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Koyun Üreme Özellikleri İçin Tanımlanan Aday Genlerden Biri Olan Rora (Rar-Related Orphan Receptor Alpha) Reseptörlerinin Koyun Üremesindeki Rolü

The Role Of Rora (Rar-Related Orphan Receptor Alpha) Receptors, One Of The Candidate Genes Identified For Sheep Reproductive Traits, In Sheep Reproduction

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Anahtar Kelimeler
RORA (RAR-Related Orphan Reseptör Alfa) 23-bp nükleotit Sekans mutasyonu
İntron 1
Bafra koyunu
MAS (işaretleyici destekli seçim)

Öz

RORA (RAR-Related Orphan Receptor Alpha) steroid ile ilişkili genleri transkribe ederek östrojen sentezini düzenler. Yavru büyüklüğü, östrojen senteziyle ilişkili önemli bir üreme özelliğidir. Bu nedenle RORA geni ile koyun yavru büyüklüğü arasındaki ilişkinin araştırılması kritik önem taşımaktadır. Bu çalışmada, RORA adı verilen bir gende 23 baz çifti nükleotid dizilim mutasyonu ve bu mutasyonun koyun yavrularının verimliliği üzerindeki etkisi araştırılmıştır. Bu çalışmada 35 dişi Bafra koyununun RORA geninin intron 1'inde 23 bp'lik bir nükleotid dizi mutasyonu bulunmuştur. Çalışmaya katılan 35 örneğin her birinden 10 ml süt toplanmıştır. DD genotipinin yavru büyüklüğü, ikinci doğan yavru büyüklüğünde ID genotipinden ve II genotipinden önemli ölçüde daha yüksek saptanmıştır ($p>0.05$). Kombine genotipler ile ortalama yavru büyüklüğü arasındaki ilişki, homozigot (DD) genotipli koyunların heterozigot (ID) genotipli koyunlardan daha büyük kuzulara sahip olduğunu göstermiştir. Birinci ve ikinci yavrulardaki çoğul gebelik oranları, ortalama ölü doğum oranından daha yüksek saptanmıştır ($p> 0.05$). Özetlemek gerekirse, bu çalışma RORA geninin işlevi ve Bafra koyunlarının ıslahı ile ilgili daha ileri araştırmalar için teorik referanslar sağlamıştır. 23-bp indel varyantları, MAS (marker destekli seleksiyon) ıslahında koyunların birinci ve ikinci doğan yavrularının büyüklüğü için moleküler belirteçler olarak kullanılabilir.

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Abstract

RORA (RAR-Related Orphan Receptor Alpha) regulates estrogen synthesis by transcribing steroid related genes. Litter size is an important reproductive trait associated with estrogen synthesis. it is critical to investigate the relationship between the RORA gene and sheep litter size. In this study, a 23 base pair nucleotide sequence mutation in a gene called RORA and the effect of this mutation on the productivity of sheep offspring were investigated. In this study, a 23 bp nucleotide sequence mutation was found in intron 1 of the RORA gene in 35 female Bafra ewes. Ten ml of milk was collected from each of the 35 samples. The litter size of the DD genotype was significantly higher than the ID genotype and the II genotype in the second born litter size ($p> 0.05$). The relationship between combined genotypes and average litter size showed that ewes with homozygous (DD) genotype had larger lambs than ewes with heterozygous (ID) genotype. Multiple pregnancy rates in the first and second litters were higher than the average stillbirth rate ($p> 0.05$). To summarize, this study provided theoretical references for further research on the function of RORA gene and breeding of Bafra sheep. The 23-bp indel variants can be used as molecular markers for the size of first and second born offspring of ewes in MAS (marker assisted selection) breeding.

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1. INTRODUCTION

Sheep breeding is an important aspect of animal husbandry in Turkey due to the country's climatic conditions, topography, and socio-cultural structure. Crossbreeding studies were initiated in 1982 at Bafra Karaköy Agricultural Enterprise to combine the high fertility and milk yield characteristics of Bafra sheep Karayaka and Sakız breeds. As a result, Chios x Karayaka G1 crosses were obtained and bred among themselves to create a new breed called Bafra sheep, which has approximately 75% Chios and 25% Karayaka genotype (Yakan & Ünal, 2010).

Bafra sheep is primarily cultivated in Central and Western Black Sea regions, Central Anatolia, Eastern Anatolia, Aegean, and Mediterranean regions. This breed has high prolificacy, which is inherited from the Sakiz sheep breed. Studies have shown that the litter size of Bafra ewes ranges between 1.78 and 2.20, while the litter size of Akkaraman ewes is between 1.20 and 1.30 (AKÇAPINAR et al., 2008). Overall, Bafra sheep has become an important breed in Turkey due to its unique characteristics and adaptability to different regions.

In order to improve reproductive performance in sheep breeding, various strategies can be applied, such as selecting for traits that are correlated with fertility and using reproductive technologies such as artificial insemination and embryo transfer. However, it is important to note that reproductive traits often have low heritability values, which means that genetic selection may have limited impact in the short term. Nevertheless, by implementing long-term breeding programs and focusing on selecting for other important traits such as milk yield, body weight, and disease resistance, it may be possible to indirectly improve reproductive performance in the herd. Additionally, proper nutrition, management practices, and veterinary care can also play a significant role in improving reproductive efficiency and overall productivity in sheep farming.

The retinoic acid receptor-related orphan receptors (ROR) are a subfamily of nuclear receptors encoded by the RORA-C (or NR1F1-3) genes. These receptors play important roles in various physiological processes such as metabolism, development, and immunity. Nuclear receptors are ligand-dependent transcription factors that translate endocrine and dietary signals into differential gene expression patterns. Although an endogenous ligand for RORs has not been unequivocally confirmed, intermediates and metabolites of cholesterol metabolism have been proposed as potential candidates. RORs have been implicated in a range of diseases, including autoimmune disorders, metabolic disorders, and cancer, making them potential therapeutic targets (Jetten & Cook, 2020).

The AF2 domain is a highly conserved region among nuclear receptors and is responsible for the ligand-dependent transcriptional activation of target genes. Upon binding of a ligand to the LBD, a conformational change occurs that results in the recruitment of co-activator proteins to the AF2 domain, leading to the initiation of transcription. Co-repressor proteins, on the other hand, are recruited to the AF2 domain in the absence of a ligand, leading to the repression of target gene transcription. The AF1 domain, located in the N-terminus, is less conserved and has been shown to be involved in ligand-independent transcriptional activation, protein-protein interactions, and receptor dimerization. The hinge region, located between the DBD and LBD, plays a role in facilitating the movement of the receptor between the cytoplasm and nucleus and is also involved in receptor dimerization. Overall, the structural features of nuclear receptors allow for precise and regulated transcriptional responses to a variety of endocrine and dietary signals. (Y. Zhang et al., 2015). Recent studies have shown that RORs play a critical role in regulating lipid and glucose metabolism, making them potential targets for the treatment of metabolic disorders such as obesity and type 2 diabetes. RORs have also been implicated in the regulation of the immune system, with RORγt playing a key role in the differentiation of Th17 cells, which are involved in autoimmune and inflammatory diseases such as multiple sclerosis and rheumatoid arthritis. In addition, RORs have been linked to cancer, with RORα and RORγt both showing both oncogenic and tumor-suppressive roles depending on the cancer type. The identification of ROR ligands with therapeutic potential has led to the development of several ROR-targeting drugs, some of which are currently in clinical trials (Marciano et al., 2014).

RORA has also been linked to neurodevelopmental disorders such as autism spectrum disorder (ASD) and intellectual disability (ID). Several studies have reported altered RORA expression in individuals with ASD and ID, and genetic variants in RORA have been associated with an increased risk of developing ASD. RORA has been shown to regulate the expression of genes involved in synapse formation and function, neuronal migration, and differentiation, which are all critical processes in neurodevelopment. Furthermore, RORA has been implicated in the regulation of the immune system, and dysregulation of RORA expression has been linked to autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (Sarachana & Hu, 2013). In addition to its potential role in animal fertility and infertility, RORA has also been implicated in a variety of other physiological processes. For example, it has been shown to play a role in lipid and glucose metabolism, and its dysregulation has been linked to the development of obesity and type 2 diabetes. RORA has also been found to be involved in the regulation of inflammatory responses and autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis. Moreover, recent studies have suggested that RORA may have a role in regulating the sleep-wake cycle, with dysregulation of RORA expression leading to sleep disorders. Thus, RORA is a promising target for the development of therapeutics for a wide range of diseases and disorders (Findlay et al., 2010).

Marker-assisted selection (MAS) is a breeding method that uses genetic markers, such as single nucleotide polymorphisms (SNPs), to identify and select animals with desirable traits. This method can help to accelerate the breeding process and increase the accuracy of selection, particularly for traits that are difficult to measure directly or have low heritability.

In sheep breeding, MAS has been used to improve a variety of traits, including reproductive performance, wool quality, and meat quality. For example, several studies have identified SNPs associated with litter size and other reproductive traits in sheep, which can be used to select animals with higher fertility.

In addition to MAS, other molecular breeding technologies, such as genomic selection and gene editing, have also shown promise in improving the hereditary character of sheep. These methods can help to identify and manipulate specific genes that affect desirable traits, allowing for more targeted and efficient breeding.

Overall, the development of molecular marker technology has provided new opportunities for improving the genetic potential of sheep and other livestock species, leading to more efficient and sustainable animal production systems (Platten et al., 2019). SNP (Single Nucleotide Polymorphism), CNV (copy number variation), and indel (insertion/deletion) are the most common MAS methods. Indel is gradually becoming a common method in MAS that is widely used for breeding because it is easily and quickly identified (Hui et al., 2020). To sum up, RORA is a nuclear receptor that plays a crucial role in various physiological processes, including circadian rhythm, neuronal cell development, and immune cell differentiation. It is also linked to several pathologies, including autoimmune, inflammatory, and metabolic diseases. RORA has been identified as a potential candidate gene that may affect animal reproductive traits, and its expression has been found to regulate the synthesis of androgens and estrogens. In addition, various MAS methods, such as SNP, CNV, and indel, have been used to identify molecular markers associated with sheep reproductive traits, providing a foundation for future research into the functions of RORA (Wang et al., 2020).

2. MATERIALS AND METHODS

10 ml of milk was collected from each of the 35 samples participating in the study. DNA was obtained from all samples using a commercial DNA extraction kit (invitrogen) according to the manufacturer's protocol.

The Rora gene 23bp indel variant were genotyped in all the subjects by the polymerase chain reaction analysis. The total reaction mixture (20 ul) contained 2 ul of genomic DNA (10 ng/ul), 1 ul of each primer (10 uM), 1 ul of d NTP mix (10 uM), 1.5ul $MgCl^2$, and 0.2 ul Taq (1 units of Taq DNA polymerase) and 13.3 ul ddH2O. A set of primers, P1-rs604927153 Intron 1 F1: GGATGGGGCTTGGTGGATTA and R1: CAGGTGGTGAGCCATCTTGG was used to amplify the Rora gene inton 1 variant 23 bp indel site.

The amplified products were carried out by electrophoresis using a 2.5% agarose gels stained with ethidium bromid. The insersion alleles yielded 195 bp. The deletion allele gave a band of 172 bp. All of the samples were taken and reworked. No difference in results was observed and no bias in genotyping.

2.1. Statistical Analysis

SPSS v22 for Windows (IBM Corp., Armonk, NY, USA). Categorical variables are presented as n (percentage frequency) and continuous variables as mean±standard deviation. Chi-square test was used for comparisons of categorical data. P values less than 0.05 were considered statistically significant.

3. RESULTS

The study included 35 female Bafra sheep. Descriptive data of these sheep are given in Table1.

Table 1. Descriptive data

Parameter		**Female Bafra sheep n:35**
Mean age X±SD		3.02±0.92
Body height (cm) X±SD		71.66±3.52
Hip height (cm) X±SD		72.17±3.84
Hip width (cm) X±SD		20.49±1.56
Abdominal circumference (cm) X±SD		95.69±12.52
Chest circumference (cm) X±SD		89.74±7.33
History of mastitis	**available n (%)**	3 (08.57)
	none n (%)	32 (91.43)
RORA_23bp_indel genotype frequencyn (%)	**DD**	21 (60.00)
	DI	13 (37.14)
	II	1 (02.86)
RORA_23bp_indel allele frequency n (%)	**D**	55 (78.57)
	I	15 (21.43)
2021 (1st parity) X±SD	**Average multiple pregnancy**	2.14±1.26
	Average stillbirth	1.74±0.56
	Average lamb weight (kg)	3.71±0.76
2022 (2nd parity) X±SD	**Average multiple pregnancy**	2.14±1.24
	Average stillbirth	1.80±0.53
	Average lamb weight (kg)	4.06±0.69

The relationship between genotype frequencies and the number of pregnancies in the first and second litterings was evaluated in Table 2.

Table 2. Relationship between genotype frequencies and number of pregnancies in thefirst and second litter

Parity	**Number of pregnancies**	**Genotype n**			x^2	**DF**	**P**
		DD	**DI**	**II**			
1. parity	**Singular**	7	4	0	0.496	2	0.780
	Plural	14	9	1			
2. parity	**Singular**	7	4	0	0.496	2	0.780
	Plural	14	9	1			

There was no significant relationship between genotype frequencies and number of pregnancies in both the first and second litters.

Stillbirth averages and lamb average weights in the first and second calving were evaluated according to genotype frequencies in Table 3.

Table 3. Comparison of stillbirth averages and lamb average weights according togenotype frequencies in the first and second calving

Parity		**Genotype**			**P**
		DD	**DI**	**II**	
1. parity	**stillbirth averages**	1.78	2.00	2.00	0.083
	lamb average weights	3.64	3.90	3.80	0.514
2. parity	**stillbirth averages**	1.83	1.85	2.00	0.848
	lamb average weights	4.23	3.89	3.65	0.442

There was no significant difference between the mean stillbirths and mean lamb weights according to genotype frequencies in the first and second calving.

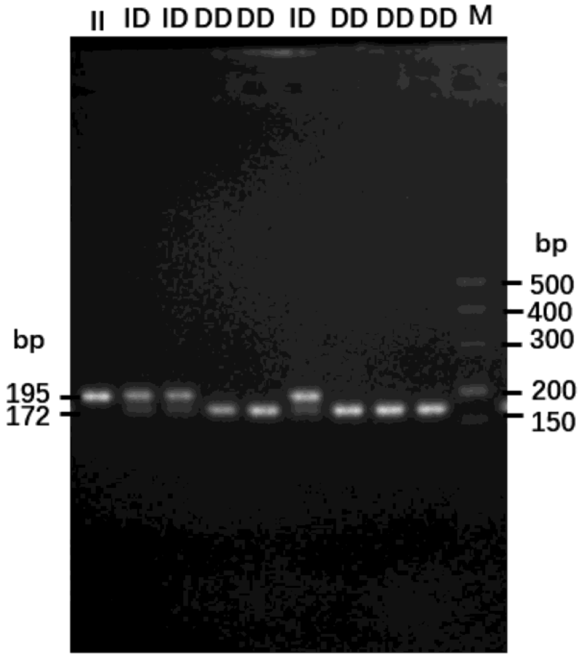

Figure 1. Genotyping of primer pair P1 23-bp indel deter- mined by PCR amplification (3.5 % agrose gel). *Note*: II, inser- tion/insertion. ID: insertion/deletion; DD: deletion/deletion.

4. DISCUSSION

Bafra sheep is a crossbreed of Chios (75%) and Karayaka (25%) sheep breeds. Body-color is white and covered with fleece. Black spots are seen around the mouth, eyes, and ears. They are generally raised in the middle of the Black Sea Region and are well suited to the harsh climate, poor pasture, and severe climatic conditions. Lambs of Bafra breeds are grown for meat production .Average live weight is 62 kg, height at withers 68 cm, body length 71 cm (Yakan & Ünal, 2010).

Sheep play a vital role in the livestock industry, and improving their growth and reproductive traits is essential for efficient breeding. Increasing litter size is particularly important for the sheep industry. Previous research has suggested that the RORA gene, which regulates sex hormones and is associated with autism, may also influence animal fertility. However, few studies have explored the relationship between sheep genetic mutations and litter size. Therefore, the aim of this study was to identify the RORA gene as a potential molecular marker for further research, which could lead to the use of MAS in sheep breeding. The study identified five transcript variants of the RORA gene in sheep, and it was found that different variants were related to the development of sheep tuberculosis and the protective immune response to infection. The RORA gene was found to be expressed in various tissues, including the testis, kidney, fat, cerebellum, liver, as well as normal breast, prostate, and ovarian epithelial cells (Jetten & Ueda, 2001). The RORA gene is known to regulate the expression of genes associated with autism spectrum disorder (ASD), including A2BP1, CYP19A1, HSD17B10, ITPR1, NLGN, and NTRK2. Among these, CYP19A1 and HSD17B10 are responsible for the conversion of androgens to estradiol, crucial for the growth of follicles and development of oocytes. Thus, RORA plays a critical role in regulating steroid metabolism in sheep, which affects the levels of androgens and estrogens. Downregulation of CYP19A1 and HSD17B10 due to reduced RORA expression can result in decreased levels of androgens and estrogens in sheep. (Lardone et al., 2017).

There are no studies on Bafra sheep and genotyping in the literature. For this reason, this study is important in terms of being the first study in the literature.

A research study was conducted to investigate the potential association between the Inhibin βB Gene Exon 2 region and FSHB gene Exon 3 region with offspring production in Akkaraman and Bafra Sheep Breeds. The results of the study revealed that no significant correlation was found between the identified polymorphisms in INHBB and FSHB genes and offspring production in either Bafra or Akkaraman sheep (Unlusoy & Ertugrul, 2016).

In a study conducted by Zhang et al., a polymorphism was identified in exon 3 of the FSHB gene in goats, resulting in a change from glutamine (Gln) to arginine (Arg). The genotypes of this polymorphism were denoted as AA, AB, and BB in four goat breeds, and it was found to have a significant effect on litter size. Additionally, Chu et al. discovered a SNP at the intron of the INHBB gene in prolific Hu sheep, and the genotypes of this SNP were classified as AA, AB, and BB. Their results indicated that the BB genotype had a significant impact on litter size in comparison to the AA genotype (C. Y. Zhang et al., 2011).

A study analyzing genetic polymorphism in Turkish domestic sheep breeds using microsatellite analysis found that Turkish Merino had the highest level of heterozygosity. The shortest genetic distance was observed between Sakız and Morkaraman breeds, indicating a close genetic relationship between these two breeds. In contrast, the farthest genetic distance was found between Turkish Merino and Ivesi breeds, suggesting a greater genetic distance between these two breeds (Yildiran & Cakir, 2012).

Yang et al. conducted a study on 532 female Australian White Sheep and identified a 23-bp nucleotide sequence mutation in intron 1 of the RORA gene. The locus had a polymorphic information content (PIC) value of 0.219. The ID genotype was found to have a significantly better second born litter size compared to the II and DD genotypes ($p<0.05$), and the ID genotype was dominant in relation to the third born litter size ($p<0.05$). The study also revealed that sheep with heterozygous (ID) genotypes had larger litters on average compared to homozygous (DD and II) genotypes (Yang et al., 2022).

Our study found a higher frequency percentage of DD genotype compared to both ID and II genotypes in RORA_23bp_indel. This finding is consistent with previous studies in the literature. Additionally, our study also found a low frequency of II genotype, which is consistent with other studies. When evaluating the RORA_23bp_indel allele frequency, we observed a higher frequency of the D allele compared to the I allele.

Our study did not find any statistically significant association between genotype frequencies and the number of pregnancies in the first and second litters. However, there was a higher number of multiple pregnancies compared to singleton pregnancies. Interestingly, although not statistically significant, the DD, ID, and II genotypes were more frequent in multiple pregnancies compared to singleton pregnancies. These findings suggest a potential association between RORA_23bp_indel genotype and multiple pregnancies in sheep, which warrants further investigation.

The non-significant results in our study regarding genotype frequencies may be attributed to the limited sample size. Thus, we propose that our study can serve as a preliminary groundwork for future research with larger sample sizes.

5. CONCLUSION

The non-significant results in genotype frequencies in our study could be attributed to the limited sample size. Therefore, we propose that our study can serve as a preliminary investigation and recommend conducting future studies with larger sample sizes to confirm our findings**.**

Author contributions: SY and RA designed the experiment, performed the experiments and drafted the manuscript. SMU and RA contributed to the experimental studies and drafted the manuscript. All authors reviewed and approved the final manuscript.

Conflict of interest disclosure: The authors declare that they have no conflict of interest

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Conflict Of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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