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Melatonin receptor 1A gene *Rsa*I and inhibin alpha subunit gene *Hae*II polymorphisms in Honamli and Hair goat breeds reared in Western Mediterranean region of Turkey

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Abstract: The melatonin receptor 1A (*MTNR1A*) and inhibin alpha subunit (*INHA*) genes play a significant role in the reproductive characteristics of animals. Blood samples were collected from 371 goats (Honamli and Hair) reared in Antalya and Burdur. The polymerase chain reaction (PCR) products were digested by *Rsa*I for the *MTNR1A* gene and *Hae*II for the *INHA* gene. Two alleles (A and G) and three genotypes (AA, AG, and GG) were observed for the *INHA* gene, while two alleles (R and r) and two genotypes (RR and Rr) were observed for the *MTNR1A* gene. The highest allelic frequency value for G (91.8%) was found in Honamli goat breeds for the *INHA* gene while the highest value for R (98.1%) was found in Hair goat breeds for the *MTNR1A* gene. The GG genotype for the *INHA* gene and the RR genotype for the *MTNR1A* gene were identified as the most common genotypes of the Honamli and Hair goat breeds. The rr genotype for the *MTNR1A* gene could not be determined in the breeds. Both Honamli and Hair goat breeds were in Hardy–Weinberg equilibrium for the genes that were studied. In conclusion, this study confirms the existence of genetic polymorphism in the *MTNR1A* and *INHA* genes as detected by PCR-RFLP analysis in Honamli and Hair goat breeds.

Key words: Goat, Honamli, Hair, *INHA*, *MTNR1A*

1. Introduction

There are many potential genes known to be related to the economic traits in farm animals and that can be used for selection criteria. Economic traits are quantitative characters controlled by several genes and also are strongly affected by environmental conditions. Since molecular genetic technologies have become more powerfully applicable in industry in recent years, it has become possible to identify which genes have an effect on variations that can be observed in these quantitative traits. This helps to speed up and improve the effectiveness of desired selections. In this regard, there has been a significant increase in the number of studies on the polymorphism of genes that affect the economic traits of livestock species (1–4). Reproductive traits are the most important economic characters in farm animal breeding. There are a number of genes that affect reproduction and that can be employed in selection programs. Two of these genes are inhibin alpha subunit (*INHA*) and melatonin receptor 1A (*MTNR1A*), which play a significant role in the reproductive process in animals (1,2). Inhibins are dimeric glycoproteins that are made up of a common inhibin alpha subunit (INHA) that is covalently linked to one of two related subunits, inhibin beta A or inhibin beta B (INHbA and INHbB) (5). Inhibin subunits are encoded by INHA, INHbA, and INHbB. Inhibin A inhibits FSH secretion by suppressing its receptor expression in granulosa cells, thus affecting the recruitment and development of ovarian follicles during folliculogenesis (6,7). The Ala257Thr missense mutation of the *INHA* gene has been shown to play an important role in receptor binding. Furthermore, the *INHA* gene has been suggested as a very likely cause of premature ovarian failure (8). Hou et al. (3) identified the polymorphisms in the 5′ promoter region of the *INHA* gene and concluded that these polymorphisms could be potential genetic markers for determining the litter size of goats. In Boer goats, *INHA* 651A/G polymorphism can have a significant effect on the mean litter size of parity-two animals (2). Goat breeds with both seasonal and year-round estrus also have different genotype distributions of the *INHA* gene, which points to a relationship between the *INHA* gene and fecundity (8). Tang et al. (9) found *Msp*I polymorphism

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in the bovine *INHA* gene as well as a correlation with the features of superovulation. Considerable influence on reproductive traits makes the *INHA* gene a prominent candidate for consideration (10). Melatonin is secreted from the pineal gland and has two receptors, which are classified as subtypes MTNR1A and MTNR1B. MTNR1A influences the regulation of seasonal reproductive activity (11). Chu et al. (12) reported that a polymorphic site in position 53 (GenBank AF419334) in Asian goat breeds has a correlation with seasonal reproduction. It has been found that Sarda, Saanen, Chamois Coloured, Maltese, and Nubian goat breeds have *MTNR1A* gene polymorphism (13). Furthermore, Mateescu et al. (1) identified a link between the *MTNR1A* gene and lambing frequency. These findings indicate that the *MTNR1A* gene is potentially an important DNA marker for breeding.

Hair goats are most frequently raised in the Mediterranean and Aegean regions and at higher altitudes in villages and small towns in and near the forested regions of Central Anatolia (14). On the other hand, Honamli goats are usually bred in the provinces of Antalya, Burdur, and Konya, which are located near the foothills of the Taurus Mountains in the western part of the Mediterranean region (14,15). The Honamli goat is a combined productive goat raised for meat, milk, and hair (14). Although there are limited reproductive studies about Honamli goats, the findings of some studies (15,16) revealed that the Honamli breed was superior to other local goat breeds in terms of various reproductive characteristics. Similarly, the number of reproductive studies on Hair goats is considerably limited (17–19). Determination of desirable genotypes of genes that have effects on reproduction traits is critically important in animal breeding programs. Therefore, determination of the *MTNR1A* and *INHA* gene polymorphisms, previously reported to have effects on reproductive characteristics, in these breeds will contribute to the literature.

The goal of this study was to investigate polymorphisms of *INHA* and *MTNR1A* genes, which have been reported to affect reproduction, using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

2. Materials and methods

2.1. Samples

Blood samples were randomly collected from 371 goats belonging to the Honamli (n = 183) and Hair (n = 188) goat breeds reared in Antalya and Burdur. Blood samples were collected in tubes with K_3-EDTA.

2.2. DNA isolation and genotyping

DNA was isolated using a DNA isolation kit (GeneJET Genomic DNA Purification Kit). Quantity and quality of DNA samples were examined using a NanoDrop 2000 (Thermo Scientific). DNA amplification of the *MTNR1A* and *INHA* genes was carried out by PCR and all PCR reactions were performed on an Amplitronyx Series 6 thermal cycler. All procedures were carried out at the Molecular Genetics Research Laboratories of the Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Animal Science.

The PCR amplification reaction was carried out in a total volume of 25 µL consisting of $MgCl^{2+}$ (2.5 mM for *INHA* and 1.5 mM for *MTNR1A*), dNTP (200 µM), primers (5 pmol) (Table 1), 1X buffer, Taq DNA polymerase (1 U/µL), and DNA (~100 ng). The PCR conditions including an initial denaturing step of 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s for *INHA* and 60 s for *MTNR1A*, 58.4 °C for 30 s for *INHA* and 60 s for *MTNR1A*, and 72 °C for 45 s, with a final cycle at 72 °C for 10 min. The PCR products of the *INHA* and *MTNR1A* genes were digested with *Hae*II (Thermo Scientific, #FD2184) and *Rsa*I (Thermo Scientific, #FD1124) restriction endonuclease enzymes according to instructions from the manufacturer (Fermentas), respectively. Digested PCR products were electrophoresed on 3% (*MTNR1A*) or 4% (*INHA*) agarose gels and then visualized under a UV-transilluminator.

2.3. Data analysis

Allele and genotype frequencies, observed and expected heterozygosity values, and Hardy–Weinberg equilibrium were calculated using the PopGene32 (www.ualberta.ca/~fyeh/Pop32.exe) program.

3. Results

The amplified PCR product of the *INHA* and *MTNR1A* genes produced 217-bp and 824-bp fragments, respectively.

Table 1. Primer sequence and restriction endonucleases.

Gene		Primer	PCR product size	RE	Reference
INHA	Forward Reverse	5'-CCACACAGGACTGGACAGACA-3' 5'-GCAGGAACAGAGAGGACAACG-3'	217 bp	*Hae*II	(20)
MTNR1A	Forward Reverse	5'-TGTGTTTGTGGTGAGCCTGG-3' 5'-ATGGAGAGGGTTTGCGTTTA-3'	824 bp	*Rsa*I	(21)

*Hae*II digestion of the *INHA* gene resulted in fragments of 27 (not appear on gel photo) and 190 bp for the GG, 27, 190 and 217 bp for the AG, and 217 bp for the AA genotype (Figure 1).

Restricted PCR products for the *MTNR1A* gene are given in Figure 2. Digestion with *Rsa*I enzyme produced five fragments (23, 53, 70, 267, and 411 bp); however, a site in position 53 was polymorphic. The presence of this cleavage site produces two fragments of 53 and 267 bp (R allele), while the absence of this site produces only one fragment of 320 bp (r allele). Restriction digestion of PCR products with *Rsa*I enzymes revealed two genotypes (Figure 2) of RR (267 bp) and Rr (320 and 267 bp), but no rr (320 bp/320 bp) genotype was detected.

Two alleles (A and G) and three genotypes (AA, AG and GG) were observed for the *INHA* gene, while two alleles (R and r) and two genotypes (RR and Rr) were observed for the *MTNR1A* gene. The results of statistical analysis are presented in Tables 2 and 3. The highest allelic frequency value for G (91.8%) was found in Honamli goats for the *INHA* gene while the highest value for R (98.1%) was found in Hair goats for the *MTNR1A* gene. The GG genotype for the *INHA* gene and the RR genotype for the *MTNR1A* gene were identified as the most common genotypes of the Honamli and Hair goat breeds. Based on genotype frequency values, the *MTNR1A* rr genotype was not determined in these breeds. The observed heterozygosity value was 0.04 and 0.10 for the *MTNR1A* gene in Hair and Honamli goat breeds, while the observed heterozygosity value was 0.21 and 0.13 for the *INHA* gene in Hair and Honamli goat breeds, respectively. The expected heterozygosity for the *MTNR1A* and *INHA* genes of Hair and Honamli goats is shown in Tables 2 and 3. According to the Hardy–Weinberg equilibrium test, both Honamli and Hair goat breeds were in equilibrium for these genes.

4. Discussion

Genetic improvement in reproductive traits associated with seasonal reproduction in livestock such as goats, sheep, and buffalo is difficult for the following reasons. First, these traits have low heritabilities (22); second, they are generally not expressed until puberty; third, they are usually important only in females; and fourth, they are only monitored in different birth seasons and in different locations depending on changes in the length of the day (23). Therefore, especially for goat and sheep breeders, seasonal reproduction is the primary factor that limits economic production. For this reason, work has been focused on improvements out of breeding season in goats and sheep. However, due to the aforementioned reasons, success has been limited with conventional improvement programs in these species. The effect of seasonality on reproduction in small ruminants can be limited by implementing marker-assisted selection programs using genetic markers. Genes are selected to either increase the ovulation rate or eliminate the limiting effect of seasonality. The information that is currently available indicates that the *MTNR1A* gene can be used to carry out more efficient selection for animal reproduction in the nonbreeding season. In addition, the *INHA* gene may be used in superovulation studies in small ruminants. In the present study, the genetic polymorphisms of the *MTNR1A* and *INHA* genes in two Turkish local goat breeds (Honamli and Hair) were examined using the PCR-RFLP method.

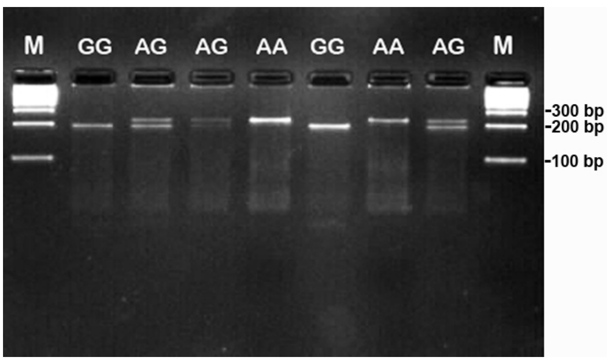

Figure 1. Gel image for the *INHA* genotypes by PCR-RFLP analysis. Lane M, molecular marker (100-bp DNA ladder).

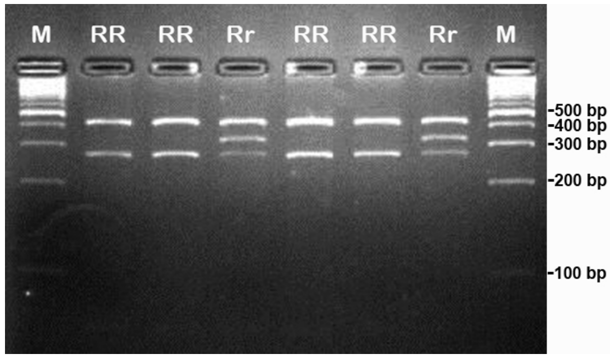

Figure 2. Gel image for the *MTNR1A* genotypes by PCR-RFLP analysis. Lane M, molecular marker (100-bp DNA ladder).

In mammals, the secretion of melatonin is triggered by the elongation and shortening of the day. In turn, seasonal reproductive activity for livestock such as goats, sheep, and buffalo is significantly influenced by melatonin (24). On the other hand, MTNR1A is thought to be the main receptor involved in the regulation of seasonal reproductive activities in mammals (11). Moreover, *MTNR1A* gene polymorphism has been found to be significantly related to seasonal reproduction in sheep (25), goats (13,26), and buffalo (27). Therefore, *MTNR1A* gene polymorphism can be used to regulate seasonal and nonseasonal reproductive activities in goats.

In this study, the 824-bp PCR products of exon II of the *MTNR1A* gene were digested with the restriction

Table 2. Allele and genotype frequencies of *INHA* gene for *Hae*II site in Hair and Honamli goat breeds.

Breed	n	Allele f. (%)		Genotype f. (%)			Heterozygosity		χ^2 (df = 1)
		A	G	AA	AG	GG	Ho	He	
Hair	188	12.77	87.23	2.12	21.28	76.60	0.21	0.22	0.43^{ns}
Honamli	183	8.20	91.80	1.64	13.11	85.25	0.13	0.15	3.24^{ns}

f.: frequency, ns: nonsignificant.

Table 3. Allele and genotype frequencies of *MTNR1A* gene for *Rsa*I site in Hair and Honamli goat breeds.

Breed	n	Allele f. (%)		Genotype f. (%)			Heterozygosity		χ^2 (df = 1)
		R	r	RR	Rr	rr	Ho	He	
Hair	188	98.1	1.9	96.28	3.72	0.00	0.04	0.04	0.06^{ns}
Honamli	183	94.8	5.2	89.62	10.38	0.00	0.10	0.09	0.52^{ns}

f.: frequency, ns: nonsignificant.

endonuclease *Rsa*I in Honamli and Hair goat breeds. The results indicated that the 267-bp and 320-bp fragments were polymorphic. However, the RR genotype was found to have a higher frequency than the Rr genotype in Hair (3.72%) and Honamli (10.38%) goat breeds. No rr homozygotes were detected in the two Turkish local goat breeds. Similar to our results, the homozygote rr genotype was not found in Chinese local goat breeds (Jining Grey, Liaoning Cashmere, Inner Mongolia Cashmere, Wendeng milk, and Beijing native goats) and Boer goats in China (26). Similarly, this genotype was not found in original European goat breeds such as Sarda, Saanen, Chamois Coloured, and Maltese goats, as well as Nubian goats that originated from Africa. In addition, the Rr genotype was only found in the Sarda breed, while the other five goat breeds (Saanen, Chamois Coloured, Maltese, Nubian) were found to be monomorphic (only the RR genotype) in terms of the *MTNR1A* gene (13).

Polymorphism at the *Rsa*I site of the *MTNR1A* gene was associated with year-round estrus and seasonal anovulatory activity in Small Tailed Han sheep (26). Similarly, the *Rsa*I site of the *MTNR1A* gene was found to be polymorphic and RR genotype frequency (0.13) was found to be lower than Rr (0.43) and rr (0.44) genotypes in Dorset sheep, which represents year-round estrus (1).

However, an association was found between the RR genotype and year-round estrus in Jining Grey and Boer goats, and an association between the Rr genotype and seasonal estrus was reported in goats (Liaoning Cashmere, Inner Mongolia Cashmere, Wendeng milk. and Beijing native goats) in China (12).

The *Rsa*I-Rr genotype was found in some breeds that have seasonal reproduction while only the RR genotype was found in Jining Grey goats, which is not a seasonal breed (26). It was reported that the Rr genotype, even if it was found in only a few breeds, showed a strong link with reproductive activity in goats. On the other hand, it has been suggested that the absence of *Rsa*I polymorphism in some breeds may be associated with different origins of breed groups, because in European breeds (Saanen and Chamois Coloured) there is no polymorphism, while in Asian and African groups, some breeds exhibit polymorphism (13). Similarly, the *Rsa*I site of the *MTNR1A* gene was found to be polymorphic in the breeds that were examined (Hair and Honamli) in this study, which are raised in Western Mediterranean region of Turkey. Neither Hair nor Honamli goats have planned selection programs conducted to increase production and achieve better control over reproductive activity. In these breeds, sexual activity has been always influenced by the photoperiod, which ensures seasonal lambing based on climatic conditions. Thus, it is reasonable to think that in Hair and Honamli breeds, low selective pressure has led to the existence of the r allele. In these goat breeds, lambing in a favorable climatic period is absolutely necessary to guarantee the survival of the offspring.

Another way to increase efficiency in small ruminants is to increase fecundity. Several fecundity genes have been described in some sheep breeds, including *INHA* (28,29). However, studies on these genes in goats are limited. Nevertheless, the *INHA* gene has been reported to have a positive correlation with litter size in Boer goats

(2). In this study, all three genotypes were detected in the two native Turkish breeds. It was determined that there is still sufficient genetic diversity in Hair and Honamli goat breeds. However, more studies are needed to investigate the relationship between the *INHA* gene and litter size in goat breeds.

In conclusion, results obtained for the two Turkish native goat breeds examined in this study show the existence of genetic polymorphism in the *MTNR1A* and *INHA* genes. Future studies are required to evaluate the relationship between different *MTNR1A* and *INHA* genotypes and reproductive seasonality and offspring in goats. Considering the cultural, historical, and environmental importance of goat production in Turkey, the data obtained here could be used as an initial guide for developing rational breeding strategies for increasing goat production as well as for preserving and utilizing local goat breeds in the region. The relationships between the *Rsa*I-RR genotype and polyestrus as well as the *Rsa*I-Rr genotype and seasonal estrus were reported (12,24). In this study, higher RR genotypic frequencies were found in Honamli and Hair goats, although these animals are known as seasonal polyestrous breeds. Therefore, studies should be planned for investigation of correlation between genotypes of *MTNR1A-Rsa*I and animals showing estrus out of season, and between polymorphism of *INHA-Hae*II and multiple pregnancies. The data obtained from these studies may have potential for studies to increase fertility traits of Honamli and Hair goats.

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