

The Association of MTNR1A Gene Alleles with the Response to Estrus Induction Treatments in Improved and Non-Improved Greek Indigenous Sheep Breeds [†]

Danai Antonopoulou ^{1,2}, Ioannis A. Giantsis ^{1,*} , George K. Symeon ³ and Melpomeni Avdi ²

¹ Division of Animal Science, Faculty of Agricultural Sciences, University of Western Macedonia, 53100 Florina, Greece; dsantono@agro.auth.gr

² Department of Animal Production, Faculty of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; avdimel@agro.auth.gr

³ Research Institute of Animal Science, HAO-Demeter, 58100 Giannitsa, Greece; gsymeon@elgo.gr

* Correspondence: igiantsis@uowm.gr

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Abstract: Seasonality in sheep reproduction and related limitations make milk production challenging throughout the year. In the present study, we investigated the response to estrus induction treatments in three indigenous breeds, Florina, Chios, and Karagouniko, as well as the melatonin receptor 1A gene variants in relation to this response. The three distinct synchronization methods were A: intravaginal sponges, B: GNRH use, and C: male effect. In group A, fertility was 85%, and Florina ewes expressed estrus at 90% in July. Ewes from Karagouniko and Chios had fecundity rates of 95% and 99%, respectively, and 100% estrus expression. The Florina ewes in group B expressed estrus at a percentage of 60%, with a fecundity rate of 57%, the Karagouniko ewes at a percentage of 65%, with a fecundity rate of 54%, and the Chios breed animals at a percentage of 87%, with a fecundity rate of 85%. Twenty to twenty-five days after ram induction, 68% of the Florina breed in group C showed signs of estrus, compared to 84% and 94% of Karagouniko and Chios breeds, respectively. In both Florina and Karagouniko breeds, all treatments showed a substantial difference in the frequency of the four identified SNPs in the MTNR1A gene between ewes who expressed estrus and ewes who did not. The genetic improvement based on the alleles analyzed in the current study is expected to decrease seasonality rates in indigenous sheep breeds.

Keywords: reproduction; sheep; MTNR1A gene



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1. Introduction

In sheep, milk production is often not feasible throughout the year due to the seasonality of reproduction. The goal of the current study was to compare how indigenous Greek sheep breeds (Florina, Chios, and Karagouniko) respond to various estrus synchronization treatments, as well as to associate this response with their genetic composition. This was accomplished by molecularly analyzing the melatonin receptor 1A (MTNR1A) gene in order to determine the alleles that are associated with those treatments. Exon 2 of the MTNR1A gene influences the seasonality of reproduction in small ruminants, with particular alleles linked with long anestrus periods [1–4]. A total of 450 ewes from three different indigenous breeds were examined, with each breed being divided into three different groups, where each group was treated with a different synchronization treatment.

2. Materials and Methods

The experimental procedures were carried out using 450 ewes (150 of Chios breed, 150 of Florina breed, and 150 of Karagouniko breed). Three groups of 50 ewes each were created

for each breed. The intravaginal progesterone sponges in Group A (sponges) contained 20 mg of flurogestone acetate FGA and were given for 14 days. At the conclusion of the treatment, 300 IU of chorionic gonadotrophin was then infused. In Group B (GnRH), 0.0084 mg buserelin acetate was given twice between D0 and D9, and 0.263 mg of prostaglandin was given seven days later. The male (or ram) effect, which states that sexually active males should be introduced to females that have been isolated for three months via visual contact from males at a geographic distance of more than 500 m, was used in group C. Blood samples were collected from each ewe. Using the PureLink Genomic DNA Mini Kit, DNA was extracted from 150 mL of collected blood. A partial segment of the MTNR1A gene located in the exon 2 was amplified as described in Giantsis et al. [3] and was sequenced using the Sanger methodology.

3. Results and Discussion

After the implementation of the first synchronization treatment (group A), Chios ewes had a fecundity rate of 99%, Karagouniko ewes 95%, and Florina ewes 85%. In group B, Chios ewes had a fecundity rate of 85%, Karagouniko ewes 54%, and Florina ewes 57%. In the third synchronization treatment (group C), Chios ewes had a fecundity rate of 94%, Karagouniko ewes 84%, and Florina ewes 68%.

The estrus expression rate of the sponge-treated Florina ewes (group A) was 90% in July, and their fecundity rate was 85% when they gave birth in December. Following the removal of the intravaginal sponges, Karagouniko and Chios ewes who were given the same treatment displayed 100% estrus expression, with fecundity rates of 95% and 99%, respectively. Ewes of the Florina breed expressed estrus at a percentage of 60%, with fecundity at 57%, Karagouniko ewes expressed estrus at a percentage of 65%, with fecundity at 54%, and Chios breed animals expressed estrus at a percentage of 87%, with a fecundity rate of 85% with regard to GnRH-PGF2a-GnRH (GnRH Protocol). Last but not least, 68% of the Florina breed displayed estrus 20–25 days after ram induction following the implementation of the male effect technique (group C), compared to 84% and 94% for the Karagouniko and Chios breeds, respectively. Chios ewes had a multiplicity (prolificacy) of 1.9, Karagouniko ewes had 1.3, and Florina ewes had 1.4.

The MTNR1A gene's amplified aligned sequence was 824 bp long and corresponded to the bases 285–1108 of the reference melatonin receptor mRNA haplotype in *Ovis aries* with the GenBank accession number U14109. In the Florina and Karagouniko breeds, all frequencies of the four identified alleles were statistically significantly different in ewes that expressed estrus compared to ewes who did not express estrus for all treatments. Only few animals from the groups that belonged to the Chios breed showed any statistical relationship with the allele frequencies. Therefore, in the two seasonal indigenous breeds, but not in the Chios breed, the scored alleles were statistically significantly related with the response to the treatments for out-of-season reproduction.

4. Conclusions

In conclusion, estrus synchronization can be achieved in indigenous sheep breeds from temperate latitudes using all three treatments. However, it has been demonstrated that some breeds are more receptive than others, and this trait is directly linked to their genetic composition. An efficient method to achieve the best estrus synchronization for milk production throughout the year is marker-assisted selection based on the MTNR1A gene.

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