ice.

2. Dilution of labeling mix

The labeling mix provided in the kit was diluted five fold as follows:

5 X Labeling mix	-	0.5μl
Water	-	2.0μl

3. Dilution of enzyme

The enzyme (Sequenase version 2.0) was diluted eight fold with Sequenase enzyme dilution buffer as follows:

Sequenase enzyme (13 U/μl) -		0.5μl
Sequenase dilution buffer	-	3.5μl

4. Labeling reaction

To the ice cold annealed DNA mixture, the following components were added.

Dithiothreitol (DTT 0.1M)	-	1μl
Diluted labeling mix	-	2μl
Diluted enzyme	-	2μl
α^{32} P dATP (10μCi/μl)	-	2μl

The contents were mixed well, spun and incubated at room temperaure for two to five minutes.

5. Four tubes labeled G, A, T and C were filled with 2.5µl of each dideoxy termination mixtures (ddGTP, ddATP, ddTTP and ddCTP).

6. To each termination tubes (G, A, T, C), 3.5µl of labeling reaction mixture was transferred mixed well and incubated at 37°C for five minutes.

7. The reaction was stopped by addition of 4µl of stop solution provided in the sequencing kit and stored at –20°C.

3.2.5 Denaturing polyacrylamide gel electrophoresis

Denaturing polyacrylamide gel electrophoresis was performed on a Vertical Sequencer (Consort, Belgium) using 6 per cent denaturing polacrylamide gel (6 per cent urea, 0.5 X TBE). The gels were set between two glass plates (41 x 33 cm) separated by 0.35 mm thick spacers.

3.2.5.1 Casting the gel

The glass plates were cleaned thoroughly with soap and water and air dried. Traces of grease and oil were removed by repeated wiping with alcohol. The plates were assembled with spacers in between and the sides and bottom sealed with sealing tape.

The gel was prepared by mixing 60 ml of 0.5X TBE gel mix (6 per cent Acrylamide, 6 M urea, 0.5X TBE) and 125 µl each of 10 per cent Ammonium persulphate solution and TEMED (N, N, N', N', Tetra Methyl Ethylene Diamine) in a beaker. The mixture was poured between the glass plates avoiding air bubbles. The plates were clamped and the comb (Shark toothed comb) inserted on top with the toothed surface facing upwards. The gel was allowed to set for 30 minutes

before electrophoresis. The tapes and clamps were removed, plates were cleaned and assembled in the sequencer. The upper and lower electrode tanks were filled with IX TBE (pH 8.3) buffer (TBE 0.045 M Tris borate, 0.001 M EDTA) to the required level. The comb was removed, wells were cleaned with buffer solution and comb was then reinserted in opposite direction with the toothed surface now facing downwards to form sample-loading wells.

3.2.5.2 Loading of samples

The PCR products were mixed with 3.5 µl formamide loading buffer (0.02 per cent Xylene Cyanol, 0.02 per cent Bromophenol Blue, 10 mM EDTA, 98 per cent deionised formamide), denatured at 95°C for 5 min 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.

3.2.5.3 Electrophoresis

The gels were electrophoresed at 40 W for three hours maintaining a temperature of around 50° C. The bromophenol dye in the loading buffer acted as indicator of the mobility of DNA fragments and had a mobility equivalent to a 25 base fragment and the Xylene cyanol dye had a mobility equivalent to approximately 100 base fragment.

3.2.5.4 Drying of gels

After electrophoresis the glass plates were removed from the sequencer, and carefully separated. The gel adhering to one of the plates was transferred to a filter paper. The position of the first well was marked by cutting out a small portion of the corresponding corner. The gel was covered with klin film and dried in a gel drier connected with the motor pump at 80° C for one and a half hours.

3.2.6 Autoradiography

The klin film was removed after drying and the gel was set for autoradiography with X-ray film (Kodak, 35.6 x 43.2cm) 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.

3.2.7 Development of X-ray film

The X-ray film was developed in the dark room. Developing was done 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. The developed film was washed thoroughly in running water and dried.

3.2.8 Microsatellite typing

The genotypes of animals were determined for each microsatellite loci by comparing the sizes of alleles with M 13 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.

3.3 STATISTICAL ANALYSIS

3.3.1 Comparison of gene frequencies, genotype frequencies in different types of birth

Chi- square test for proportions (Rangaswamy,1995) was conducted to test the significance of different allele frequencies and genotypic frequencies of microsatellite markers BM1329 and TGLA68 between different types of birth.

3.3.2 Comparision of litter size of different genotypes

Student't test (Snedecor and Cochran, 1985) was used to compare the genotype mean of litter size with that of population mean.

3.3.3 Effect of season of birth on litter size

The association between seasons and type of birth was tested by Chi-square test (Snedecor and Cochran, 1985).

3.3.4 Heterozygosity (He)

Heterozygosity is a measure of usefulness of the marker. Heterozygosity was calculated by the method of Ott (1992)

$$He = 1- \sum_{i=1}^{n} p_i^2$$

Where p_i is the frequency of ith allele at a locus.

3.3.5 Polymorphic Information Content (PIC)

The polymorphic information content expresses informativeness or usefulness of a marker for linkage studies. The PIC values of the markers were calculated as;

$$PIC = 1 - (\sum_{i=1}^{n} Pi^2) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 Pi^2 Pj^2$$

Where p_i and p_j are the frequencies of ith and j^{th} alleles respectively. (Botstein *et al.*, 1980)

3.4 ASSOCIATION BETWEEN SEASONALITY AND TYPE IF BIRTH

The information regarding the litter size of 120 does of Malabari goats and seasonality of kidding were collected from the records maintained in the project on AICRP on Malabari goat improvement. One year was classified into three different seasons viz., January to April (season-1), May to August (season- 2) and September to December (season -3).

Results

4. RESULTS

4.1 INCIDENCE OF MULTIPLE BIRTHS

Incidence of multiple births in the areas of operation of All India Coordinated Research Project (AICRP) on Malabari goat improvement, from where the samples were collected is given in the Table 4.1. The three areas were Tanur in Malappuram district, Tellechery in Kannur district and Badagara in Kozhikode district.

The per cent of birth of singles, twins, triplets and quadruplets were 38.33 per cent, 51.67 per cent, 9.17 per cent and 0.83 per cent respectively, in the total population under study. Among the three regions, the twin birth was more than singles in all areas followed by triplets and quadruplets. The overall incidence of multiple births is more in Tanur compared to Tellechery and Badagara.

4.2 MICROSATELLITE ANALYSIS

4.2.1 Isolation of Genomic DNA

A total of 120 DNA samples were isolated from whole blood collected from Malabari does reared in the field area of AICRP on Malabari goat improvement, viz., Tanur, Tellechery and Badagara. The DNA was isolated using phenol chloroform extraction procedure with some modifications.

4.2.2 Yield and quality of DNA

The yield and purity of DNA isolated from 120 Malabari goats are presented in Table 4.2. 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 and good

deproteinisation. The mean value of yield of DNA was found to be 357± 23.057 µg per 5 ml of blood. The quality of DNA samples was checked by agarose gel electrophoresis. The presence of single, clear band with out shearing indicated the quality of high molecular weight DNA.

4.2.3 PCR analysis

The microsatellite markers OarAE101, BMS2508 and BM1329 linked to the Booroola fecundity gene and microsatellite markers TGLA54 and TGLA68 linked to the Inverdale prolificacy gene found in sheep were used for the study. The primers for these markers were custom synthesized. Polymerase Chain reaction (PCR) was performed on all the 120 DNA samples using the primer pairs of the selected loci.

4.2.4 Optimisation of PCR

The composition of PCR reactions and the PCR conditions like temperature, time, number of cycles were standardised separately for different primer pairs for the efficient amplification and accurate typing of the products. The reaction components and cycle parameters for each primer pair is presented in Table 4.3.

Table 4.1 Incidence of multiple births in total population of Malabari
goats and in three different regions

Type of Birth	Regions*						Total Population (Percent)
	Tanur		Tellechery		Badagara		
	No. of births	Per cent	No. of births	Per cent	No. of births	Per cent	
Single	9	30	14	38.89	23	42.59	38.33
Twins	15	50	18	50.00	29	53.70	51.67
Triplets	5	16.67	4	11.11	2	03.70	09.17
Quadru plets	1	3.33	0	0	0	0	00.83

*Non Significant

|72575|

Table 4.2 Yield and purity of DNA isolated from Malabari goats

Parameter	Mean ± S.E
Yield/5 ml	357± 23.057 μg
O.D Ratio (260 nm/280 nm)	1.652 ± 0.017

Table 4.3 General conditions for PCR assay

Parameter	Level
Template DNA	50 ng
10X Reaction buffer	1 μl
Forward primer concentration	5 pM
Reverse primer concentration	6 pM
dNTP concentration	200 μM each
MgCl$_2$ concentration	1.5 mM
Taq DNA polymerase	0.3 units
Reaction volume	10 μl
Initial denaturation	94°C for three minutes
Denaturation for PCR cycles	94°C for one minute
Primer annealing*	60°C for one minute
Primer extension	72°C for one minute
Number of cycles	35
Final extension	72°C for five minutes

* Annealing temperature for OarAE101- 60°C, BMS2508 - 59°C, BM1329 - 58°C, TGLA54 - 60°C and TGLA68- 60°C

4.3 MICROSATELLITE ANALYSIS

The microsatellite markers OarAE101, BMS2508 and BM1329 linked to the Booroola gene (Fec^B) and the Microsatellite markers TGLA54 and TGLA68 linked to Inverdale ($FecX^I$) gene were selected for the study of polymorphism in Malabari goats.

4.3.1 OarAE101

All the animals typed were found to be monomorphic for this locus with allele size of approximately 80 bp. (Plate1).

4.3.2 BMS2508

This microsatellite locus was found to be monomorphic in the Malabari goat population after typing forty animals. So further analysis was not carried out. The animals typed were found to be of the genotype 100/100. (Plate 2).

4.3.3 BM1329

The BM1329 locus was found to be highly polymorphic with 15 alleles ranging in size from 167-195 bp. (Plate 3). The allele sizes and their frequencies are given in table 4.4. The allelic frequency in different groups based on the type of kidding is given in table 4.5.

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 ($P \le 0.01$) and the alleles 179 bp and 185 bp ($P \le 0.05$) for the locus BM1329 were found in different types of births. But the highest frequency was observed for the allele 177 bp in all groups. 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 0.1049 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.

A total of thirty-four genotypes were detected at the BM1329 loci. The genotypes and genotypic frequency are presented in table 4.6. The genotypic frequency in different groups based on the type of kidding is given in table 4.7.

The genotype 173/177 was present in highest frequency followed by 173/179. The genotypes 171/189, 171/185, 185/193, 177/183, 171/195, 173/195, 173/173, 171/173, 173/181, 189/195, 167/173 and 175/175 were present in lowest frequency in the population.

The genotype 177/191 was found to be significantly different in different types of births (P≤0.01). The highest frequency (0.1957) was observed in singles followed by twins (0.0323) and absent in triplets.

The mean litter size for different BM1329 genotypes is presented in the table 4.8. The litter sizes in genotypes 177/191 and 175/185 were significantly different when compared with average litter size. The genotype 175/185 is found to be significantly different with a higher litter size when compared with the population average in Malabari goats (P≤0.01). The average litter size of 2.0909 was observed for the genotype 175/185 as against a population average of 1.733. The results indicated that the microsatellite locus BM1329 has a significant effect on litter size in Malabari goats.

Table 4.4 Allele sizes and their frequencies at BM1329 locus in Malabari goats.

Sl. No	Allele Size (bp)	Allelic frequency
1	167	0.004167
2	169	0.008333
3	171	0.062500
4	173	0.175000
5	175	0.145830
6	177	0.233330
7	179	0.104167
8	181	0.045830
9	183	0.012500
10	185	0.066670
11	187	0.020800
12	189	0.045833
13	191	0.058333
14	193	0.004167
15	195	0.01250

Table 4.5 Alleles and their frequencies at BM1329 locus in different types of kidding in Malabari goats

Allele No.	Allele Size (bp)	Allelic frequency in Malabari goats given birth to							
		Singles		Twins		Triplets		Quadruplets	
		Frequency	No. of alleles	Frequency	No. of alleles	Frequency	No. of alleles	Frequency	No. of alleles
1	167	0.00000	0	0.00000	0	0.045455	1	0.0000	0
2	169	0.010870	1	0.008065	1	0.000000	0	0.0000	0
3	171	0.032609	3	0.096774	12	0.000000	0	0.0000	0
4	173	0.184783	17	0.161290	20	0.136364	3	1.00000	2
5	175	0.097826	9	0.185484	23	0.136364	3	0.0000	0
6	177	0.206522	19	0.241935	30	0.318182	7	0.0000	0
7	179*	0.173913	16	0.064516	8	0.045455	1	0.0000	0
8	181**	0.065217	6	0.008065	1	0.181818	4	0.0000	0
9	183	0.010870	1	0.016129	2	0.00000	0	0.0000	0
10	185*	0.021739	2	0.104839	13	0.045455	1	0.0000	0
11	187	0.032609	3	0.016129	2	0.00000	0	0.0000	0
12	189	0.032609	3	0.056452	7	0.045455	1	0.0000	0
13	191**	0.119565	11	0.016129	2	0.045455	1	0.0000	0
14	193	0.000000	0	0.008065	1	0.00000	0	0.0000	0
15	195	0.010870	1	0.016129	2	0.00000	0	0.0000	0
Total		1	92	1	124	1	22	1	2

*P≤0.05

**P≤0.01

Table 4.6. Genotypic frequency at the Microsatellite locus BM1329 in Malabari goats.

Sl. No.	Genotype	Genotypic frequency	Sl. No.	Genotype	Genotypic frequency
1	171/189	0.008333	18	175/177	0.058333
2	177/189	0.041667	19	177/187	0.016667
3	177/191	0.091667	20	171/195	0.008333
4	171/185	0.008333	21	173/195	0.008333
5	177/185	0.025000	22	179/189	0.016667
6	175/185	0.091667	23	175/189	0.016667
7	173/177	0.133333	24	173/175	0.016667
8	173/179	0.108333	25	175/179	0.050000
9	185/193	0.008333	26	173/173	0.008333
10	169/171	0.016667	27	171/173	0.008333
11	171/175	0.025000	28	175/181	0.016667
12	173/187	0.025000	29	173/181	0.008333
13	171/181	0.016667	30	189/195	0.008333
14	177/183	0.008333	31	181/191	0.025000
15	171/177	0.033333	32	177/181	0.025000
16	177/179	0.033333	33	167/173	0.008333
17	173/183	0.016667	34	175/175	0.008333

Table 4.7 Genotypes and frequencies at the microsatellite locus BM1329 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	171/189	0.00000	0	0.01613	1	0.00000	0	0.00000	0
2	177/189	0.00000	0	0.06452	4	0.09091	1	0.00000	0
3	177/191**	0.19565	9	0.03226	2	0.00000	0	0.00000	0
4	171/185	0.00000	0	0.01613	1	0.00000	0	0.00000	0
5	177/185	0.04348	2	0.01613	1	0.00000	0	0.00000	0
6	175/185	0.00000	0	0.16129	10	0.09091	1	0.00000	0
7	173/177	0.06522	3	0.19355	12	0.09091	1	0.00000	0
8	173/179	0.17391	8	0.06452	4	0.09091	1	0.00000	0
9	185/193	0.00000	0	0.01613	1	0.00000	0	0.00000	0
10	169/171	0.02174	1	0.01613	1	0.00000	0	0.00000	0
11	171/175	0.00000	0	0.04839	3	0.00000	0	0.00000	0
12	173/187	0.04348	2	0.01613	1	0.00000	0	0.00000	0
13	171/181	0.02174	1	0.01613	1	0.00000	0	0.00000	0
14	177/183	0.00000	0	0.01613	1	0.00000	0	0.00000	0
15	171/177	0.00000	0	0.06452	4	0.00000	0	0.00000	0
16	177/179	0.06522	3	0.01613	1	0.00000	0	0.00000	0
17	173/183	0.02174	1	0.01613	1	0.00000	0	0.00000	0
18	175/177	0.02174	1	0.06452	4	0.18182	2	0.00000	0
19	177/187	0.02174	1	0.01613	1	0.00000	0	0.00000	0
20	171/195	0.00000	0	0.01613	1	0.00000	0	0.00000	0
21	173/195	0.00000	0	0.01613	1	0.00000	0	0.00000	0
22	179/189	0.02174	1	0.01613	1	0.00000	0	0.00000	0
23	175/189	0.02174	1	0.01613	1	0.00000	0	0.00000	0
24	173/175	0.02174	1	0.01613	1	0.00000	0	0.00000	0
25	175/179	0.08696	4	0.03226	2	0.00000	0	0.00000	0
26	173/173	0.00000	0	0.00000	0	0.00000	0	1.00000	1
27	171/173	0.02174	1	0.00000	0	0.00000	0	0.00000	0
28	175/181	0.04348	2	0.00000	0	0.00000	0	0.00000	0
29	173/181	0.02174	1	0.00000	0	0.00000	0	0.00000	0
30	189/195	0.02174	1	0.00000	0	0.00000	0	0.00000	0
31	181/191	0.04348	2	0.00000	0	0.09091	1	0.00000	0
32	177/181	0.00000	0	0.00000	0	0.27273	3	0.00000	0
33	167/173	0.00000	0	0.00000	0	0.09091	1	0.00000	0
34	175/175	0.00000	0	0.01613	1	0.00000	0	0.00000	0

**P≤0.01

No. is the number of genotypes

Table 4.8. The mean litter size of different BM1329 genotypes in Malabari goats.

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

4.3.4 TGLA54

All the animals typed were found to be monomorphic for the locus with allele size of 109bp (Plate 4).

4.3.5 TGLA68

At this locus eight different alleles could be detected with a size range of 98-114 bp (Plate 5). The different alleles and their frequencies are given in Table 4.9. A total of 12 different genotypes could be detected at this locus after typing 120 animals. The allelic frequency in different groups based on the type of kidding is given in Table 4. 10.

The allele 104 bp was found to be significantly different in different types of births (P≤0.01). The highest frequency (0.4545) was observed in triplets followed by twins (0.2177) and singles (0.1848).

The different TGLA68 genotypes and their frequencies are presented in Table 4.11. The genotypic frequency in different groups based on the type of kidding is given in table 4. 12. The genotype 104/106 was found to be significantly different in different types of births (P≤0.01). The highest frequency (0.4545) was observed in triplets followed by singles (0.1087) and twins (0.0333).

The mean litter size for different TGLA68 genotypes is presented in the Table 4.13. No significant difference between litter size of different genotypes of the microsatellite marker TGLA68 could be detected when compared with the population average of 1.733 in Malabari goats (P≥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.

Table 4.9 Allele size and their frequencies at TGLA68 locus in Malabari goats

Allele No	Allele size (bp)	Allelic frequency
1	98	0. 004170
2	100	0.108330
3	102	0.075000
4	104	0.225000
5	106	0.287500
6	108	0.195800
7	110	0.091667
8	114	0.012500

Table 4.10 Alleles and their frequencies at TGLA68 locus in different types of kidding in Malabari goats

Allele No.	Allele size (bp)	Allelic frequency in Malabari goats given birth to							
		Singles		Twins		Triplets		Quadruplets	
		Frequency	No. of alleles	Frequency	No. of alleles	Frequency	No. of alleles	Frequency	No. of alleles
1	98	0.00000	0	0.00807	1	0.00000	0	0.00000	0
2	100	0.06522	6	0.13709	17	0.13636	3	0.00000	0
3	102	0.07609	7	0.07258	9	0.09091	2	0.00000	0
4	104**	0.18478	17	0.21774	27	0.45454	10	0.00000	0
5	106	0.33696	31	0.25807	31	0.27273	6	0.50000	1
6	108	0.23913	22	0.18548	23	0.04546	1	0.50000	1
7	110	0.09783	9	0.10484	13	0.00000	0	0.00000	0
8	114	0.00000	0	0.02419	3	0.00000	0	0.00000	0
Total		1	92	1	124	1	22		1

**P≤0.01

Table.4.11 Genotypic frequency at the microsatellite locus TGLA68 in Malabari goats.

Sl. No.	Genotype	Frequency	Number
1	102/106	0.066667	8
2	100/104	0.191667	23
3	106/110	0.116667	14
4	108/110	0.066667	8
5	104/108	0.066667	8
6	102/104	0.066667	8
7	104/106	0.116667	14
8	106/108	0.258333	31
9	100/102	0.016667	2
10	106/114	0.016667	2
11	104/114	0.008333	1
12	98/100	0.008333	1

Table. 4.12 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

**$P \leq 0.01$

No. is the number of genotypes

Table.4.13 The mean litter size of different TGLA68 genotypes in Malabari goats.

Sl. No.	Genotype	Litter size (Mean ± SE)
1	98/100	2.000
2	102/106	1.38 ±0.1711
3	100/104	1.87 ± 0.1276
4	106/108	1.53 ± 0.1025
5	104/108	1.5 ± 0.17680
6	104/106	2 ± 0.225800
7	102/104	2 ± 0.250000
8	106/110	1.57 ± 0.1323
9	108/110	1.63 ± 0.1711
10	100/102	1.000
11	106/114	2.000
12	104/114	2.000

4.4 HETEROZYGOSITY AND POLYMORPHIC INFORMATION CONTENT (PIC)

The number of alleles, allele sizes, heterozygosity and polymorphic information content (PIC) of microsatellite markers are given in table 4.14. 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 indicate the level of information about a marker. The PIC values obtained were 0.8526 for BM1329 and 0.7823 for microsatellite marker TGLA68.

4.5 ASSOCIATION BETWEEN SEASONALITY AND TYPE OF BIRTH IN MALABARI GOATS

The incidence of multiple births in different seasons is presented in Table 4.15. No significant association could be detected between season of kidding and type of birth in Malabari goats (P≥0.05). There was no significant difference between seasons with respect to number of kidding (P≥0.05). The highest per cent of kidding (42.5 per cent) took place in season-2 followed by season-3 (32.5 per cent) and season-1 (25 per cent). The average litter size was highest in season-1 (2.0689) followed by season-3 (1.74) and season-2 (1.678)

Table. 4.14 Number of alleles, allele sizes, heterozygosity and polymorphic information content of microsatellite markers in Malabari goats

Marker	BM1329	TGLA68
Number of observations	120	120
Number of alleles	15	8
Allele size (bp)	167-195	98-114
Heterozygosity (He)	0.8660	0.8024
PIC value	0.8526	0.7823

Table. 4.15 Per cent of multiple birth and mean litter size of Malabari goats kidded
in different seasons.

Type of birth	Season of kidding		
	Season-1 (January-April	Season-2 (May-August)	Season-3 (September-December)
Single	33.33 (10)	43.14 (22)	40.00 (14)
Twins	56.67 (17)	47.06 (24)	46.67 (21)
Triplet	6.670 (2)	9.800 (5)	15.00 (4)
Quadruplets	3.330 (1)	0.000 (0)	0.000 (0)
Overall	25.00 (30)	42.50 (51)	32.50 (39)
Mean litter size	2.0689	1.678	1.74

*Figures in parenthesis are observed number of kidding
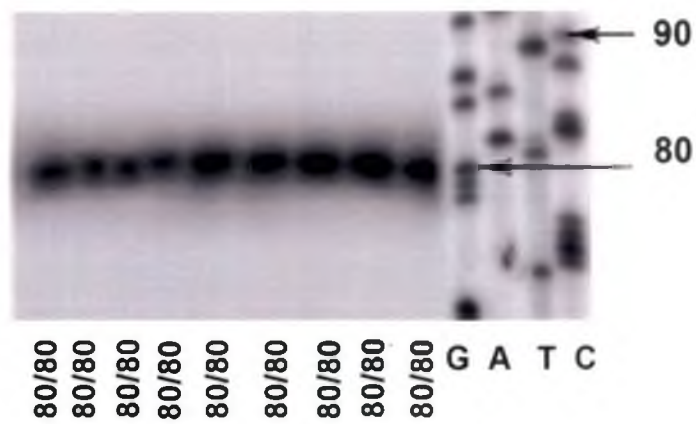
Plate 1. Autoradiograph of the gel showing PCR products of OarAE101 locus in Malabari goats

GATC represents M13 sequence used as marker

Plate 2 Autoradiograph of the gel showing PCR products of BMS2508 locus in Malabari goats
GATC represents M13 sequence used as marker

Plate 3. Autoradiograph showing polymorphism at BM1329 locus in Malabari goats

GATC represents M13 sequence used as marker

Plate 4. Autoradiograph of the gel showing PCR products of TGLA54 locus in Malabari goats GATC represents M13 sequence used as marker

Plate 5. Autoradiograph showing polymorphism at TGLA68 locus in Malabari goats
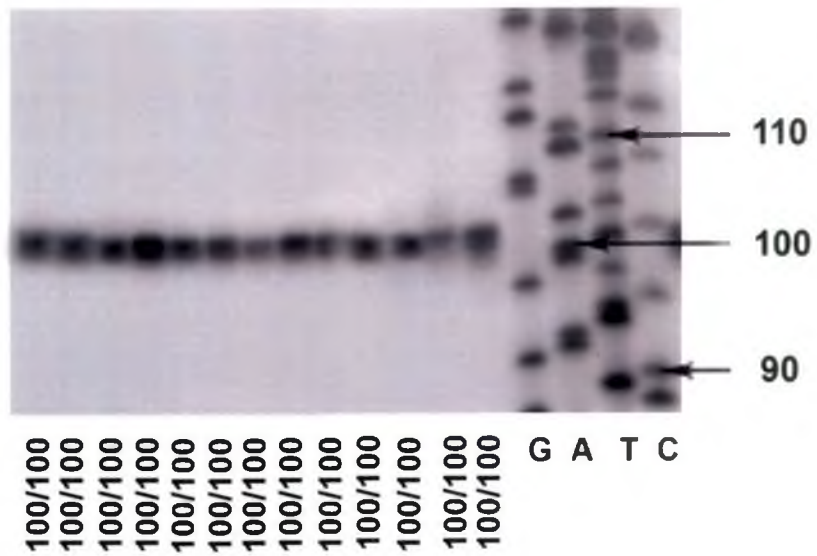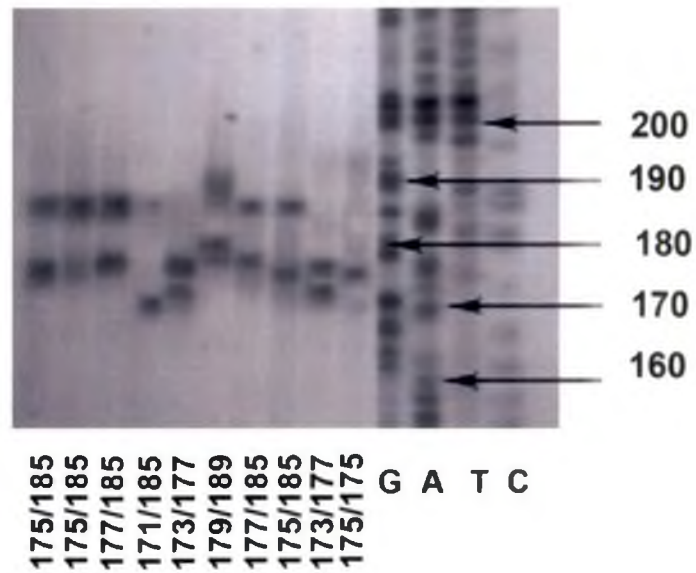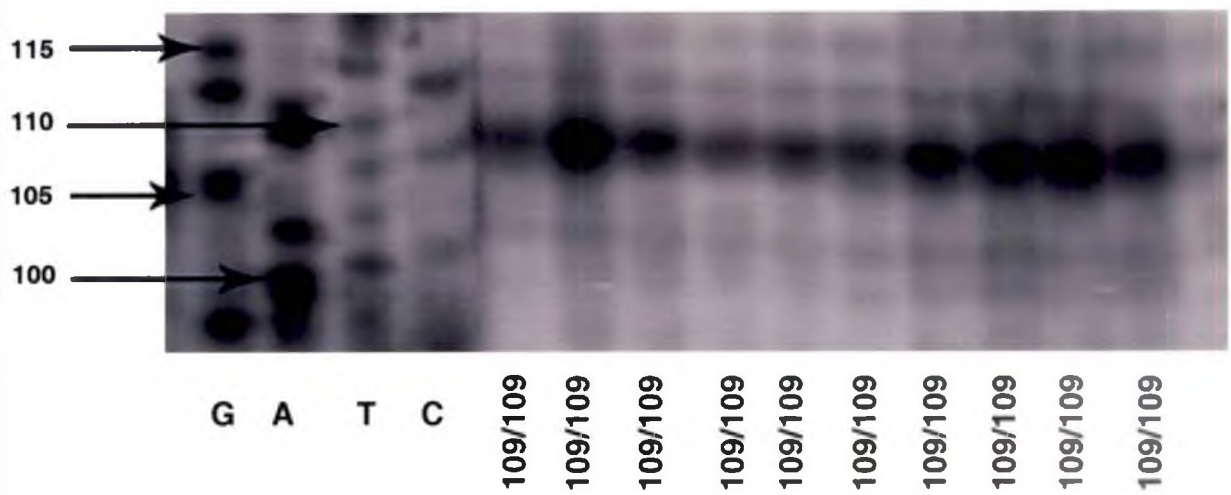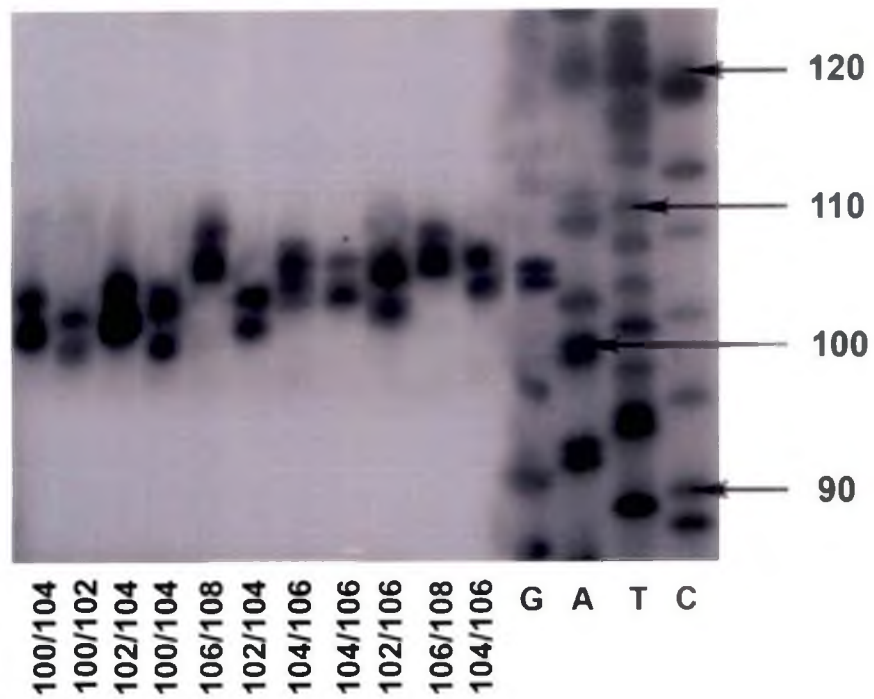GATC represents M13 sequence used as marker

Discussion

5. DISCUSSION

Goat is a prolific ruminant under tropical and subtropical conditions with a very high twinning rate. The triplets and quadruplets are also frequent (Devendra and Burns, 1970). The Malabari goat is no exception to this and a mean litter size at birth of 1.583 ± 0.081 has been reported (Raghavan *et al.*, 1999).

5.1 INCIDENCE OF MULTIPLE BIRTHS

The incidence of multiple births in Malabari goat population observed in the present study were 38.33 per cent, 51.67 per cent, 9.17 per cent and 0.83 per cent singles, twins, triplets and quadruplets, respectively. The overall per cent of multiple births recorded in Malabari goats maintained in the field area of the All India Coordinated Research Project (AICRP) on Malabari Goat Improvement was 31.59, 54.35, 12.79 and 1.28 per cent for singles, twins, triplets and quadruplets respectively (Anon., 2005). The differences observed can be attributed to the small sample size. Acharya (1982) reported the values as 50.5, 42.4, 6.6 and 0.5 per cent for singles, twins, triplets and quadruplets, respectively in Malabari goats. Sundaresan and Raja (1973) observed 47 per cent of singles, 35.29 per cent of twins and 17.65 percent of triplets; where as Mukundan and Rajagopalan (1971) reported the values as 47, 42.4 and 10.6 per cent in the same breed.

5.2. MICROSATELLITE ANALYSIS

5.2.1 Isolation of Genomic DNA

DNA for the present study was isolated using phenol-chloroform extraction method with some modifications. This method was found to be suitable for isolation of DNA by Aravindakshan *et al.* (1997), Suprabha (2003), Anilkumar (2003),

Uthaman (2005) and Thomas (2005) from cattle blood, and by Chitra (2002), Mathew (2004) and Jacob (2005) from goat blood.

5.2.2 Yield and quality of DNA

The mean value of yield of DNA from Malabari goats in the study was found to be 357± 23.057 µg per 5 ml of blood. The yield obtained in this study is more than that reported by Chitra (2002) and Mathew (2004), who have obtained yields of 230.97 ± 11.65 µg and 350.05 ± 10.05 µg, respectively, from 5 ml of blood using the same procedure in Malabari goat.

Senthil *et al.* (1996) and Aravindakshan *et al.* (1997) had reported average yields of 444.58 ± 21.54 µg and 394.50 ± 34.26 µg by phenol chloroform method from 10 to15 ml of cattle blood. The high value obtained in goats, compared to that recorded in cattle may be due to increased leukocyte count in goats (Swenson, 1996). The ratio between optical density (OD) at 260 nm and 280 nm was 1.652 ± 0.017, which indicated good deproteinisation.

Relatively high yield was obtained from a small amount of blood sample. The purity of DNA obtained was considerably high in this method. The DNA produced was of good quality and was suitable for PCR amplification.

5.2.3 PCR analysis

5.2.3.1 Optimisation of PCR

PCR conditions were optimized for the primers selected to obtain specific products. Annealing temperature and $MgCl_2$ concentration are the two important factors determining the stringency of primer hybridization.

The annealing temperature of 60°C was used for the microsatellite markers OarAE101, TGLA54 and TGLA68, 59°C for the microsatellite locus BMS2508 and

58°C for the locus BM1329. Bishop *et al.* (1994) used an annealing temperature of 58°C for the microsatellite marker BM1329. Yang *et al.* (2004) used annealing temperature of 57°C for the microsatellite marker OarAE101.

Don *et al.* (1991) reported that the occurrence of spurious bands can be reduced by adjusting the [Mg^{2+}] concentration or increasing the annealing temperature of the PCR. The concentration of 1.5 mM $MgCl_2$ used in this study has yielded specific product, which is in agreement with the report of Chu *et al.* (2003).

The other conditions affecting the specificity of the polymerase chain reaction include concentration of enzyme and primers as well as the annealing time, extension time and number of cycles. A concentration of 0.3 U/ reaction of *Thermus aquaticus* DNA polymerase and 5 pM of forward primers and 6 pM of reverse primers per reaction gave satisfactory results. Gyllensten and Erlich (1988) used unequal molar amounts of two amplification primers in a single step to amplify a single copy gene by PCR, and produced an excess of single stranded DNA of chosen strand. Xu *et al.* (1999) performed the PCR with the reaction mixture containing unequal concentrations of forward (50 ng) and reverse primer (75 ng) in 25 µl reaction.

The concentration of dNTPs used was 200 µ*M* each in every 10 µl reaction. The use of limiting dNTP concentrations may influence the fidelity of DNA replication by Taq DNA polymerase (Esposito *et al.*, 1998).

The PCR conditions were optimized separately for different microsatellite markers.

5.3 MICROSATELLITE MARKERS LINKED TO THE PROLIFICACY GENE

5.3.1 Selection of markers

Microsatellites are excellent genetic markers because of their high polymorphism and abundant distribution throughout the genome (Chu *et al.*, 2003; Ihara *et al.*, 2004). Shiue *et al.* (1999) found that microsatellite based markers are more useful when compared to RAPD markers.

In the present study, the microsatellites markers OarAE101, BMS2508 and BM1329 linked to fecundity gene Booroola (Fec^B) in Booroola sheep and TGLA54 and TGLA68 linked to Inverdale ($FecX^1$) in Romney sheep were used to study the relationship between these markers and prolificacy in Malabari goats. The fecundity gene (Fec^B) has been marked to ovine chromosome 6 (Montogomery *et al*, 1993; 1994). They also found that microsatellite marker OarAE101 was linked to Fec^B gene at a distance of 13 cM. Lord *et al.* (1998) mapped the Fec^B gene to a 10 cM region between microsatellite markers BM1329 and OarAE101. Mulsant *et al.* (1998) reported that the closest flanking markers of the Fec^B gene were bovine microsatellite BMS2508 and caprine microsatellite LSCV043 which were situated about 2cM on either side of the gene.

The Inverdale gene ($FecX^1$) was identified in a prolific family of Romney ewes. This gene affects ovulation rate and located on the sheep X chromosome. (Davis *et al.* 1991; 1992a). The Inverdale gene was located in 28.7 cM region flanked with microsatellites TGLA54 and TGLA68 (Galloway *et al.*, 1999).

Malabari goat is a highly prolific animal coming next to Black Bengal goats among Indian breeds. There exists a great similarity between sheep and goat genomes. This was evident from the studies of Yang *et al.* (2004) who observed the existence of conservation of microsatellite markers between sheep and goat in

different microsatellite loci. Further, Kim *et al.* (2004) found that about 55.2 per cent of the bovine microsatellite loci amplified in domestic goats was polymorphic. Maddox *et al.* (2001) reported that the sheep linkage map contains strong links to both the cattle and goat maps. A total of 209 loci positioned on the sheep linkage map had also been placed on the goat linkage map. Sun *et al.* (2004) reported that there were no significant differences in microsatellite DNA sites in sheep and goat, and Maddox (2005) reported genome similarity in sheep and goats and a good agreement between the maps in terms of loci mapping to equivalent chromosomes in the two species. So the study was conducted using markers found to be polymorphic in sheep, to analyse association between microsatellite markers linked to Fec^B and $FecX^1$ gene and prolificacy in Malabari goats.

5.3.2 Microsatelite markers linked to Booroola gene

5.3.2.1 OarAE101

This microsatellite locus was found to be monomorphic in the Malabari goat population under study. The allele size of 80 bp and genotype of 80/80 was observed for this locus. Chu *et al.* (2003) observed a total of 9 alleles for the microsatellite marker OarAE101 with a size range of 97 to 135 bp and Yang *et al.* (2004) observed a size range of 93-113 bp for the same marker in sheep. The monomorphic nature of this locus may be due to the fact that the degree of polymorphism displayed by a particular microsatellite can vary with the species (Moore *et al.*, 1991).

5.3.2.2 BMS2508

This microsatellite locus was found to be monomorphic in the Malabari goat population under study. Similar reason as cited above could be ascribed for monomorphism of this marker also (Moore *et al.*, 1991). Only one allele with the size of 100 bp was detected.

5.3.2.3 BM1329

For BM1329 locus, 15 alleles with a size range of 167-195 bp were detected with 34 genotypes. This is slightly different from the product size range of 160- 180 bp with 6 alleles as reported by Chu *et al.* (2003). In the present study, highest frequency of 0.233 was observed for the allele 177, followed by 173 with a frequency of 0.175 and the lowest frequency of 0.0042 was observed for the allele 167 and 193.

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 triplets had the highest frequency of allele 177 bp (0.3182) 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. Where as in the case of 185 bp, it was twins followed by triplets and singles. The frequency of 191 bp was highest in singles, followed by triplets and twins. A significant correlation between this marker and litter size was also reported by Chu *et al.* (2001) in Small Tail Han sheep. He found that the allele 146 bp had a significant positive correlation with litter size and the allele 148 bp had significant negative correlation with litter size.

The highest frequency of 0.1333 was observed for the genotype 173/177 followed by 173/179 with a frequency of 0.1083 in Malabari goats.

The genotype 177/191 was found to be significantly different with regard to types of births in Malabari goats ($P \leq 0.01$). The frequency was higher in singles than in twins. No triplets were recorded in this category. The low frequency in twins was ascribed to the low sample size. There were nine single births but only three twin births in this category. Only triplets were recorded in 177/181 genotype. In 175/177

genotype triplets had a higher frequency when compared with singles and twins. Chu *et al.* (2003) reported that the genotype 164/174 was the highest among BM1329 genotypes and 162/164 was the lowest in Small Tail Han sheep. Nowak *et al.* (2001) identified a total of 5 alleles from 162 bp to 174 bp in microsatellite BM1329 and the marker of the Fec^B gene seemed to be an allele of 162 bp in sheep.

The litter sizes in genotypes 177/191 and 175/185 were significantly different when compared with average litter size ($P \leq 0.01$). An average litter size of 2.0909 was observed for the genotype 175/185. The average for the population was 1.732. The results indicated that the microsatellite locus BM1329 had a significant effect on litter size in Malabari goats. The 175/185 genotypes had a significantly higher litter size over the population average ($p < 0.01$). Higher litter size than the average was reported by Chu *et al.* (2003) in Small Tail Han sheep using the same marker in which the genotype 164/174 had the highest litter size of 3.17. Lord *et al.* (1998) reported that the microsatellite marker BM1329 was linked to the Booroola fecundity gene at a distance of 10 cM region. A similar association between prolificacy and microsatellite locus BM1329 may also exist in Malabari goats as there is a high degree of homogeneity between sheep and goat genome.

The above results indicated a significant association between the microsatellite locus BM1329 and litter size in Malabari goats. The animals with genotype 175/185 of the microsatellite marker BM1329 can be used for the selection of Malabari goats for high prolificacy. The allele 177 bp of the microsatellite marker BM1329 had recorded highest frequency in triplets in Malabari goats and goats with this allele had a higher chance of giving birth to triplets when compared to other alleles.

5.3.3 Microsatellite markers linked to Inverdale gene

5.3.3.1 TGLA54

This microsatellite locus was found to be monomorphic in the Malabari goat population under study. This differs from the reports of Chu *et al.* (2003), who observed 5 alleles with a size range of 116 to 136 bp in Small Tail Han sheep. The difference in polymorphism may be due to species variation as reported by Moore *et al.* (1991)

5.3.3.2 TGLA68

Eight alleles with a size range of 98-114 bp and 12 genotypes were observed for the microsatellite marker TGLA68 in the Malabari goat population. Chu *et al.* (2003) reported 2 alleles and allele size range of 98-100 for this locus in Small Tail Han sheep.

The highest frequency of 0.2875 was observed for the allele 106 bp followed by 104 bp with a frequency of 0.225. 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) ($P \leq 0.01$). The goats with this allele have a high probability of giving birth to triplets, when compared to twins and singles. The highest genotype frequency of 0.258 was observed for the genotype 106/108. The genotype 104/106 was found to be significantly different in different types of birth with triplets having a frequency of (0.4545), followed by singles (0.1087) and twins (0.0333). Chu *et al.* (2003) reported no significant effect on litter size for the same microsatellite locus TGLA68 in Small Tail Han sheep. Nowak *et al.* (2001) identified a total of 6 alleles from 97 bp to 119 bp.

The higher litter size of above 2 was observed in the genotypes 98/100, 104/106, 102/104, 106/114 and 104/114. The average litter size of the population

under study was 1.7332. 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.

From the above findings, it can be concluded that allele 104 bp and the genotype 104/106 of the microsatellite marker TGLA68 can be used as a genetic marker for the selection of Malabari goats for high prolificacy.

5.4 HETEROZYGOSITY AND POLYMORPHIC INFORMATION CONTENT (PIC)

The highest heterozygosity (He) was observed for the microsatellite marker BM1329 (0.8660) followed by TGLA68 (0.8024). The highest heterozygosity obtained for BM1329 was found to be higher than the heterozygosity (0.516) obtained by Chu *et al.* (2003) and slightly lower than the heterozygosity (0.8864) reported by Martinez *et al.* (2004). Chu *et al.* (2003) observed the heterozygosity of 0.497 for the microsatellite marker TGLA68.

A marker is highly informative if its PIC is greater than 0.5 (Botstein *et al.*, 1980). In this study, the microsatellite markers BM1329 and TGLA68 were highly informative. The highest PIC value was obtained for the microsatellite marker BM1329 (0.8526) followed by TGLA68 (0.7823). Martinez *et al.* (2004) reported a PIC value of 0.80 for the locus BM1329 in Blanca Andaluza goat and this is higher than the value obtained in the study. A lower PIC value was observed by Chu *et al.* (2001) in Small Tail Han sheep (0.54), Hu sheep (0.67), Charollais sheep (0.59), Ujumquin sheep (0.66) and in Dorset sheep (0.67) for the same microsatellite marker.

The difference in the heterozygosity and PIC value of the microsatellite markers BM1329 and TGLA68 between sheep and goats might be due to the species variation. Both these markers are highly polymorphic and informative. So they can be used for molecular studies in Malabari goats in future.

5.5 ASSOCIATION BETWEEN SEASON OF BIRTH AND PROLIFICACY IN MALABARI GOATS

Association between season of birth and prolificacy in Malabari goats was studied by dividing the year into three different seasons, viz., January to April (season- 1), May to August (season -2) and September to December (season -3).

No significant association between season of kidding and type of birth could be observed. Singh and Singh (1974) reported that the month of kidding could not influence the frequency of single and twin births based on the distribution of kidding. No significant difference existed between the number of kidding and season of birth in Malabari goats. The incidence of kidding was highest in September and lowest in January and November. Non significant effect of season of birth on prolificacy was reported by Raghavan *et al.* (1999) in Malabari goats. Sharma (1985) reported a higher rate of multiple births during June- July season than the October- November season in Jamanapari goats. The average litter size was higher in season-1 (2.069) followed by season-3 (1.74) and season-2 (1.678) in the study. Singh *et al.* (1987) reported an average litter size of 1.34, 1.33 and 1.35 in summer, monsoon and winter season respectively, in Black Bengal goats.

The present study on relationship between microsatellite markers linked to Booroola and Inverdale gene and prolificacy has revealed that the genotype 175/185 of the microsatellite marker BM1329 and the allele 104 bp and genotype 104/106 of the microsatellite marker TGLA68 are associated with significantly higher litter size in Malabari goats. The animals with this genotype can be selected for future breeding

program for increased prolificacy in Malabari goats. The speed and efficiency of selection is expected to increase by use of these molecular markers in selection of Malabari goats. 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.

Summary

6. SUMMARY

Malabari goats are the indigenous goat breed of northern Kerala. They are also known as 'Tellechery' and 'Kutch' and are well known for their prolificacy. They are medium sized dual purpose animals, reared for meat and milk and their skin is popular in the tanning industry.

High prolificacy is one of the most important economic traits that should be given attention for profitable goat farming. This being a lowly heritable trait, scope for improving by traditional ways of selection is limited. The advances in molecular biology have opened up ways for selection of better performing animals by utilization of genetic markers. Microsatellite markers have emerged as the best tool for delineating polymorphisms at DNA level. Marker assisted selection (MAS) using the microsatellite markers has become an important tool for selection by utilizing markers linked to quantitative trait loci (QTL). So in this study an attempt was made to analyse association between prolificacy gene linked microsatellite markers with litter size in Malabari goats.

Blood samples were collected from the field areas of the All India Coordinated Research Project (AICRP) on Malabari Goat Improvement viz., Tanur in Malappuram district, Tellechery in Kannur district and Badagara in Kozhikode district of Kerala. The DNA samples from 120 Malabari goats which had given birth to singles, twins, triplets and quadruplets in the second and subsequent parities were utilized for the study.

Microsatellite markers linked to Booroola fecundity gene such as OarAE101, BMS2508 and BM1329 and those linked to the Inverdale fecundity gene viz., TGLA54 and TGLA68 in sheep were selected for the study. These markers were

selected because of the reported high degree of homology between sheep and goat genome.

The per cent of births of singles, twins, triplets and quadruplets were 38.33 per cent, 51.67 per cent, 9.17 per cent and 0.83 per cent respectively in the total Malabari goat population selected for the study. The incidence of multiple births was high in Tanur area followed by Tellechery and Badagara. The twin births were more than singles in all areas, followed by triplets and quadruplets.

The whole blood collected from the Malabari goats was used as the source of DNA. Phenol chloroform extraction procedure with some modification was practiced for the isolation of genomic DNA. The average yield of DNA obtained from 5 ml of whole blood sample was 357 ± 23.057 µg. The mean ratio between optical density (OD) at 260 nm and 280 nm was observed to be 1.652 ± 0.017.

The DNA samples were amplified by polymerase chain reaction (PCR) using the specific primers of the microsatellite markers OarAE101, BMS2508 and BM1329 linked to Booroola gene, and the microsatellite markers TGLA54 and TGLA68 linked to Inverdale gene. Among the five microsatellite markers only two markers BM1329 (linked to Fec^B gene) and TGLA68 (linked to $FecX^1$ gene) were found to be polymorphic in the Malabari goat population under study.

The microsatellite markers OarAE101 and BMS2508 (linked to Fec^B gene) and microsatellite marker TGLA54 (linked to $FecX^1$ gene) of sheep were found to be monomorphic in Malabari goats.

With respect to the BM1329 locus, 15 alleles with 34 genotypes were observed in the population. The heterozygosity observed for this marker was 0.8660 and the PIC value calculated was 0.8526.

A significant difference in the alleles 181 bp and 191 bp (P≤0.01) and the alleles 179 bp and 185 bp (P≤0.05) for the locus BM1329 were found in different types of births. The highest frequency (0.3182) for 177 bp was observed in triplets, followed by twins (0.2419) and singles (0.2065). In 179 bp allele, the highest frequency was observed in singles followed by twins and triplets. A highest frequency of 0.1818 was recorded for 181 bp in triplets followed by singles and twins. Whereas in the case of 185 bp, it was twins followed by triplets and singles. The frequency of 191 bp was highest in singles, followed by triplets and twins.

The genotype 177/191 was found to be significantly different in different types of births (P≤0.01). The genotype 175/185 was found to be significantly related to a higher litter size when compared with the population average of 1.733 in Malabari goats (P≤0.01).

A total of eight alleles with a size range of 98 to 114 bp were observed for the microsatellite marker TGLA68. The heterozygosity and PIC value obtained were 0.8024 and 0.7823 respectively, for the locus TGLA68.

The allele 104 bp of the locus TGLA68 was found to be significantly different in different types of births (P≤0.01). The highest frequency (0.4545) was observed in triplets followed by twins (0.2177) and singles (0.1848).

The genotype 104/106 was found to be significantly different in different types of births (P≤0.01). The highest frequency (0.4545) was observed in triplets, followed by singles (0.1087) and twins (0.0333).

There was no significant difference between litter size and different genotypes of the microsatellite marker TGLA68 when compared with the population average of 1.733 in Malabari goats.

The effect of season on type of birth and per cent of kidding was also studied. The results indicate the absence of any significant association between season of kidding, type of birth, and season with respect to number of kidding in Malabari goats.

The results of the study revealed that microsatellite locus BM1329 had a significant effect on litter size in Malabari goats. The genotype 175/185 of the microsatellite marker BM1329 and the allele 104 bp and the genotype 104/106 of the microsatellite marker TGLA68 are associated with significantly higher litter size in Malabari goats. So the animals with these genotypes can be selected for breeding for high prolificacy in Malabari goats.

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Annexures

ANNEXURE – 1

COMPOSITION OF REAGENTS AND BUFFERS USED IN THE STUDY

40% Acrylamide

Acrylamide	380g
N$_1$N – Methylene bisacrylamide	20 g
Water to	1000 ml

1.5% Agarose

0.9g of agarose powder weighted and mixed with 60ml of 1 X TAE buffer in a conical flask. Solution heated in a microwave oven until boiling and cooled slowly.

10% Ammonium persulphate

APS	100mg
Water to	1 ml

6% Denaturing Acrylamide Gel

0.5 X TBE Gelmix	60ml
TEMED	0.125ml
10% APS	0.125ml

Mixed well without air bubbles

EDTA (0.5m, p^H 8.3)

Dissolved 18.61g of EDTA (disodium, dihydrate) in 80ml of distilled water by bringing the p^H to 8.3 with NaOH solution and volume made upto 100 ml. Stored at room temperature after filtration and autoclaving.

Ethidium Bromide (10mg/ml)

100mg ethidium bromide dissolved in10ml distilled water. Solution stored at 4°C in dark coloured bottle.

Formamide dye/ Stop buffer

Deionised formamide	98%
Xylene cyanol	0.025%
Bromophenol blue	0.025%
0.5 M EDTA	10mM

Gel loading buffer

Bromophenol	0.25%	50mg
Xylene cyanol	0.25% ·	50mg
Sucrose	40%	8g

Components stirred well in 20ml distilled water and stored at 4°C.

Phenol (Saturated, p^H 7.8)

Commerically available crystalline phenol melted at 65°C in a waterbath. Hydroxyquinolone added to a final concentration of 0.1 percent. 0.5M Tris HCl (p^H 8.0) added to molten phenol in equal volume. Mixture stirred for 30 min. on a magnetic stirrer and contents transferred into a separating funnel. Lower phenolic phase colleced, mixed with equal volume of 0.1M Tris HCl (p^H 8.0) and stirred again for 30 min. The phenolic phase was collected and extraction repeated with 0.1M Tris HCl (p^H 8.0) until the p^H of phenolic phase was more than 7.8. Finally 0.1 volume of 0.01M Tris HCl (p^H 8.0) added and stored in dark bottle at 4°C.

Phosphate Buffered Saline (PBS, p^H 7.4)

Sodium chloride (NaCl)	138mM	8.0647g
Sodium Dihydrogen Phosphate ($NaH_2PO_22H_2O$)	4mM	0.6240g
Disodium hydrogen phosphate ($Na_2HPO_412H_2O$)	6mM	2.1488g

Contents weighted and mixed 800ml distilled water on a magnetic stirrer and P^H adjusted to 7.4 using dilute HCl. Final volume made upto 1000ml.

RBC lysis buffer

Ammonium chloride	150mM	8.0235g
Potassium chloride	10mM	0.7455g
EDTA	0.1mM	0.0372g

Dissolved the contents in distilled water and volume made upto 1000ml. Stored at 4°C after filtration and autoclaving.

Sodium acetate (3 M, p^H 5.5)

Dissolved 40.824g of sodium acetate in 70ml distilled water and P^H adjusted to 5.5 with glacial acetic acid. Volume made utpo 100ml, autoclaved and stored at room temperature.

Sodium chloride (5M)

Dissolved 29.22g of sodium chloride in 80ml distilled water and volume made upto 100ml. Solution filtered and stored at room temperature.

Sodium chloride – EDTA (SE) buffer (p^H 8.0)

Sodium chloride	75mM	4.383g
EDTA	35mM	9.306g

Dissolved in 900ml distilled water and P^H adjusted to 8.0. Made up the volume to 1000ml, filtered, autoclaved and stored at 4°C.

Sodium dodecyl sulphate (SDS) 20%

SDS	20g
Distilled water to make upto	100 ml

Stirred, filtered and stored at room temperature.

Tris Acetate EDTA (TAE) buffer (50X)

Tris base	48.4g
Glacial acetic acid	11.42ml
0.5M EDTA (P^H 8.0)	20ml
Distilled water upto 1000ml	

Autoclaved and stored at room temperature.

Tris-Borate EDTA (TBE) buffer(p^H 8.3) 10X

Tris base	108.0g
Boric acid	55.0g
EDTA	9.3g

Dissolved in 700ml of distilled water and P^H adjusted to 8.3. Volume made upto 1000ml, autoclaved and stored at room temperature.

0.5X TBE Gel mix

40% Acrylamide	150ml
10X TBE buffer	50ml
Urea	450g
Mixed well in 700ml distilled water	

Volume made upto 1000ml and stored at 4°C.

Tris Buffered Saline (TBS) p^H 7.4

Sodium chloride	140mM	8.18g
Potassium chloride	0.5mM	0.0373g

Tris base   0.25mM   0.0303g

Dissolved in 900ml distilled water and p^H adjusted to 7.4. Made up the volume to 1000ml, filtered, autoclaved and stored at 4°C.

Tris EDTA (TE) buffer (p^H 8.0)

Tris base   10mM   1.2114g

EDTA   0.1mM   0.3722g

Dissolved in 900ml distilled water and adjusted the P^H to 8.0. Made up the volume to 1000ml, filtered, autoclaved and stored at 4°C.

Tris 1M (p^H 8.0)

Tris base   121.14g

Distilled water upto 1 litre. pH adjusted to 8.0, filtered and stored at room temperature.

ANNEXTURE – II

SOURCES OF IMPORTANT CHEMICALS AND ENZYMES USED IN THIS STUDY

(A) CHEMICAL

Acrylamide (Molecular Biology grade)	-	SRL, Bombay
Agarose (Low EED)	-	Bangalore Genei Pvt. Ltd.
Ammonium chloride	-	SRL, Bombay
Ammonium per sulphate	-	SRL, Bombay
N-N-Methylene Bis acrylamide	-	SRL, Bombay
Boric acid	-	SRL, Bombay
Chloroform	-	Merck
Crystalline phenol	-	Merck
Di-sodium hydrogen orthophosphate	-	SRL, Bombay
dNTPs.	-	Finn Enzymes
EDTA	-	SRL, Bombay
Ethanol	-	Merck
Ethidum bromide	-	BDH lab, England
6 X gel loading buffer	-	Bangalore Genei Pvt. Ltd.
Glacial acetic acid	-	BDH-E, Merck (India) Ltd.
Hydroxy quinolone	-	Qualigens Chemicals, Mumbai
Isoamyl alcohol	-	Merck

Methanol	-	SRL, Bombay
Potassium chloride	-	SRL, Bombay
Sodium acetate	-	SRL, Bombay
Sodium chloride	-	SRL, Bombay
Sodium dodecyl sulphate (SDS)	-	SRL, Bombay
TEMED	-	SRL, Bombay
Tris base	-	SRL, Bombay
Urea	-	SRL, Bombay

(B) PRIMERS
Imperial bio medics.

(C) MOLECULAR MARKERS

pBR322 DNA /Hae III digest	-	Bangalore Genei Pvt. Ltd.
M13 sequencing ladder	-	Amersham Pharmacia Biotech, USA.

(D) ENZYMES

Taq DNA polymerase	-	Bangalore Genei Pvt. Ltd.
Proteinase-K	-	Bangalore Genei Pvt. Ltd.
PNK	-	Bangalore Genei Pvt. Ltd.

(E) KITS

DNA-End-labelling kit	-	Bangalore Genei Pvt. Ltd.
Sequenase version 2.0 DNA sequencing kit	-	Amersham Pharmacia Biotech, USA.

(F) ISOTOPES

γ^{32} P-ATP	-	BRIT,(Jonaki), Hyderabad
α^{32} P -dATP	-	BRIT(Jonaki), Hyderabad.

ANNEXURE – III

ABBREVIATIONS

RFLP	Restriction Fragment Length Polymorphism
PCR	Polymerase Chain Reaction
VNTR	Variable Number of Tandem Repeat
RAPD	Random Amplified Polymorphic DNA
DNA	Deoxy Nucleic Acid
PAGE	Polyacrylamide Gel Electrophoresis
STR	Short Tandem Repeat
PIC	Polymorphic Information Content
QTL	Quantitative Trait Loci
EDTA	Ethylene Diamine Teraacetic Acid
DTT	Dithiothretiol
TEMED	N, N, N, N Tetramethylethylenediamine
APS	Ammonium Persulphate
PNK	Polynucleotide Kinase\
PE	Probability of Exclusion
CPE	Combined Probability of Exclusion
He	Heterozygosity
cM.	Centimorgan
µl	microlitres
µg	microgram
mg	milligram
mM.	millimolar

cm	centimeter
nm	nanometer
mCi	millicurie
Kb	Kilo basepair
Rpm	Revolutions per minute
SDS	Sodium Dodecyl Sulphate
dNTP.	Deoxy Nucleotide Triphosphate
ddATP.	Dideoxy Adenosine Triphosphate
ddCTP	Dideoxy Cytosine Triphosphate.
ddGTP.	Dideoxy Guanosine Triphosphate
ddTTP.	Dideoxy Thymidine Triphosphate

POLYMORPHISM OF OVINE FECUNDITY GENE LINKED MICROSATELLITE MARKERS IN MALABARI GOATS

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ABSTRACT

The objective of the present study was to explore the polymorphism of ovine fecundity gene linked microsatellite markers in Malabari goats. Malabari goats are one of the most prolific breeds in India. The microsatellite markers OarAE101, BMS2508 and BM1329 linked to the Booroola gene (Fec^B) and the microsatellite markers TGLA54 and TGLA68 linked to Inverdale ($FecX^I$) gene in sheep were selected for the study.

The DNA samples from 120 Malabari goats which had given birth to singles, twins, triplets and quadruplets in the second and subsequent parities were utilized for the study. DNA was isolated by phenol chloroform extraction procedure with some modifications. The DNA samples were amplified by PCR using the radioactively labeled microsatellite primers. The amplified products were resolved by polyacrylamide gel electrophoresis followed by autoradiography. The genotypes of animals were determined for each microsatellite loci by comparing the sizes of alleles with M13 phage DNA sequencing ladder.

The microsatellite markers OarAE101, BMS2508 and TGLA54 were found to be monomorphic in the population under study. The microsatellite markers BM1329 and TGLA68 were found to be highly polymorphic in Malabari goats.

A total of 15 alleles with 167-195 bp for the locus BM1329 and 8 alleles with a size range of 98-114 bp were observed for the locus TGLA68. The total number of genotypes observed was 34 for BM1329 locus and 12 for TGLA68 locus. Heterozygosity of 0.8660 for the locus BM1329 and 0.8024 for the locus TGLA68 were observed. The polymorphic information content (PIC) computed was 0.8526 and 0.7823 for the loci BM1329 and TGLA68 respectively.

A significant difference in the alleles 181bp and 191 bp (P≤0.01) and the alleles 179 bp and 185 bp (P≤0.05) and the genotype 177/191 (P≤0.01) for the locus BM1329 were found in different types of births. The genotype 175/185 of the microsatellite marker BM1329 was found to be significantly related to a higher litter size when compared to the mean litter size of the population in Malabari goats (P≤0.01).

The allele 104 bp of the locus TGLA68 was found to be significantly different in different types of births (P≤0.01). The highest frequency (0.4545) was observed in triplets followed by twins (0.2177) and singles (0.1847). The genotype 104/106 was found to be significantly different in different types of birth with triplets having a frequency of (0.4545), followed by singles (0.1087) and twins (0.0333).

The season of birth had no significant effect on the type of birth and number of kidding in Malabari goats.

Identification and selection of individuals that carry the alleles and genotypes associated with high prolificacy is possible in Malabari goats based on the above result. So new breeding strategies involving selection for high prolificacy can maximize the net profit of farmers. This study has brought to light important information improving the reproductive performance of Malabari goats by marker assisted selection.