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Melatonin Receptor 1A (MTNR1A) Gene Polymorphism in Cross-Bred Hamdani Sheep: A Preliminary Study

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Abstract
Melatonin plays a key role in regulating several vital physiological processes, including the maturation and functioning of the reproductive system, pubertal development, seasonal reproduction, and adaptation. The activation of the melatonin hormone is facilitated by melatonin receptors. This study aimed to investigate melatonin receptor 1A (MTNR1A) gene polymorphism in cross-bred Hamdani sheep. A total of 102 cross-bred Hamdani ewes were used as animal material. DNA was extracted from whole blood. The 824 bp PCR products from the exon II region of the MTNR1A gene underwent analysis for restriction fragment length polymorphism (RFLP) using the *RsaI* restriction enzyme. In the study, RR, Rr, and rr genotypes were detected for MTNR1A gene in cross-bred Hamdani sheep. Genotype frequencies for RR, Rr, rr were 0.69, 0.27, and 0.04, respectively. Allele frequencies were detected as 0.82 and 0.18 for R and r alleles, respectively. In conclusion, more comprehensive research investigating association between MTNR1A genotypes and reproductive traits should be carried out in cross-bred Hamdani sheep.

Research Article

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1. Introduction

The sheep industry is of vital importance for Türkiye as it has contributed to animal production for centuries. According to novel data, Turkey is one of the top countries in the case of the number of sheep worldwide (TUIK, 2023). However, this is not reflected in the production data as expected. The main reasons for this are the insufficient recording systems, traditional breeding strategies, high feeding cost, and low production ability of animals (Tamer and Sarıözkan, 2017; Karadaş, 2018; Barığ and Çelikyürek, 2023).

Sheep breeds raised in Türkiye are mainly native breeds that are well adapted to their region. Although these breeds have good adaptation ability, their reproduction depends on photoperiod (Fındık, 2017). Therefore, reproductive activities are seasonal in most native sheep breeds of Türkiye (Doğan and Kutlu, 2022). These limit the number of offspring and eventually sustainability of animal products such as milk and meat (Doğan and Kutlu, 2022). However, consumers demand access to animal products throughout the year (Kan et al., 2010). Therefore, seasonality in sheep is one of the major limitations for consumers' demands.

In sheep, seasonal reproduction is activated by Melatonin (MT) secretion in autumn when the daylight gets shorter. Melatonin secretion from the pineal gland triggers gonadotropin-releasing hormone (GnRH). Eventually, GnRH regulates the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). MT uses its receptor, melatonin receptor 1A (MTNR1A), to regulate reproductive activities in reproductive organs (Chemineau et al., 2010). Current researches reveal that seasonality in sheep has genetic origins. Accumulative findings from these researches indicate that genetic variations may affect seasonality and also reproductive traits in sheep. (Cosso et al., 2021; Antonopoulou et al., 2023). At this point, the MTNR1A gene is one of the best-characterized genes. The findings across previous studies consistently pointed to an association between the MTNR1A gene and its role in regulating seasonal influences on ewes' reproduction in sheep (Martínez-Royo et al., 2012; Luridiana et al., 2020; Starič et al., 2020; Cosso et al., 2021; Antonopoulou et al., 2023). Therefore, the MTNR1A gene is an important candidate to improve reproductive traits by decreasing seasonality in native sheep breeds of Türkiye.

Cross-bred Hamdani sheep are raised in Siirt province and its surroundings and constitute the majority of sheep raised in Siirt in Türkiye (Bakır and Mikail, 2019; Turgut et al., 2023). In this study, it was aimed to investigate MTNR1A gene polymorphism in cross-bred Hamdani sheep.

2. Materials and Methods

2.1. Animals and sample collection

In this study, 102 cross-bred Hamdani ewes were used as animal material. Blood samples were collected from 14 flock located in Siirt, Kurtalan, Tillo, Eruh, Şirvan, Pervari. Blood samples were collected from the jugular vein into 9 mL K_3EDTA tubes (BD Vacutainer®, Becton Dickinson, Turkey) and stored at -20°C until analysis.

2.2 DNA extraction

DNA was extracted from blood using a genomic DNA isolation kit (Hibrigen, Hydra Biotechnology, Türkiye) following the manufacturer's instructions. DNA quantity and purity were assessed via spectrophotometry (Allsheng, Hangzhou, China), and integrity was evaluated via %1 agarose gel electrophoresis.

2.3. Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP)

PCR reactions were conducted in 25 µL volumes, including genomic DNA (50-100 ng), PCR 2XTaq Master Mix (12.5 µL) (Hibrigen, Hydra Biotechnology, Türkiye), primers (4 pmol each), and water up to 25 µL. PCR conditions included initial denaturation (95°C for 5 min), followed by cycles of denaturation (94°C for 30 sec), annealing (64°C for 45 sec), extension (72°C for 45 sec), and final extension (72°C for 7 min) Kyratec SC300G thermal cycler (Kyratec, Queensland,

Australia). PCR products of exon II region of MTNR1A gene were visualized on a 2% agarose gel stained with SYBR Safe under UV light. Primer pair used for PCR amplification was given in Table 1.

Table 1. Primers and PCR product size

Gene	Region	Primers (5'→3')	Product size (bp)	Reference
MTNR1A	Exon II	F: TGTGTTTGTGGTGAGCCTGG R: ATGGAGAGGGTTTGCGTTTA	824	Messer et al., (1997)

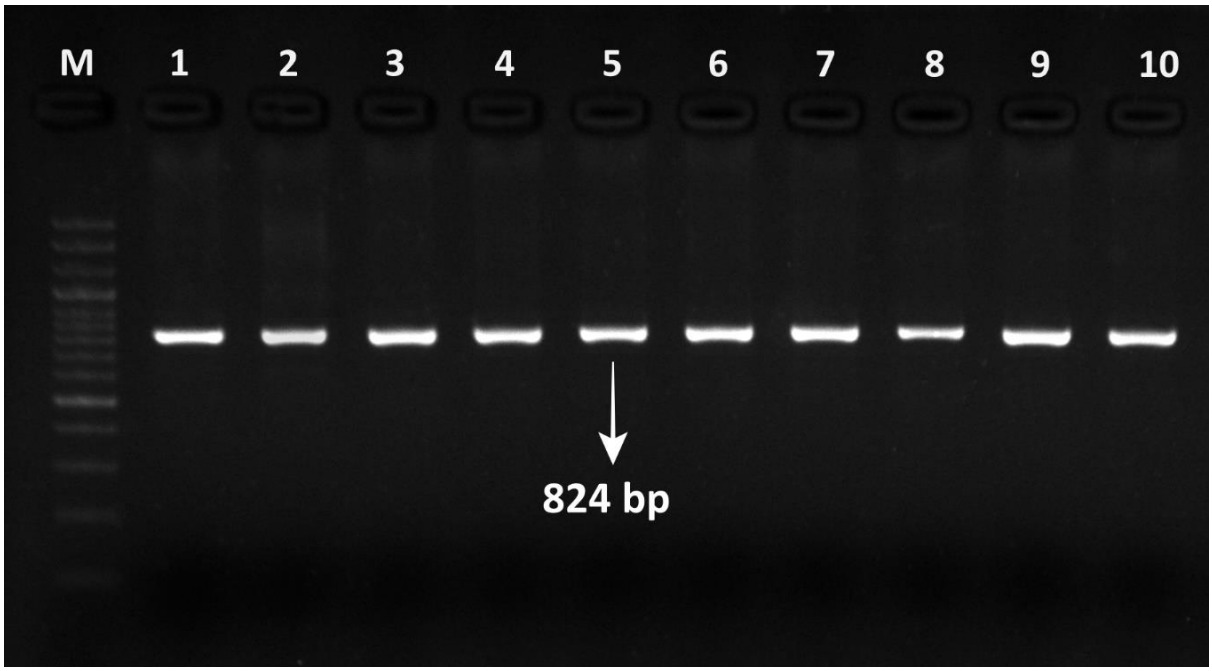

Figure 1. Lines 1-10 show 824 bp PCR products of exon II region of MTNR1A gene. M: 100 bp DNA ladder

MTNR1A genotypes were identified using the restriction fragment length polymorphism (RFLP) method. RFLP reactions were performed in 25 µL; PCR products (10 µL), *RsaI* restriction enzyme (10 units) (NEB, UK), 10X rCutSmart buffer (2.5 µL) (NEB, UK), and water up to 25 µL. Fragment analysis was carried out on a 4% agarose gel stained with SYBR Safe under UV light using a gel imaging system (Gene-Box, ER Biotech, Türkiye).

2.4. Statistical analysis

Statistical analyses were conducted using Minitab® 16 software. Allele and genotype frequencies of MTNR1A gene were calculated by direct counting method. Hardy-Weinberg equilibrium for MTNR1A genotypes and alleles was evaluated using the Chi-square test.

3. Results

Result for PCR reaction is shown in Figure 1. Following RFLP reaction, genotype discrimination was performed based on fragment size on the agarose gel.

In the study, RR, Rr, and rr genotypes were identified in exon II region of MTNR1A gene (Figure 2). Observed genotype frequencies for RR, Rr, and rr were 0.69, 0.27, and 0.04, respectively. Regarding genotypes, R and r allele frequencies were 0.82 and 0.18, respectively. Allele distribution of MTNR1A gene was in HWE ($p>0.05$) (Table 2).

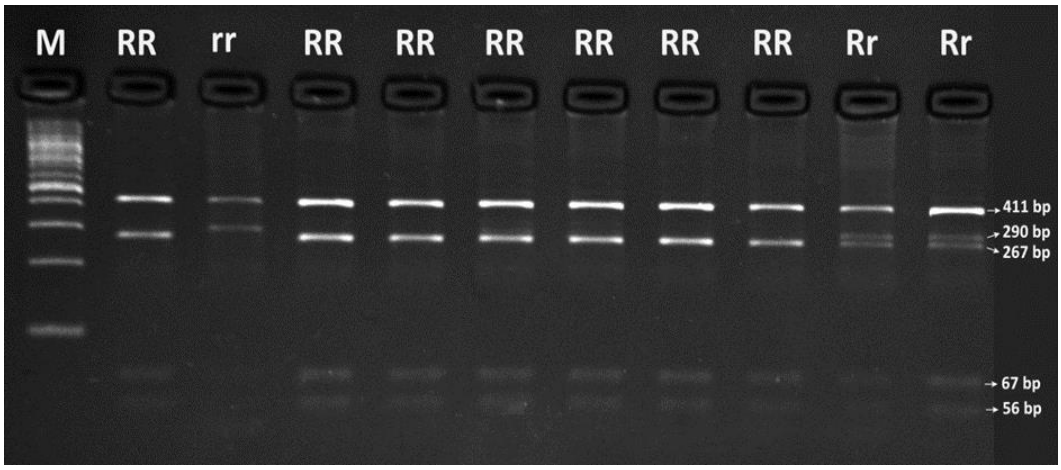

Figure 2. PCR-RFLP restriction pattern of exon II region of MTNR1A gene for *RsaI* enzyme. Three genotypes: RR (411 bp, 267 bp, 67 bp, and 56 bp), Rr (411 bp, 290 bp, 267 bp, 67 bp, and 56 bp), and rr (411 bp, 290 bp, 67 bp, and 56 bp) were detected. The smallest fragments (23 bp) of RR and Rr genotypes are not visible on the agarose gel. M: 100 bp DNA ladder

Table 2. Allele and genotype frequencies exon II region of MTNR1A gene

		Allele Frequency		Genotypes (Observed)					
MTNR1A	n	R allele	r allele	RR	Rr	rr	Ho	He	p-value
	102	0.82	0.18	70	28	4	0.274	0.290	$>0.05^{NS}$

NS: Non-significant, Ho: Observed heterozygosity, He: Expected heterozygosity

4. Discussion

In this study, it was detected polymorphism in exon II of MTNR1A gene in Cross-bred Hamdani sheep. In the study, we have detected RR, Rr and rr genotypes. Allele frequencies for R and r alleles were 0.82 and 0.18, respectively. Allele frequencies for MTNR1A gene were in HWE equilibrium. In Kurdi sheep, Varanlou et al. (2017) detected R and r allele frequency 0.49 and 0.51, respectively. In another study, Saxena et al., (2015) detected polymorphism at the same point in MTNR1A gene in different Indian sheep breeds. They reported R and r allele frequency as 0.93 and 0.07 in Malpura breed; 0.72 and 0.18 in Patanwadi breed; 0.46 and 0.54 in Sandyno breed and 0.56 and 0.44 in Nilgiri breed. However, Vibha et al., (2018) reported that MTNR1A gene exhibits a monomorphic restriction pattern for *RsaI* in Suwarna and Mandya sheep breeds. On the other hand, Luridiana et al., (2016) also reported that 824 bp amplicons of exon II region of MTNR1A gene show polymorphic patterns for *RsaI*. Arjoune et al., (2023) found to be polymorphic MTNR1A gene in two different Mediterranean sheep breeds. They reported R and r allele frequency as 0.37 and 0.63 in Barbarine sheep, and 0.67 and 0.33 in Queue Fine de l'Ouest sheep. Similarly, Fathy et al., (2018) detected polymorphism in MTNR1A gene in different sheep breeds raised in Egypt. The findings of this study were consistent with previous studies in different breeds.

This polymorphism was also investigated in Native Turkish sheep breeds. Avanus and Altınel (2016) detected polymorphism in MTNR1A gene for this SNP in Kıvırcık ewes. They reported R and r allele frequencies as 0.68 and 0.32, respectively. In another study Elmacı et al., (2013) detected R and r allele frequency as 0.73 and 0.27 in Turkish Awassi, 0.60 and 0.40 in Dağlıç, 0.64 and 0.36 in Akkaraman, and 0.41 and 0.58 in Sakız sheep breeds. However, Şeker et al., (2011) reported as monomorphic for this SNP in Turkish Awassi, Akkaraman and Sakız sheep breeds. Our findings show that R allele frequency is higher than in r allele frequency consistent with previous studies conducted in native sheep breeds of Türkiye.

Comprehensive studies revealed that there were many polymorhism in MTNR1A gene. Cosso et al., (2021) reported new polymorphisms in promoter, exon I, intron I, exon II, and 3'UTR regions of MTNR1A gene in Awassi sheep. Similarly, Mura et al., (2022) and Arjoune et al., (2023) reported new polymorphisms in MTNR1A gene in sheep. Furthermore, these studies also indicate that MTNR1A gene polymorphisms are related to fertility rate, days from ram introduction to lambing (DRIL) (Mura et al., 2022), litter size, lambing rate, days to lambing (Cosso et al., 2021; Pulinas et al., 2022; Arjoune et al., 2023). According to our acknowledgement, this is the first report showing MTNR1A gene polymorphism in cross-bred Hamdani sheep. A preliminary study implies that fertility may be affected due to individual differences between ewes in cross-bred Hamdani ewes (Turgut and Koca, 2024). Considering the results of previous studies in different sheep breeds, our findings in this study may be valuable in detection of genetic markers for selection programs in cross-bred Hamdani sheep.

5. Conclusions

In conclusion, we detected polymorphism in exon II region of MTNR1A gene in cross-bred Hamdani sheep in this study. Due to the effect of MTNR1A gene polymorphisms on reproductive parameters in sheep, further research investigating association between MTNR1A genotypes and reproductive traits should be carried out in cross-bred Hamdani sheep.

Declaration of Author Contributions

Idea / Concept: Ali Osman TURGUT, Davut KOCA, Data Collecting and / or Processing: Ali Osman TURGUT, Davut KOCA, Analysis and / or Interpretation: Ali Osman TURGUT, Writing the Article: Ali Osman TURGUT, Davut KOCA

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

Ethical Committee Approval

This study was conducted with the permission of Siirt University Animal Experiments Local Ethics Committee with the number 2023/07/51.

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