GROWTH AND SURVIVABILITY OF GH/*Msp* I GENOTYPES IN MALABARI GOATS

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

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2004

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DECLARATION

I hereby declare that this thesis entitled **"GROWTH AND SURVIVABILITY OF GH/*Msp* I GENOTYPES 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.

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Certified that the thesis entitled **"GROWTH AND SURVIVABILITY OF GH/*Msp* I GENOTYPES IN MALABARI GOATS"** is a record of research work done independently by **Dr. Bindu Mathew**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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ACKNOWLEDGEMENTS

It is with immense pleasure that I record my sincere and heartfelt gratitude to the Chairman of the Advisory Committee, ***Dr. K.C. Raghavan*** *for the meticulous guidance, personal attention, keen interest, affectionate encouragement, persuasion and help offered to me from the initiation of work to the shipshaping of the manuscript. Without his strong support and co-operation, the successful completion of this work would not have been possible.*

I deem it my privilege to pay respect and deep sense of gratitude to ***Dr. K.V. Raghunandanan****, Director, Centre for Advanced Studies in Animal Genetics and Breeding and Member of the Advisory Committee. I wish to express my gratefulness to* ***Dr. T.V. Aravindakshan****, Assistant Professor, Department of Animal Breeding and Genetics and Member of Advisory Committee for sharing his professional experience and constructive review of my manuscript. I am grateful to* ***Dr. G. Krishnan Nair****, Associate Professor, Department of Microbiology and Member of Advisory Committee for his valuable advice and sound comments.*

I am in short of words to express my sincere thanks to ***Dr. K.A. Bindu****, Assistant Professor, Department of Animal Breeding and Genetics, for her wholehearted help and constant support. I wish to express my thankfulness to* ***Dr. T.V. Raja,*** *Assistant Professor, Department of Animal Breeding and Genetics, for the help he rendered in sample collection and statistical analysis. I would like to express my utmost gratitude to* ***Dr. A.P. Usha****, Associate Professor, Department of Animal Breeding and Genetics for her timely advice and concrete suggestions. I am grateful to* ***Dr. K. Anilkumar****, Assistant Professor, Department of Animal Breeding and Genetics, for his constant encouragement. I wish to express my sincere thanks to* ***Dr. Meera V. Menon****, Assistant Professor, Department of Agronomy, for her valuable help in correction of the thesis.*

I am grateful to the Dean, College of Veterinary and Animal Sciences for providing necessary facilities for the work.

*My sincere thanks are due to **Dr. Preethy. M.S**. for her timely support and encouragement during the whole period of course work and research. I remember with gratitude, **Dr. Chitra, Dr. Suprabha, Dr. Kowsigaraj** and **Dr. Jeeva** for sharing their experiences in research. I would like to express my gratitude to **Dr. Naicy, Dr. Amrita, Dr. Jeeba** and **Dr. Shymaja** for their support and timely help.*

*No words are sufficient to express my thanks to **Mrs. Ampily, Mr. Shajeer, Mr. Kunjiraman, Mrs. Rakhi** and **Mr. Babu**, for their help in sample collection. It gives me pleasure to record my sincere and heartfelt thanks to **Dr. Lydia, Miss. Sindhu, Mr. Varghese, Mrs. Sindhu, Mrs. Nisha, Mrs. Jolly** and **Mrs. Smitha** for their constant encouragement and wholehearted help. I express my sincere thanks to all other staff members of the Department for their help and encouragement.*

*With great fondness, I wish to express the salutary influence of my friends **Dr. Ambili, Dr. Rani, Dr. Seema, Dr. Dhanya, Dr. Josemi, Dr. Ashalatha, Dr. Smitha** and **Dr. Deepa**, for their warm friendship, affectionate encouragement and unfailing response, which enabled a fairly strenuous task to remain a pleasure throughout.*

*I appreciate with thanks the care and skill with which the typing and compiling of the thesis was done by **Mr. O.K. Ravindran**, M/s Peagles, Mannuthy.*

*Last but not least I owe a special appreciation and affection to my beloved **parents** and other **family members** for their unfailing fortitude provision and encouragement. Without their blessings and prayers it would not have been possible for me to complete the course in a successful manner.*

*Above all, I bow my head before the **Almighty** for the blessings showered on me and being with me in all my endeavors.*

BINDU MATHEW

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Introduction

1. INTRODUCTION

Goats constitute a very important species of livestock in India, mainly on account of the short generation interval, higher rate of prolificacy and the ease with which their products can be marketed. They contribute greatly to the agrarian economy, especially in areas where crop and dairy farming are becoming less economical. Thus goat farming plays an important role in the livelihood of a large proportion of small, marginal farmers and landless labourers.

Goats are the main meat animals in India (Pal and Agnihotiri, 1996). Their meat is the most preferred and represents almost 37 per cent of total meat produced in the country. India has a goat population of 124.5 million, which is around 17 per cent of global goat population (FAO, 2003).

India's vast genetic resources in goat are reflected by the existence of 20 breeds of goats (Acharya, 1982). Most of the breeds of goat have evolved mainly through natural selection, adaptation to agro-ecological conditions and, to a limited extent, by artificial selection for specific needs. The breeds are generally named after their place of origin or on the basis of prominent characteristics.

Malabari is one of the accredited goat breeds of southern India. Mixing of Jamunapari, Surti and Arab goats with local goats centuries ago on the northern Kerala coast led to the evolution of this prolific and highly adapted breed (Pattabhiraman, 1955). It is a dual-purpose breed, and is highly adapted to the tropical climate of Kerala. Even though a lot of research has been done to improve the breed based on the phenotypic characteristics, there has been little effort to study, conserve and further improve the breed using newer molecular technologies.

Molecular markers are capable of detecting variation at the DNA sequence level and possess unique genetic properties that make them more useful than other markers. Molecular markers are numerous and are distributed ubiquitously throughout the genome. They follow Mendelian inheritance and are multiallelic, with a mean heterozygosity of more than 70 per cent (Mitra *et al*., 1999) and are unaffected by environmental factors, age and sex. Their most important property is that the entire genome including the non-coding region can be visualized. These properties of the molecular markers have removed the limitations of the earlier markers such as morphological, chromosomal and biochemical markers.

There are several types of molecular markers, including RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), VNTR (Variable Number Tandem Repeats) and SNP (Single Nucleotide Polymorphism).

Molecular markers are "landmarks" at the genome that can be chosen for their proximity to Quantitative Trait Loci (QTL). The inheritance at the QTL itself cannot be observed, but that of a linked marker can be observed. Selection of animals by this method is known as marker assisted selection (MAS).

Genes involved in the biology of a trait are candidates for this purpose. Undoubtedly, genes coding for growth hormone (GH) fall into this category. Growth hormone, also referred as somatotropin, is a protein hormone produced in a subclass of pituitary acidophilic cells called somatotrophs. Growth hormone has somatogenic effect and lactogenic effect. It has a major impact on growth, lactation and mammary gland development in dairy animals. It has also been well documented that administration of either pituitary derived or recombinant DNA derived bovine growth hormone enhances milk production and milk components, including milk fat in dairy cows. Therefore the growth hormone

gene is a potential target for studies of molecular variation because of the possible direct or indirect effects upon growth and milk production.

Absence of certain growth hormone genotypes in the population is a strange phenomenon observed by some workers (Hoj *et al*., 1993; Lagziel *et al*., 1999; Chitra, 2002). The results have indicated the absence of GH/*Msp*1 (-/-) genotype in the population studied.

This could be due to many factors including sampling error, non-viability of GH/*Msp*1 (-/-) genotype and mortality at early ages of (-/-) genotypes. So an investigation has been planned to conduct purposeful mating of different genotypes, including heterozygous mating, with the following objectives.

- To estimate the frequencies of different GH/*Msp*1 genotypes in Malabari goat population of northern Kerala.

- To find out the survivability of different GH/*Msp*1 genotypes in the population.

- To find the relation between GH/*Msp*1 genotypes and growth rate.

Review of Literature

2. REVIEW OF LITERATURE

2.1 GENOMIC DNA ISOLATION

The preliminary stage in any molecular marker study is the extraction of genomic DNA. The common methods for extraction of genomic DNA include phenol-chloroform extraction, high salt method and guanidine hydrochloride method.

Blin and Stafford in 1976 introduced the new method of phenol-chloroform extraction for isolation of high molecular weight DNA from eukaryotes.

Beckman *et al.* (1986) and Andersson *et al.* (1986) also used the phenol chloroform method of extraction of DNA. Even though the methods suggested, involved use of toxic and corrosive phenol and several repeated extractions with phenol and chloroform, the yield was superior to those obtained by many other methods.

The phenol-chloroform extraction method was used for isolation of genomic DNA from Angus, Brahman, Hereford, Holstein and Jersey cattle by Rocha *et al*. (1992).

Apparao *et al*. (1994) developed an optimized phenol-chloroform extraction method for isolation of genomic DNA from blood for RFLP studies in different livestock species.

Senthil *et al*. (1996) compared the phenol-chloroform method and high salt method for isolation of DNA from cattle WBC and he observed that the high salt method was simple, rapid and yielded high molecular weight DNA.

The phenol-chloroform extraction method was used for isolation of genomic DNA from Korean native cattle steers by Choi *et al. (*1997).

Aravindakshan *et al*. (1998) compared the guanidine hydrochloride method, phenol-chloroform method and high salt method of extraction of DNA and found that high salt method was as equally effective as phenol chloroform method and that guanidine hydrochloride method was unsuitable for cattle samples.

2.2 PURITY AND YIELD OF DNA

Andersson *et al*. (1986) obtained a yield of 150 - 250µg DNA from 10 ml of whole blood of Swedish Red and White breed cattle using phenol - chloroform extraction method. Beckmann *et al*. (1986) also reported a yield of 300- 500 µg DNA per 10 ml of blood of Holstein-Friesian dairy bulls using the same method.

The average yields of DNA obtained from 100 samples of 20 ml blood of sheep by Montgomery and Sise (1990) were 640±0.26 µg and 500±0.19 µg by high salt method and phenol chloroform extraction method respectively. The optical density ratio (OD_{260}/OD_{280}) was in between 1.6 and 2.0.

Apparao *et al.* (1994) obtained DNA of good quality and suitability for RFLP studies. The yield was about 250 to 300 µg from 15 ml of blood from cattle, buffalo, goats, sheep and pigs. Senthil *et al*. (1996) obtained an average yield of 444.58 ± 21.5 µg from 15 ml of cattle blood.

Aravindakshan *et al*. (1998) obtained an average yield of 394.50 ± 34.26 µg using phenol - chloroform method in cross bred cattle and the ratios of optical density at 260 and 280 nm were consistently between 1.75 and 1.90, indicating good deproteinisation.

2.3 GROWTH HORMONE

Growth hormone (GH), also referred to as somatotropin, is a protein hormone produced in a subclass of pituitary acidophilic cells called somatotrophs. In terms of weight, it is the most abundant hormone of the anterior pituitary, accounting for about 10 per cent of dry weight. The bovine GH has been isolated from the pituitary gland and its amino acid sequences has been elucidated as two different amino acid sequences containing either 190 or 191 amino acids (Wallis, 1975; Kostyo and Reagan, 1976).

The crystal structure of the hormone has been resolved. The hormone has an anti- parallel four-helix bundle core, with a characteristic up-up-down-down topology. There are two binding sites for the growth hormone receptor on each GH molecule (Sherin *et al*., 1987).

2.4 EFFECTS OF GROWTH HORMONE

In terms of physiological roles of GH, it has been known that GH exerts, either directly or indirectly, anabolic responses such as bone growth, cell division and protein synthesis, increases the oxidation of lipid and inhibits the transport of glucose into body tissues (Pell and Bates, 1990). Growth hormone orchestrates many diverse physiological processes so that more nutrients are used for lean tissue accretion, which is referred to as somatogenic effect or for milk synthesis, which is referred as lactogenic effect (Bauman and Vernon, 1993). Growth

hormone, due to its essential role in lactation and growth processes, is a perfect candidate marker associated with somatotropic axis (Parmentier *et al*., 1999).

2.4.1 Effects on Lactation

Asimov and Krouze (1937) reported for the first time that injection of crude extracts of bovine anterior pituitary glands enhanced milk production in lactating cows. Cowie *et al*. (1964) demonstrated that GH was the major galactopoietic hormone and was essential for the maintenance of normal lactation in ruminants. Bauman *et al*. (1982) conducted the first study with dairy cows using recombinant bovine growth hormone. It has been well documented that administration of either pituitary derived or recombinant DNA derived bGH enhances milk production (Bitman *et al*., 1984; Bauman *et al*., 1985; Eppard *et al*., 1985; Eppard *et al.,* 1991; Jordan *et al.*, 1991; Gibson *et al.,* 1992; Lynch *et al*., 1992; Hansen *et al*., 1994; Bauman *et al.*, 1999).

The positive effect of recombinant bovine growth hormone in goat milk yield was studied by Malveiro *et al*. (2001) and Boutinaud *et al*. (2003).

2.4.2 Effect on Growth

The growth promoting effects of GH in pituitary extract was first described in the early 1930's when it was observed that treatment of rats with a crude preparation of GH significantly enhanced body weight gain (Evans and Simpson, 1931; Lee and Schaffer, 1934). The effect of exogenous growth hormone on body growth has been extensively studied and many workers have reported an increase in body weight gain (Brumby, 1955; Bauman *et al*., 1982).

2.5 GROWTH HORMONE GENE

The bovine growth hormone gene is a part of a multiple gene family that contains prolactin and the placental lactogens. The gene is approximately 1800 bp with five exons (I through V) and four introns (A through D) (Woychick *et al*., 1982; Gordon *et al*., 1983). Growth hormone gene, a member of synteny 21 (Fries *et al*., 1989) has been assigned by *in situ* hybridization to bovine chromosome 19q 26 qter and sheep chromosome 11 q 25 qter (Hediger *et al*., 1990). The caprine growth hormone gene has been assigned to chromosome 19q 22 qter. The porcine GH gene is localized in chromosome 12 within a regional assignment 12p14 (Chowdhary *et al*., 1994). It comprises five exons with a total transcribed length of 1.7 kb.

The ovine growth hormone gene is similar to that found in bovines and has a primary transcript of 1792 bp, with five exons and with intron sizes of 264 bp, 231 bp, 227 bp and 273 bp. The gene is flanked by artiodactyl-specific middle repetitive DNA, consisting mainly of elements belonging to the 'C-A3' family of repeated DNA (Byrne *et al.*, 1987). Yato *et al*. (1988) had sequenced goat growth hormone gene and found that it encoded 217 amino acids.

On the basis of studies on a restriction fragment length polymorphism, Valinsky *et al*. (1990) concluded that there are two alleles at the growth hormone gene locus in sheep and goats. In sheep, in one allele (Gh1), the growth hormone gene is represented by a single copy (GH1), while in the other (Gh2) the gene is duplicated (GH2-N (5') and GH2-Z (3') genes) (Wallis *et al.*, 1998).

Yamano *et al*. (1991) investigated growth hormone genes in a goat genomic library and found two types of fragments, one containing a single GH gene (gGH1) and the other containing two genes arranged at tandem (gGH2 and

gGH3). The tandem arrangement of the gGH2 and gGH3 genes is similar to that seen for the ovine GH2-N and GH2-Z genes.

2.5.1 Species Homology

The sequence of GH from Indian species of cattle, buffalo and goat are identical (Mukhopadhyay and Sahni, 2002). The amino acid sequence for bovine GH and ovine GH differs only by a single position and thus partly explains why bGH is biologically active in sheep. However growth hormones from other species differ moderately from each other. Bovine GH and porcine GH share 90 per cent sequence homology. Both bovine GH and porcine GH have only 65 per cent homology with human GH. This partly explains the ineffectiveness of bovine GH and porcine GH on human growth.

The growth hormone amino acids sequence homologies between goat and bovine, rat and human were 99, 83 and 66 per cent respectively (Yamano *et al.* 1988). The species homology of growth hormone gene in cattle, rat and human being are presented in Table 1.

Table 1. Amino acid and nucleotide homologies

Amino acid and nucleotide	**bovine/ rat (%)**	**bovine/ human (%)**	**rat/ bovine (%)**
Amino acid sequence			
Identical	83.5	66.8	64.4
Conservative differences	75.0	58.9	64.1
Nucleotides			
Identical nucleotide	83.9	76.5	74.9
Identical codon	61.0	48.6	42.0
Two identical nucleotides / codon	30.7	36.8	43.8
Not expressed	70.1	48.1	50.0

(Miller *et al*., 1980)

2.6 POLYMORPHISM AT GROWTH HORMONE GENE LOCUS

Allelic variation in the structural or regulatory sequences of growth hormone would be of interest because of possible direct or indirect effects on milk production and growth performance. Also variations in introns or flanking sequences have potential usefulness as genetic markers. Consequently because GH is involved in lactation, GH gene has potential, as marker for genetic variation in milk production traits, and linkages to RFLP around these genes with QTL for milk traits may be possible.

Several polymorphic regions of the growth hormone gene have been reported.

Lucy *et al*. (1993) reported a polymorphic region in exon V at position 2141 that results in a change in amino acid from valine to leucine. Zhang *et al*. (1993) reported a polymorphic site in intron C at position 1547.

These two polymorphic regions were detected with PCR-RFLP techniques and the positions reported were according to the sequence published by Gordon *et al*. (1983).

Gootwine *et al.* (1993) had observed a *Pvu* II polymorphism in the second intron in the duplicate copy of ovine GH gene.

Investigation of bovine GH has resulted in detection of two RFLPs –an insertion/deletion of approximately 0.9-kb in 3' region of the gene and a polymorphic *Msp*1 restriction site in the third intron. These RFLPs have been shown to be present in several breeds of cattle (Hallerman *et al.,* 1987; Cowan *et al*., 1989; Hilbert *et al*., 1989; Hoj *et al.,* 1993; Aravindakshan *et al.,* 1997) and in goats (Chitra, 2002).

According to the sequence of the growth hormone gene published by Woychick *et al*. (1982), Yao *et al*. (1996) mapped the polymorphic *Msp*1site to position 1547-1548 and opined that it was due to C to T transition at position 1547.

The porcine GH gene reveals some single nucleotide polymorphism in exons, introns as well as in the regulatory sequences. Among the last category, Nielsen *et al*. (1993) found two variants of TATA box sequence – TATAAA (allele TATA1) and TATATA (allele TATA2).

2.7 INCIDENCE OF DIFFERENT GH/*Msp* I GENOTYPES

Hoj *et al.* (1993) observed an absence of -/- genotype in the Red Danish and Red Norwegian cattle. The insertion/deletion polymorphism was used for selection of high fat lines.

In the Polish Holstein-Friesian cattle only *Msp* I (-) allele of the GH gene was identified by Zwierzchowki (1994). Among 56 Sahiwal and Zebu cattle typed, Mitra *et al.* (1995) observed all the three genotypes out of which 42 were of (-/-) genotype.

The GH/*Taq*I polymorphism which corresponded to the GH/*Msp* I polymorphism revealed two genotypes AA and AB. The BB genotype was not reported in Holstein-Friesian cattle by Falaki *et al.* (1996).

Aravindakshan *et al.* (1997) observed all the three genotypes in Jersey crossbred and Ongole cattle with a higher frequency of heterozygotes in Jersey. However Murrah and Surti were monomorphic for the (+/+) genotype, indicating species character.

Lagziel *et al.* (1999) observed only 2 genotypes, the +/- and +/+. Out of the 523 Israel Holstein cattle typed, 246 were (+/-) and 277 were homozygous (+/+) for the polymorphic *Msp* I site.

Aggrey *et al.* (2001) reported the presence of three genotypes in Holstein bulls. The absence of one of the homozygous genotypes has been described in several commercial lines of pigs with regard to most genes examined in studies on the relationship between genotype and carcass quality traits (Kulig *et al.*, 2001).

Dybus (2002) obtained the three genotypes on GH/*Msp* I polymorphism in Polish Black and White cattle with a higher frequency of homozygotes. Urban *et al.* (2002) observed all the three genotypes on GH/*Msp* I polymorphism in pigs.

Sorensen *et al*. (2002) reported that VV genotype did not exist in Danish Holstein and Danish red cattle.

GH/*Alu* I polymorphism in Sahiwal and Holstein-Friesian revealed the presence of only two genotypes, LL and LV. The heterozygotic percentage was high in Jersey cattle (Biswas *et al.*, 2003).

Kuryl *et al.* (2003) studied GH/*Hae* II polymorphism in exon 2 and GH/*Msp* I polymorphism in intron 2. All the pig breeds under study had a higher heterozygotic frequency. In GH/*Hae* II polymorphism, Pietrain and Stambock breed did not possess one of the homozygous genotypes.

In Gyr breed, a higher frequency of the (-/-) genotype was noted by de Mattos et *al.* (2004).

2.8 GENE AND GENOTYPE FREQUENCY OF GH/*Msp* I GENOTYPES

Gene frequency of 0.26 was noted for C allele in 35 Holstein bulls by Zhang *et al.* (1993). High gene frequency of 0.81 and 0.95 was obtained for (+) allele in Red Danish and Norwegian line (Hoj *et al.*, 1993).

Mitra *et al.* (1995) reported a frequency of 0.14 and 0.86 respectively for the (+) and (-) *Msp* I allele in Sahiwal cattle. Murrah and NiliRavi buffalo were monomorphic at the site with a (+) allele frequency of 1.0000.

Falaki *et al.* (1996) obtained a genotype frequency of 0.7033 for the AA genotype and 0.2970 for the AB genotype. Sabour *et al.* (1996) had reported a frequency of 0.9 for the (+) allele in Holstein cattle. Aravindakshan *et al.* (1997) reported a higher frequency of heterozygotes (0.43) in Jersey crossbred and a frequency of 0.87 was noted in Ongole breed for +/- genotype.

Chung *et al*. (1998) reported an allele frequency of 0.76 and 0.78 for the A allele in Korean cows and bulls. A frequency of 0.97 was obtained in Holstein for the (+) allele by Vukasinovic *et al.* (1999).

Faria *et al.* (1999) obtained a gene frequency of 0.13 and 0.87 for the (+) and (–) allele respectively. Lagziel *et al*. (2000) reported a low frequency of *Msp* 1 (-) allele in breeds of northern Europe.

The GH (+/+) genotype was found to be most frequent in Polish Black and white cattle studied by Dybus *et al.* (2002). This was followed by (+/-) (0.118 – 0.388) and the least frequent was (-/-) genotype (0.013 – 0.030). Frequency of 0.81 was noted for *Msp* (-) allele in Gyr bulls by de Mattos *et al.* (2004).

2.9 TESTING OF HARDY WEINBERG EQUILIBRIUM

Falconer and Mackey (1996) described the method to test whether the population under study was in Hardy-Weinberg equilibrium. This method of testing the population was used by Moody *et al*. (1996) and Yao *et al*. (1996).

2.10 GENE AND GENOTYPE FREQUENCY IN DIFFERENT TYPES OF MATING

Limited information is available on GH/*Msp*1gene frequencies in parental and progeny generations. Lagziel *et al.* (1999) had typed 523 daughters of the sires heterozygous for haplotype E (+/-). Of the 523 daughters, 246 were +/- genotype and the remaining 277 were of (+/+) genotype in Holstein-Friesian cattle.

2.11 INCIDENCE OF ABORTION, STILL BIRTH AND MORTALITY

The overall mortality rate was found to be about 5 per cent in Malabari goats (Raghavan *et al.*, 2004). Literature regarding mortality and GH polymorphism were not available.

2.12 INCIDENCE OF MULTIPLE BIRTH AND KIDDING PERCENTAGE

Pattabhiraman (1955) observed a general tendency for twin births in Malabari goats. The number of kids per 100 kidding was reported to be 197.14, 171.33 and 176.18 respectively for Malabari goats of Thanur, Badagara and Thalassery (Raghavan and Raja. 2004). Raghavan *et al*. (2004) reported that under field conditions the percentage of single, twins, triplets and quadruplets were 32.15, 53.14, 12.86 and 1.45 respectively in Malabari goats.

2.13 RELATIONSHIP BETWEEN GROWTH TRAITS AND GH POLYMORPHISM

Rocha *et al*. (1992) reported an association between birth weight as a maternal trait and a genomic DNA *Taq* 1 allele that hybridized to a GH cDNA probe.

Schlee *et al*. (1994) determined genotypes for polymorphism in exon V of Bavarian Simmental bulls and reported that there was significant effect of the heterozygous genotype on carcass gain and meat value.

Moody *et al*. (1996) reported that in GH/*Alu*1polymorphism, GH-B allele was associated with a significant increase in 180-day gain. He also reported that the effect of allele substitution on gain from weaning to yearling was significant for the GH polymorphism.

An attempt was made by Aravindakshan *et al.* (1997) to correlate the different GH/*Msp*I genotypes and birth weight and adult body weights in Jersey crossbred animals. But no significant variation ($P \leq 0.05$) in the mean birth and adult body weights could be observed between different GH/*Msp*I genotypes.

Pierzchala *et al.* (1999) studied associations of polymorphism of GH/*Hae*II and GH/*Msp*1 loci and reported that the haplotypes differed significantly in percentages of lean meat and in several traits of carcass fatness. The highest weight of ham with or without shank and weight of ham meat was in pigs of genotype BB at GH/*Hae*II. Weight of ham with and without shank as well as weight of ham meat was significantly higher in pigs of genotypes AB at locus GH/*Msp*1.

Cheng *et al.* (2000) studied *Taq*1 and *Ara*1 polymorphism and intensity of growth in pigs of Duroc, landrace and Tao-Yuan breeds. A significant effect of GH gene was found only in Tao-Yuan breed.

In majority of the studies with GH/*Alu*1 polymorphism, genotype VV demonstrated lower growth rate than individuals with LL/LV genotypes. (Switanski, 2002)

di Stasio *et al*. (2002) suggested a lack of association between GH gene and meat production traits in Piedmontese cattle.

The average daily gain of weight was high in *Msp*1 (+/-) heterozygote in Duroc pigs in a study conducted by Urban *et al.* (2002).

In Holstein-Friesian cattle, the LV heterozygous animals had significantly higher birth weight ($P \leq 0.01$) than LL genotype (26.75 vs 22.00 kg). In higher birth weight group of Holstein-Friesian cattle, the frequency of V allele was found to be significantly higher than lower birth weight group (Biswas *et al.*, 2003).

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Animals for the study consisted of 32 bucks, 241 does mated to those bucks and their 297 progenies of Malabari goats belonging to three areas of Thalasseri, Badagara and Thanur as well as Malabari goat conservation unit of Regional Agricultural Research Station, Pilicode.

3.2 COLLECTION OF SAMPLES

Genomic DNA was isolated from blood. About 5 ml of blood was collected from jugular vein using sterile disposable syringes and transferred immediately to sterile disposable centrifuge tubes containing EDTA (1 mg/ml of blood) as anticoagulant. The samples were brought under refrigerated conditions to the laboratory and stored at 4°C till processed.

3.3 EXTRACTION OF GENOMIC DNA

Genomic DNA from whole blood was extracted using phenol chloroform extraction method (Andersson *et al*., 1986) with some modifications.

3.3.1 Phenol Chloroform Extraction Method

1. Double quantity of ice-cold RBC lysis buffer was added to 5 ml of blood sample and kept in ice with occasional mixing for 10 minutes for complete lysis of RBCs.

2. The leucocytes were spun down at 4000 rpm for 15 minutes and the supernatant containing lysed RBCs was discarded.

3. Steps one and two were repeated with minimal quantity of RBC lysis buffer, till the pellet was clear without any unlysed erythrocytes.

4. The pellet was washed twice with TBS buffer by centrifugation at 3000 rpm for 10 minutes.

5. Five millilitres of SE buffer was added and the pellet was re-suspended by vigorous vortexing.

6. Twenty five microlitres of proteinase K (20 mg/ml) and 0.25 ml of 20 per cent SDS were added, mixed well and incubated at 50°C in a waterbath for 3 hours, with occasional shaking.

7. The digested samples were cooled to room temperature and 300 µl of 5*M* NaCl was added and mixed by vortexing.

8. The digested sample was extracted with an equal volume of saturated phenol (pH 7.8) and the aqueous phase was collected in fresh tubes.

9. The aqueous phase was then extracted with phenol: chloroform: isoamyl alcohol (25:24:1) and the aqueous phase was collected in fresh tubes.

10. The aqueous phase containing DNA was then extracted with chloroform: isoamyl alcohol (24:1).

11. The supernatant was transferred to a sterile beaker and one-tenth volume of 3*M* sodium acetate (pH 5.5) was added.

12. An equal volume of isopropyl alcohol was added and the precipitated DNA was spooled out on a clear micropipette tip, rinsed in 70 per cent ethanol, air-dried and re-suspended in 0.5 ml of TE buffer and stored at –20°C.

The composition and source of various reagents and buffers used in the study are presented in the annexure I and II.

3.3.2 Quantification and Quality Check of DNA

From the stock solution, 20 µl of sample was diluted with 1980 µl of distilled water. Optical densities were measured at 260 and 280 nm wavelength in an UV spectrophotometer.

3.3.2.1 Yield

At 260 nm, an absorbance of 1 measured in a cuvette with a 1 cm path length is indicative of double stranded DNA at a concentration of approximately 50 µg/ml.

Concentration of DNA stock solution in µg/ml = OD 260 value x 50 x dilution factor.

The total yield of DNA was calculated by multiplying the concentration and the volume of DNA stock solution.

3.3.2.2 Purity

Purity was assessed by estimating the ratio between the readings at 260 nm and 280 nm wavelengths. Pure DNA samples have the ratio 1.8.

3.3.2.3 Quality Checking

The quality and molecular weight of DNA was assessed electrophoretically using 0.7 per cent agarose. 0.7 g of agarose was weighed and added to 100 ml of 1 x TAE buffer in a conical flask, mixed gently and boiled. An appropriate sized gel tray was washed and sealed. Combs were kept in position and the tray was kept on an even surface. The molten agarose was cooled, 0.5 µg/ml of ethidium bromide was added and then poured onto the tray and allowed to polymerise. After gelling, the comb and sealing were removed gently and the gel tray was immersed in the 1 x TAE buffer in the electrophoresis tank. About 0.5-1µg of DNA was mixed with one-sixth the volume of 6x gel loading buffer and then loaded into the wells. Electrophoresis was carried at 2V/cm at room temperature until the bromophenol blue dye migrated more than two third of the length of the gel. The gel was visualized under UV transilluminator.

3.4 PCR-RFLP TYPING

The polymorphism study was based on PCR-RFLP technique. The amplified product was digested with *Msp*1 restriction enzyme to analyse the genotypes.

3.4.1 Template DNA Preparation for PCR

Template DNA for PCR was prepared by diluting the DNA stock solution with sterile triple distilled water to a concentration of 50 ng/µl.

3.4.2 Selection and Dilution of Primers

Primers for the study were obtained from Invitrogen India Pvt. Ltd. and were custom synthesized. The sequence of the primer was as presented below:

5'AGAATGAGGCCCAGCAGAAATC3'
5'GTCGTCACTGCGCATGTTTG 3'

The primers obtained in lyophilized form were reconstituted in sterile distilled water to a concentration of 200 p*M*/µl. The stock solution was distributed in 10 µl aliquots and further diluted 10 folds before using for PCR.

3.4.3 PCR-setting

The master mix containing 10x PCR buffer, 50 m*M* $MgCl_2$, dNTP mix, primer pairs, *Taq* DNA polymerase and water was prepared such that a final concentration of 1 x PCR buffer, 1.5 m*M* $MgCl_2$, 200 µm dNTPs, 20 p*M* of each primer and one unit *Taq* DNA polymerase in a total volume of 25 µl was obtained. The master mix was mixed thoroughly. The reaction was carried out in 200 µl reaction tubes. To each reaction tube 24 µl of master mix and 1 µl of 50 ng DNA was added. The tubes were spun briefly and placed in a thermal cycler.

The thermal cycling profile consisted of an initial denaturation of 3 minutes at 94°C and 35 cycles of 1 minute at 94°C, 1 minute at 60°C and 1 minute at 72°C. This was followed by final extension of 7 minutes at 72°C. The amplified products were stored at –20°C till analysed.

3.4.4 Checking of Target DNA Amplification

The PCR products were checked by agarose gel electrophoresis. Three µl of the product was checked electrophoretically using 1 per cent agarose gel in 1xTAE buffer. The product size was confirmed using 100 bp ladder and ϕx174 DNA/*Hae* III digest as DNA size markers.

3.4.5 Analysis of Amplified Products

For analysis, ten µl of the amplified product was digested with 15 units of *Msp* I enzyme at 37°C for 2 hours in a final volume of 15 µl. Mastermix containing the enzyme, 10x assay buffer and distilled water was prepared and 5 µl of the mix was distributed to 10 µl of amplified products taken in separate tubes. Incubating the tubes at 65°C for 30 minutes after the digestion inactivated the enzyme. The digested products were stored at –20°C till analysed.

3.4.6 Separation of Restriction Fragments

The DNA fragments were separated by electrophoresis in 3 per cent agarose gels in 1 x TAE buffer at 2V/cm for 3 hours. 100 bp DNA ladder and ϕx 174 DNA/*Hae* III digest were used as DNA size markers. The gels were visualized and the images were documented in a gel documentation system (Bio Rad Laboratories, USA).

3.5 SCREENING OF ANIMALS FOR GH/*Msp* 1 GENOTYPES

Based on the gel readings the parents (bucks and does) were classified into the category GH/*Msp*1 (+/+) and (+/-) genotypes. The mating groups were classified as (1) heterozygous mating which included (+/-) bucks and (+/-) does, (2) heterozygous and homozygous mating consisting of two types of mating: a) (+/-) bucks x (+/+) does and b) (+/+) bucks x (+/-) does and (3) homozygous mating which included (+/+) bucks x (+/+) does. The progenies obtained were also typed for the GH/*Msp*1 polymorphism and classified according to the genotype.

3.6 COLLECTION OF DATA.

Information about each experimental animal and data on production and reproduction were collected and recorded in the data-recording sheet (Annexure 111). Body weights of the progenies from all matings were recorded at bi-weekly intervals upto six months of age.

Details regarding birth such as stillbirth, singles, twins, triplets or quadruplets and abortion and mortality rates of progeny were recorded. Kidding percentage was also recorded.

3.7 STATISTICAL ANALYSIS

The data were analysed using the methods by Panse and Sukhatme (1954).

Results

4. RESULTS

4.1 GENOMIC DNA ISOLATION

DNA was isolated using phenol -chloroform extraction method from 5 ml of venous blood collected from 32 bucks, 241 does mated to those bucks and their 297 progenies of Malabari breed in three areas of Badagara, Thalassery, Thanur as well as the Malabari Conservation Unit of Regional Agricultural Research Station, Pilicode.

4.2 YIELD AND PURITY OF DNA

The average yield and optical density (O.D) ratio of DNA in the present study are presented in the Table 2. The average yield (Mean ± SE) of DNA from 5 ml of blood was 350.056 ± 10.048 μg. The concentration of DNA averaged about 700.113 ± 20.095 μg/ml. The ratios of optical density at 260 and 280 nm were consistently around 1.7, indicating good deproteinisation. DNA appeared as single bands without sheared fragments on agarose gel electrophoresis.

4.3 PCR-AMPLIFICATION OF GH GENE

A 768 bp fragment enclosing the sequence from the third exon (position + 743) to the fifth exon (position +1511) in the GH gene was amplified (Fig.1).

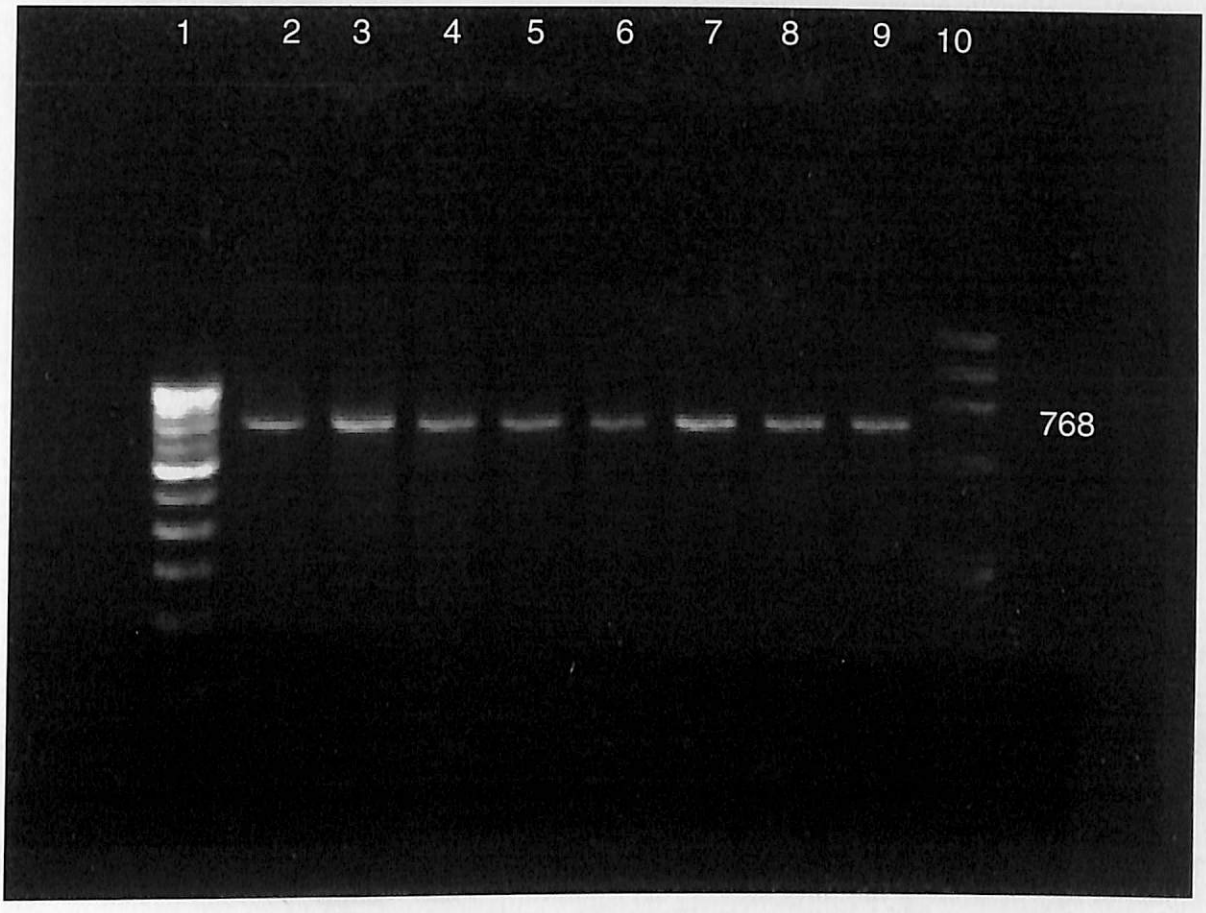

Figure1 . The 768 bp fragment of growth hormone gene amplified by PCR

Lane 1 : Marker - 100 bp DNA ladder.
Lane 2 - 9 : The 768 bp fragment of growth hormone gene.
Lane 10 : Marker ϕ x 174 DNA/*Hae* III digest.

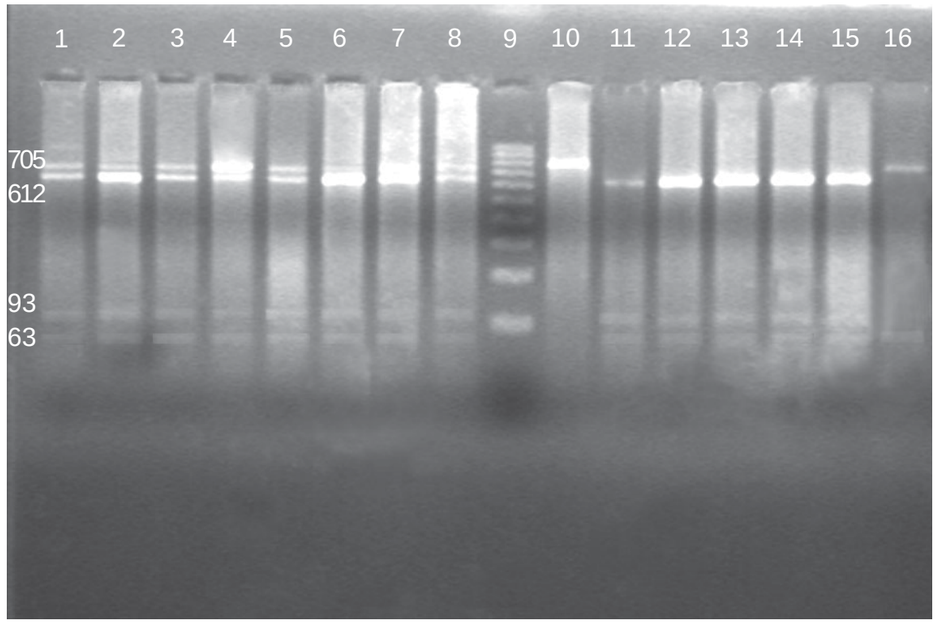

Figure 2 . The *Msp* I polymorphism of growth hormone gene detected by PCR- RFLP

Lane 1- 8 : +/- GH/ *Msp* I genotype (705, 612, 93 & 63 bp fragments).

Lane 9 : marker - 100 bp DNA ladder.

Lane 10 : 768 bp amplified product.

Lane 11 - 15 : +/+ GH/ *Msp* I genotype (612, 93 & 63 bp fragments).

Lane 16 : -/- GH/ *Msp* I genotype (705 & 63 bp fragments).

4.4 PCR-RFLP ANALYSIS AT GH GENE LOCUS USING *Msp* I

The polymorphic *Msp* I site in the third intron of growth hormone gene was detected by PCR-RFLP. Upon electrophoresis two digestion patterns could be observed. These allowed the identification of two alleles, *Msp* I (+) allele and *Msp* I (-) allele. The *Msp* I (+) allele produced 612, 93 and 63 bp fragments while the *Msp* I (-) allele produced the 705 and 63 bp fragments (Fig.2).

4.5 INCIDENCE OF DIFFERENT GH/*Msp*I GENOTYPES IN MALABARI GOAT POPULATION

Typing for GH/*Msp* I genotypes was performed for a total of 32 bucks, 241 does and their 297 progenies. Incidence of different GH/*Msp*I genotypes in Badagara, Thalassery, Thanur, Pilicode and the pooled population are presented in Table 3. Of the 570 animals typed, none of the progenies/does/bucks were homozygous for the *Msp* I (-) allele, indicating that the frequency of this allele in the general population of Malabari goats is effectively zero. The percentage of heterozygous animals was higher than expected in all the different centers. Statistical analysis using Z test revealed a significant difference in the incidence of different GH/*Msp*1 genotypes in Badagara population from the other three ($P \leq 0.05$).

4.6 GENE AND GENOTYPE FREQUENCY OF GH/*Msp* I POLYMORPHISM IN BUCKS AND DOES

The center-wise gene and genotype frequencies of bucks and does in different centers are presented in the Table 4. Of the 32 bucks typed for GH/*Msp*1 polymorphism, (+/-) genotype was more frequent. The genotype frequency of +/- genotype was 0.6875 in the pooled population. The (+) and (-) allele frequencies in the total buck population was 0.6562 and 0.3437

respectively. Among the 241 does genotyped, about 61% were of (+/-) genotype. None was of (-/-) GH/*Msp*1genotype. The gene and genotype frequencies of does of Badagara was found to be significantly different from other populations on applying the Z test ($P \leq 0.05$).

4.7 TESTING OF HARDY WEINBERG EQUILIBRIUM

The GH/*Msp*I genotypes were tested for Hardy Weinberg Equilibrium using Chi-square test. The test revealed a significant deviation from equilibrium (Table 5).

4.8 GENOTYPE AND GENE FREQUENCIES OF PROGENY IN DIFFERENT TYPES OF MATING

Table 6 shows the frequency of different genotypes and alleles in different types of mating. The matings were classified into three based on the genotypes of bucks and does. It includes (1) heterozygous mating (+/- bucks x +/- does), (2) heterozygous and homozygous mating (+/-bucks x +/+ does or +/+ bucks x +/- does) and (3) homozygous mating (+/+ bucks x +/+ does).

In the heterozygous mating, the frequency of heterozygote genotype in the progeny population was more than expected and the frequency of GH/*Msp*1 (+/+) homozygote genotype was less than expected. The allele frequency of 0.64 and 0.36 was noted for the (+) and (-) allele in the progeny population.

Allele frequencies of 0.70 and 0.30 were observed in the heterozygous-homozygous mating in the pooled population.

The homozygous (+/+) mating produced only homozygous (+/+) progenies and a frequency of 1.00 was noted for the (+) allele.

4.9 INCIDENCE OF ABORTION, STILL BIRTH AND MORTALITY IN DIFFERENT TYPES OF MATING

The major reasons for kid loss in the total population were stillbirth and mortality at early ages (Table 7). Abortions were not noticed in any of the populations. Total kid loss before one month of age was found to be more in progenies of heterozygous mating. The percentage of kid loss in the heterozygous mating was 4.27 while that of heterozygous x homozygous and homozygous matings were 0.71 and 0.35 per cent respectively.

4.10 INCIDENCE OF MULTIPLE BIRTH AND KIDDING PERCENTAGE IN DIFFERENT TYPES OF MATING

The percentage of singles, twins, triplets and quadruplets of total kids born and the kidding percentage of does in different types of mating are shown in Table 8. The percentage of singles were 20, 20 and 10 in +/- x +/-, +/- x +/+ or +/+ x +/- and +/+ x +/+ mating and it was 80, 80 and 90 for multiple births. Kidding percentage of 1.9, 1.8 and 2 was noticed in the heterozygous mating, homozygous x heterozygous and the homozygous dominant matings.

4.11 RELATIONSHIP BETWEEN GH/*Msp*I GENOTYPES AND BODY WEIGHT UPTO SIX MONTHS

The mean ± SE of body weights at birth, one, three and six months are presented in the Table 9. There was no significant difference between the +/- and +/+ genotypes in body weight at any stages. A slightly higher mean value was noted for the heterozygote population.

Table 2. Yield and purity of DNA

Parameters	**Mean± S.E**
Concentration µg/ml	700.113 ± 20.095 µg
Yield/5ml	350.056 ± 10.048 µg
O.D Ratio(260nm/280 nm)	1.69 ± 0.006

Table 3. Incidence of different GH/*Msp*1 genotypes in Malabari goat population

Population	**GH/*Msp*I genotypes (%)**		
	+/+	+/-	-/-
Badagara* (185)	29.18 (54)	70.82 (131)	0.00 (0)
Thalassery (166)	42.16 (70)	57.84 (96)	0.00 (0)
Thanur (156)	44.23 (69)	55.77 (87)	0.00 (0)
Pilicode (63)	39.68 (25)	60.32 (38)	0.00 (0)
Total (570)	38.24 (218)	61.76 (352)	0.00 (0)

Figures in parenthesis are observed numbers.
*Significantly different at 5 % level.

Table 4. Category and center - wise genotype and gene frequency of GH/*Msp*1 genotypes

Category	Centre	GH/*Msp*1 genotype frequency		Gene frequency	
		+/+	+/-	+	-
Bucks	Badagara[a] (11)	0.3636 (4)	0.6363 (7)	0.6818	0.3182
	Thalassery[a] (9)	0.3333 (3)	0.6666 (6)	0.6666	0.3333
	Thanur[a] (8)	0.3750 (3)	0.6250 (5)	0.6875	0.3125
	Pilicode[a] (4)	0.0000 (0)	1.0000 (4)	0.5000	0.5000
	Total (32)	0.3125 (10)	0.6875 (22)	0.6562	0.3437
Does	Badagara[b] (77)	0.2337 (18)	0.7662 (59)	0.6168	0.3831
	Thalassery[a] (60)	0.4833 (29)	0.5166 (31)	0.7416	0.2583
	Thanur[a] (80)	0.4625 (37)	0.5375 (43)	0.7312	0.2687
	Pilicode[a] (24)	0.4166 (10)	0.5833 (14)	0.7083	0.2916
	Total (241)	0.3900 (94)	0.6099 (147)	0.6950	0.3050

Values with same superscript do not differ
Figures in parenthesis are observed numbers.

Table 5. Testing of Hardy Weinberg Equilibrium.

Source	GH/*Msp*1 Genotypes			χ^2
	++	+/-	-/-	
Observed	218	352	0	113.613**
Expected	272	243	54	

** Significant at 1% level

Table 6. Genotype and gene frequency of progeny in different types of mating

Mating	Centre	GH/*Msp*1Genotype frequency		Gene frequency	
		+/+	+/-	+	-
+/- x +/-	Badagara (74)	0.2297 (17)	0.7702 (57)	0.6148	0.3852
	Thalassery (55)	0.3090 (17)	0.6909 (38)	0.6545	0.3454
	Thanur (32)	0.4375 (14)	0.5625 (18)	0.7187	0.2812
	Pilicode (14)	0.0714 (1)	0.9285 (13)	0.5357	0.4642
	Total (175)	0.2800 (49)	0.7200 (126)	0.6400	0.3600
+/+ x +/- or +/- x +/+	Badagara (22)	0.4090 (9)	0.5909 (13)	0.7045	0.2954
	Thalassery (36)	0.5238 (14)	0.4761 (22)	0.6944	0.3050
	Thanur (27)	0.5454 (12)	0.4545 (15)	0.7222	0.2777
	Pilicode (11)	0.2727 (3)	0.7272 (8)	0.6363	0.3636
	Total (96)	0.4605 (38)	0.5394 (58)	0.6979	0.3020
+/+ x +/+	Badagara (1)	1.0000 (1)	0.0000 (0)	1.0000	0.0000
	Thalassery (5)	1.0000 (5)	0.0000 (0)	1.0000	0.0000
	Thanur (4)	1.0000 (4)	0.0000 (0)	1.0000	0.0000
	Pilicode (0)	0.0000 (0)	0.0000 (0)	0.0000	0.0000
	Total (10)	1.0000 (10)	0.0000 (0)	1.0000	0.0000

Figures in parenthesis are observed numbers

Table 7. Incidence of abortion, still birth and mortality in different types of mating

Reasons of kid loss	+/- x +/-	+/- x +/+ or +/+ x +/-	+/+ x +/+
Abortion	Nil	Nil	Nil
Stillbirth	3	2	Nil
Mortality (below one month)	9	Nil	1
Percentage of kid loss upto one month of age of total kids studied	4.27%	0.71%	0.35%

Table 8. Incidence of multiple birth and kidding percentage in different types of mating

Mating	Centre	Incidence of multiple birth in percentage				Kidding per cent
		Single	Twin	Triplet	Quadruplet	
+/- x +/-	B(77)	27	42	31	0	1.8
	T(62)	22	58	14	6	1.8
	TR(35)	3	63	34	0	2.0
	P (29)	17	48	21	14	1.8
	TL (203)	20	51	25	4	1.9
+/+ x +/- or +/- x +/+	B(21)	19	67	14	0	1.8
	T (38)	24	53	23	0	1.7
	TR(25)	28	72	0	0	1.7
	P(17)	0	82	18	0	2.1
	TL(101)	20	65	15	0	1.8
+/+ x +/+	B(2)	0	100	0	0	1.0
	T(6)	17	33	50	0	2.0
	TR(2)	0	100	0	0	1.0
	P(0)	0	0	0	0	0.0
	TL(10)	10	60	30	0	2.0

Figures in parenthesis are observed numbers

B-Badagara, T-Thalassery, TR-Thanur, P-Pilicode, TL-Total

Table 9. Relationship between GH/*Msp*1 genotypes and body weights up to six months

GH/*Msp*1 genotypes	Body weight in months			
	Birth	**1**	**3**	**6**
+/-	2.0 ± 0.1	4.8 ± 0.3	9.9 ± 0.3	14.8 ±1.4
+/+	2.0 ± 0.2	4.75 ± 0.6	9.7 ± 0.8	13.9 ± 2.1
Test of significance ($P \leq 0.05$).	NS	NS	NS	NS

Discussion

5. DISCUSSION

5.1 GENOMIC DNA ISOLATION

DNA for the present study was isolated using phenol - chloroform extraction method with some modifications. The phenol - chloroform extraction method is routinely used for isolation of good quality and high molecular weight DNA from blood samples. The DNA samples obtained were found to be suitable for PCR-RFLP studies. This method was also reported to be suitable for molecular studies by Aravindakshan *et al*. (1997), Chitra (2002), Anilkumar (2003) and Suprabha (2003).

5.2 QUANTITY AND QUALITY OF DNA

An average yield of 350.05 ± 10.05 µg of DNA was obtained from 5 ml of goat blood in the study.

Chitra (2002) obtained an average yield of 230.097±11.65 µg from 5 ml of Malabari goat blood.

Andersson *et al*. (1986), Apparao *et al*. (1994), Senthil *et al*. (1996) and Aravindakshan *et al*. (1997) had reported average yields of 100-150 µg, 250-300 µg, 444.58 ± 21.54 µg and 394.50 ± 34.26 µg, respectively per 10-15 ml of cattle blood using phenol - chloroform extraction method.

The higher yield per unit volume of blood in goats compared to that of cattle may be due to increased leukocyte count in goats (Swenson, 1996). The higher yield may be also attributed to the better suitability of the procedure of phenol - chloroform extraction of DNA in goats.

The ratio between optical densities at 260 nm and 280 nm was consistently around 1.7, which indicated good deproteinisation. Senthil *et al*. (1996) had reported that DNA with purity of 1.7 and above was suitable for PCR-RFLP studies.

5.3 PCR-AMPLIFICATION OF THE GH GENE

The PCR conditions resorted in the study were similar to that of Hoj *et al.* (1993), Aravindakshan *et al.* (1997) and Chitra (2002). The amplification of the caprine growth hormone gene with the bovine primers indicated the homology of growth hormone gene in bovine and caprine species (Yamano *et al*., 1988). The size of the amplified product was 768 bp in all the animals studied, which was consistent with the earlier reports in cattle. This indicated conservation of the DNA sequences in related species.

5.4 PCR-RFLP OF GH GENE

The restriction patterns obtained were similar to that reported by Hoj *et al.* (1993) and Aravindakshan *et al.* (1997). The amplified 768 bp fragment has a non-polymorphic *Msp*1 site suitable for control of *Msp*1 digestion giving rise to two fragments of 705 bp and 63 bp. The 705 bp fragment was restricted into a 612 bp and a 93 bp fragment when the polymorphic *Msp*1 site was present. These allowed the identification of two alleles *Msp* I (+) allele and *Msp* I (-) allele. The *Msp* I (+) allele produced 612, 93 and 63 bp fragments, while the *Msp* I (-) allele produced the 705 and 63 bp fragments.

Sequence analysis of amplified PCR fragment showed that the *Msp* (-) allele was created by the insertion of a T at position +837 and a C-G transversion at position +838 (Hoj *et al.*, 1993). However, Yao *et al.* (1996) mapped the

polymorphic *Msp* I site to position 1547-1548 and reported that it was due to a C to T transition.

5.5 INCIDENCE OF GH/*Msp* I GENOTYPES

The overall incidence of GH/*Msp*1 +/+ and +/- genotypes was found to be 38.24 and 61.76 per cent respectively. The frequency of GH/*Msp*1 -/- genotype in different populations was found to be zero. The heterozygous population was higher than expected in all the four centers studied. The absence of GH/*Msp*1 (-/-) genotype was reported by Hoj *et al*. (1993) in Red Danish and Norwegian cattle and Lagziel *et al*. (1999) in Israel Holstein-Friesian cattle. The homozygous genotype of V/V, which corresponded to the GH/*Msp*1 -/- genotype, was seen absent in Red Danish and Danish Holstein cattle, on *Alu*I polymorphism studies at bovine growth hormone gene locus (Sorensen *et al.*, 2002).

The absence of one of the homozygous genotypes has been described in several commercial lines of pigs with regard to several genes such as growth hormone gene and leptin gene in studies on the relationship between genotype and carcass quality traits (Kulig *et al.*, 2001; Kuryl *et al.,* 2003).

5.6 GENOTYPE AND GENE FREQUENCIES OF BUCKS AND DOES

The genotype or gene frequency of mates (bucks and does) is almost in line with general population. The high genotype frequency of GH/*Msp*1 +/- bucks in Pilicode may be due to the less number of observations. The heterozygotic advantage is seen in both sexes. None of the animals were of GH/*Msp*1 -/- genotype and was in concordance with the general population.

5.7 TESTING OF HARDY WEINBERG EQUILIBRIUM

Even though the population under study was a large random mating population, it was found not to be in Hardy Weinberg Equilibrium. This was expected, as the GH/*Msp*1 -/- genotype was not found in the population studied. The chi-square value was highly significant since there was a discrepancy between the observed and expected frequencies. An excess of heterozygotes can result from selective elimination of homozygotes or from the gene frequencies being different in males and females of the parental population (Falconer and Mackey, 1996).

5.8 COMPARISON OF GENOTYPE AND GENE FREQUENCIES OF PROGENIES IN DIFFERENT TYPES OF MATINGS

The results of the specific mating conducted among heterozygotes, between +/- and +/+ and among +/+ animals revealed interesting results.

The ratio of frequencies of +/+ and +/- genotype was 46:53 in +/- bucks x +/+ does or +/+ does x +/- bucks mating, which is almost in expected lines of 50:50. In homozygous (+/+ x +/+) mating, the results were exactly in agreement with the expected (100 per cent from +/+). But in heterozygous x heterozygous (+/- x +/-) mating, the expected result was 25 per cent (+/+), 50 per cent heterozygous (+/-) and 25 per cent (-/-). But there was no -/- genotype in the population. The GH/*Msp*1 +/+ was around 25 per cent (28 per cent overall), which was as expected. But heterozygous was increased to 72 per cent. A specific advantage for heterozygote was seen. Heterozygotic advantage obtained in this study was consistent with earlier reports of Chitra (2002) in goats, Biswas *et al*. (2003) in cattle and Kuryl *et al.* (2003) in pigs.

The presence of duplicate copies of the growth hormone gene in goats may be a possible cause for this. There are two alleles at the Growth Hormone gene locus in goats. One allele (GH1) is represented by a single copy (gGH1 gene), while in the other (GH2); the Growth Hormone gene is duplicated (g GH2 and g GH3) and is arranged in tandem. This could result in three types of gene combinations such as GH1/GH1, GH1/GH2 and GH2/GH2. Animals homozygous for GH1 possess two GH like genes and those homozygous for GH2 possess four GH like genes. Heterozygous animals containing one copy of GH1 and one copy of GH2 possess three GH like genes. Hence the higher percentage of heterozygotes could be attributed to the presence of duplicate gene. It seems likely that the duplicate gene arose as a consequence of gene duplication event. It implies the existence of strong selective forces, presumably heterozygote advantage, to maintain it.

5.9 INCIDENCE OF ABORTION, STILL BIRTH AND MORTALITY

Mortality and stillbirth were noted more in heterozygous mating. Percentage of kid loss below one month of age was 4.27 per cent in heterozygous mating, while in the other types of mating; it was only below one per cent. However, the mortality percentage is well within the limits found for general population (Raghavan *et al.*, 2004). But even if the dead kids are considered as possessing the (-/-) genotype, the number does not tally with the expected 25 per cent. So mortality at early stages may not be a cause for the absence of the (-/-) genotype in the population. Hence, the heterozygotic advantage obtained may not be because of the decreased viability of -/- homozygotes.

5.10 INCIDENCE OF MULTIPLE BIRTH AND KIDDING PERCENTAGE IN DIFFERENT TYPES OF MATING

The percentage of multiple births was 80, 80 and 90 respectively for the three types of mating. The incidence of multiple births did not differ much in the different types of mating, suggesting that pre-embryonic mortality might not be the cause for the absence of the GH/*Msp*1 -/- genotype in the population. The incidence of multiple births seen is in line with the trend seen in general Malabari population (Raghavan and Raja. 2004). The kidding percentage was also similar in all the three types of mating, adding to the fact that pre-embryonic mortality might not be the cause for the absence of the GH/*Msp*1 -/- genotype in the population.

5.11 RELATIONSHIP OF GH/*Msp*1 GENOTYPES WITH BODYWEIGHTS UP TO SIX MONTHS

The genotype does not appear to have an influence on growth of the kids. This is in consistency with the observations of Aravindakshan *et al*. (1997) in Ongole and Jersey cross-bred and Chitra (2002) in Malabari goats. It was inferred that the body weight increased or decreased more or less in a similar pattern in the different populations as well as in the different GH/*Msp*1 genotypes. However, Moody *et al*. (1996) and Biswas *et al*. (2003) had reported a positive correlation of body weights to the growth hormone genotypes.

The GH/*Msp* 1 genotype had no relation with growth rate of kids. Though an increased mortality rate was observed in heterozygous matings, it was well within the limits found in the general population. So the GH/*Msp*1 genotype did not have any relationship with growth and mortality rate.

Based on the frequency of GH/*Msp*1 genotypes in different types of matings, the following conclusions were drawn.

The GH/*Msp*1-/- genotype was found to be absent in the population and there was a clear heterozygotic advantage. Based on the mortality rate, frequency of multiple births and kidding percentage, it can be inferred that the absence of the GH/*Msp*1 -/- genotype could not be due to the early embryonic mortality. In sheep and goats the growth hormone gene has duplicate copies, unlike in bovine where the gene is present as a single copy. The absence of the GH/*Msp*1-/- genotype and the heterozygotic advantage can be attributed to the presence of duplicate copies of the growth hormone gene in goats.

Summary

6. SUMMARY

India's vast genetic resources in goat are reflected by the availability of 20 breeds of goats. Malabari is one of the important goat breeds of southern India. Even though improvement based on the phenotypic characteristics was conducted efficiently, there has been little effort to further improve the breed using newer technologies like molecular markers.

Molecular markers are capable of detecting variation at the DNA sequence level. Molecular markers are landmarks at the genome that can be chosen for their proximity to quantitative trait loci, which aids in marker assisted selection. Genes involved in the biology of a trait such as growth hormone genes are candidates for this purpose. Growth hormone, also referred as somatotropin, is a protein hormone produced in a subclass of pituitary acidophilic cells called somatotrophs of pituitary gland. Growth hormone has somatogenic effect and lactogenic effect, which gives the hormone more importance in the field of animal production.

Growth hormone gene is assigned to chromosome 19q 22qter in goats. There are two alleles at the GH gene locus in goats, one allele (GH1) is represented by a single copy (gGH1 gene), while in the other (GH2); the GH gene is duplicated (gGH2 and g GH3) and are arranged in tandem.

The present investigation was undertaken to study the growth and survivability of GH/*Msp*I genotypes in Malabari goats at different centers of Badagara, Thalassery, Thanur and Pilicode, using PCR-RFLP technique.

DNA was isolated from 32 bucks, 241 does mated to those bucks and 297 of their progeny using phenol - chloroform extraction method. The average yield

(Mean ± SE) of DNA from 5 ml of blood was 350.056 ± 10.0478 µg. The ratios of optical density at 260 and 280 nm were consistently around 1.7, indicating good deproteinisation. A 768-bp fragment from position +741 to +1511 (from III intron to V exon) was amplified well using bovine primers, indicating species homology. The amplified product was digested with 15 µl of *Msp*1 restriction endonuclease. Upon electrophoresis two digestion patterns could be observed. These allowed the identification of two alleles *Msp* I (+) allele and *Msp* I (-) allele. The *Msp* I (+) allele produced 612, 93 and 63 bp fragments while the *Msp* I (-) allele produced the 705 and 63 bp fragments. The animals with GH/*Msp*1 +/+ genotype produced 612, 93 and 63 bp fragments, while those with GH/*Msp*1 +/- genotype produced 705, 612, 93 and 63 bp fragments. The GH/*Msp*1 -/- genotype would produce 705 and 63 bp fragments pattern.

Out of the 570 animals typed no homozygous (-/-) genotype could be observed. The population of Badagara was found to be significantly different from the other centers. The genotype frequencies of bucks and does were consistent with the general population. Purposeful matings of +/- bucks x +/- does, +/- bucks x +/+ does, +/+ bucks x +/- does and +/+ bucks x +/+ does were done.

In the specific (+/- x +/-) heterozygous mating, the frequencies of +/+ and +/- genotypes were 0.28 and 0.72 respectively. The GH/*Msp*1 +/+ frequency was around 25 per cent (28 per cent overall), which was as expected. But frequency of heterozygous genotype was increased from the expected 50 per cent to 72 per cent. Though twenty five per cent of GH/*Msp*1 -/- genotype was expected, none of the animals were of the -/- genotype.

The overall kid loss before one month of age was found to be high in heterozygous mating when compared to other types of mating. Percentage of kid loss before one month of age was 4.27 per cent in heterozygous mating, while in

the other types of mating; it was only below one per cent. Even if the dead kids are considered as possessing the (-/-) genotype, the number does not tally with the expected 25 per cent. If the survivability of -/- genotype is less, higher percentage mortality than the observed 4.27 could have been observed. This suggests that decreased viability of GH/*Msp*1 -/- genotype was not a cause for the absence of the GH/*Msp*1 -/- genotype.

The incidence of multiple births did not differ in different types of mating suggesting that pre-embryonic mortality may not be the cause for the absence of the GH/*Msp*1 -/- genotype.

The body weights at birth, one, three and six months for the GH/*Msp*1 +/- genotype and GH/*Msp*1 +/+ genotype were not different, indicating that body weight and GH/*Msp*1 genotypes were not related.

The results obtained indicate that the GH/*Msp*1-/- genotype was found to be absent in the population and there was a clear heterozygotic advantage. Based on the mortality rate, frequency of multiple births and kidding percentage, it can be inferred that the absence of the GH/*Msp*1 -/- genotype could not be due to the early embryonic mortality. In goats, the growth hormone gene has duplicate copies, unlike in bovine where the gene is present as a single copy. The absence of the GH/*Msp*1-/- genotype and the heterozygotic advantage can be attributed to the presence of duplicate copies of the growth hormone gene in goats. It also implies that there exists a strong selective force to maintain a heterozygotic advantage for GH/*Msp*1 genotypes in Malabari goat population studied.

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*Originals not consulted

Annexures

ANNEXURE-I
SOURCES OF IMPORTANT CHEMICALS, ENZYMES AND EQUIPMENTS USED IN THIS STUDY

A. CHEMICALS

Agarose (Low EEO)	Bangalore Genei Pvt Ltd
Ammonium chloride	SRL, Bombay
Boric acid	SRL, Bombay
Crystalline Phenol	Merck
dNTPs	Bangalore Genei Pvt Ltd
EDTA	SRL, Bombay
Ethanol	Merck
Ethidium bromide	BDH Lab, England
6 X Gel loading buffer	Bangalore Genei Pvt Ltd
Glacial acetic acid	BDH-E-Merck (India) Ltd
Hydroxyquinolone	Qualigens Fine Chemicals, Mumbai
Potassium chloride	SRL, Bombay
Sodium acetate	SRL, Bombay
Sodium chloride	SRL, Bombay
Sodium dodecyl sulphate (SDS)	SRL, Bombay
Tris base	SRL, Bombay

B. ENZYMES

Taq DNA polymerase	Bangalore Genei Pvt Ltd
Proteinase K	Bangalore Genei Pvt Ltd
Restriction endonuclease	
*Msp*I	Bangalore Genei Pvt Ltd

C. MOLECULAR MARKERS

ϕ174X RF DNA/*Hae*III digest	Bangalore Genei Pvt Ltd
100 bp ladder DNA	Bangalore Genei Pvt Ltd

D. PRIMERS	Invitrogen (India) Pvt. Ltd
E. EQUIPMENTS	
Autoclave	Labo Autoclave, Sanyo, Japan
Centrifuge	REMI Motors Ltd, Mumbai
Deep freezer	Kripscold
Disposable plastic centrifuge tubes	Tarsons
Gel documentation system	Biorad Laboratories
Hot Plate & Stirrer	Jenway 1000, UK
Laminar Flow	Labline Instruments, Cochin
Microcentrifuge	Mikro 22R-Hettich Zentrifugen, Germany
Microwave oven	BPL, India
Micropipettes	Labsystems, Finland
pH meter	Systronics, India
Spectrophotometer	Jenway, Genova, UK
Submarine Electrophoresis Unit	Bangalore Genei Pvt Ltd
Thermal cycler	PTC-200, MJ Research Inc. USA
PCR tubes (0.2ml)	Bangalore Genei Pvt Ltd
Vortex	Bangalore Genei Pvt Ltd
UV Transilluminator	MacroVue-20, Pharmacia Biotech
Water bath	KEMI Lab Equipments
Weighing balance	Sartorius, Germany

ANNEXURE – II

COMPOSITION OF REAGENTS AND BUFFERS USED IN THE STUDY

EDTA (0.5*M*, pH 8.0)

Dissolve 18.61 g of EDTA (disodium dihydrate) in 80 ml of distilled water by bringing the pH to 6.0 with NaOH and make up the volume to 100 ml. Filter, autoclave and store at room temperature.

Phenol (saturated, pH7.8)

Melt the commercially available crystalline phenol at 65ºC in a water bath. Add hydroxyquinolone to a final concentration of 0.1%. To the molten phenol, add equal volume of 0.5 *M* Tris.HCl (pH 8.0). Stir the mixture for 30 min on a magnetic stirrer. Transfer the content into a separating funnel and when the two phases have separated, collect the lower phenolic phase. Add an equal volume of 0.1 *M* Tris HCl (pH8.0) to the phenol. Stir the mixture on a magnetic stirrer for 30-min. Collect the phenolic phase as above.

Repeat the extraction with 0.1*M* Tris HCl (pH 8.0), until the pH of the phenolic phase is more than 7.8. Finally add 0.1 volume of 0.01*M* Tris. HCl (pH 8.0) and store in a dark bottle at 4ºC.

RBC Lysis Buffer

Ammoniun Chloride	150 m*M*	8.0235 g
Potassium Chloride	10 m*M*	0.7455 g
EDTA	0.1 m*M*	0.0372 g

Add distilled water up to 1000 ml, stir, filter and autoclave. Store at 4ºC

Sodium acetate (3*M*, pH5.5)

For 100 ml, dissolve 40.824 g of sodium acetate in 70 ml of distilled water. Adjust the pH to 5.5 with glacial acetic acid. Make up the volume to 100 ml, autoclave and store at 4ºC.

Sodium Chloride (5*M*)

For 100 ml, dissolve 29.22 g of sodium chloride in 80 ml of distilled water, make up the volume to 100 ml, filter and store at room temperature.

Saline EDTA Buffer (pH8.0)

Sodium Chloride	75 m*M*	4.383 g
EDTA	35 m*M*	9.306 g

Dissolve in 900 ml distilled water. Adjust the pH to 8.0. Make up the volume to 1000 ml. Filter, autoclave and store at 4ºC.

Sodium dodecyl Sulphate (SDS) 20%

SDS	20 g
Distilled water to make up to 100ml.	

Stir on a magnetic stirrer, filter and store at room temperature.

TAE Buffer (50X)

Tris base	48.4g
Glacial acetic acid	11.42 ml
0.5*M* EDTA (pH 8.0)	20 ml
Distilled water	upto 1000ml.

Autoclave and store at room temperature.

Tris-Borate- EDTA (TBE) buffer (pH 8.3) 10X

Tris base	108.0 g
Boric acid	55.0g
EDTA	9.3g

Dissolve in 700ml of distilled water and adjust the pH to 8.3. Make up the volume to 1000 ml, autoclave and store at room temperature.

Tris Buffered Saline (pH 7.4)

Sodium Chloride	140 m*M*	8.18 g
Potassium Chloride	0.5 m*M*	0.0373 g
Tris base	0.25 m*M*	0.0303 g

Dissolve in 900 ml distilled water and adjust the pH to 7.4. Make up the volume to 1000 ml, filter, autoclave and store at room temperature.

Tris-EDTA (TE) Buffer (pH 8.0)

Tris base	10 m*M*	1.2114 g
EDTA	0.1 m*M*	0.3722 g

Dissolve in 900 ml distilled water and adjust the pH to 8.0 Adjust the pH to 8.0. Make up the volume to 1000 ml. Filter and autoclave in batches of 100 ml and store at 4ºC.

GROWTH AND SURVIVABILITY OF GH/*Msp* I GENOTYPES IN MALABARI GOATS

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

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2004

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ABSTRACT

Growth hormone gene, due to its essential role in lactation and growth processes, is a perfect candidate marker associated with somatotropic axis. Selection of animals based on the growth hormone genotypes can be introduced in the animal husbandry sector for better production.

The present investigation was undertaken to study the growth and survivability of GH/*Msp*I genotypes in Malabari goats at different centers of Badagara, Thalassery, Thanur as well as Malabari conservation unit, Regional Agricultural Research Station, Pilicode.

DNA was isolated from 32 bucks, 241 does mated to those bucks and 297 of their progeny using phenol - chloroform extraction method. A 768-bp fragment from third exon to fifth exon containing the polymorphic *Msp*1 site was amplified well using bovine primers, indicating species homology. The amplified product on digestion with the *Msp*1 enzyme revealed the GH/*Msp*1 (+) and (-) alleles.

The percentage of incidence of (+/-) genotype was 61.76 and that of (+/+) genotype was 38.24. None of the animals typed were of the GH/*Msp*1 (-/-) genotype. The genotype frequencies of bucks and does were consistent with the general population. In the specific heterozygous mating (+/- x +/-), 28 per cent of the progenies were (+/+) homozygotes and the rest 72 per cent were heterozygotes (+/-).

Early embryonic mortality was not found to be a cause for the absence of the GH/*Msp*1 -/- genotype as the kidding percentage in heterozygous mating were

not different from other types of matings. There was no relationship between growth upto six months of age and GH/*Msp*1 genotypes.

The study confirms a strong heterozygotic advantage for the GH/*Msp*1 +/- genotype and also the absence of GH/*Msp*1 -/- genotype in Malabari goats. Presence of duplicate copies of the growth hormone gene in goats may be a possible reason for the above results.