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ABSTRACT

The aims of this study were 1) to investigate the effect of *MTNR1A* gene polymorphisms on reproductive performance in ewes of one Italian and two Slovenian dairy sheep breeds (Sarda, Istrian Premenka and Boska, respectively) which were located at different latitudes, and 2) to highlight if the different season of the male placement with females that was utilized in the different breeding systems in Sardinia (Italy) and Slovenia resulted in different effects of these polymorphisms on reproductive functions. Reproductively mature ewes (n = 100) from each breed were utilized to conduct the study. To evaluate the reproductive efficiency, lambing dates and number of lambs born were recorded per ewe; additionally, the duration in days from ram placement with ewes to lambing (DRPEL), litter size and the fertility rate were determined based on lambing dates. In each breed, there were eight nucleotide variations within the *MTNR1A* gene exon II, two of which (g.17355358 and g.17355171), respectively, resulted in a valine to isoleucine, and alanine to aspartic acid substitution, in amino acid sequence. The SNPs at position g.17355452 and g.17355458 were determined to have effects on reproductive performance. Genotypes C/C and C/T at g.17355452 in Bovska and Sarda and genotype A/A at g.17355458 in Istrian Pramenka were associated with a greater fertility and a lesser duration in days from ram placement with ewes to lambing. These findings confirmed that the nucleotide sequences of the *MTNR1A* gene could affect reproductive functions of Mediterranean sheep.

Keywords: MTNR1A; Polymorphism; Sheep; Reproductive Performances

1. Introduction

Melatonin is an indoleamine produced in a variety of tissues including the pineal gland (Venegas et al., 2012). Synthesis of pineal melatonin is tightly controlled by the circadian light/dark cycle, with there being inhibition of melatonin synthesis and release during the light period. The day/night oscillation of melatonin in blood represents a peripheral clock involved in the regulation

of various circadian and circannual rhythms including seasonal reproduction (Reiter, 1991). Melatonin has functions through binding and activation of specific receptors in the hypothalamus, both at the suprachiasmatic nucleus - which is the site of the circadian clock - for circadian effects, (Weaver et al., 1996), and in premammillary hypothalamus (PMH) for reproductive effects (Migaud et al., 2005).

Melatonin has effects by binding to high-affinity, G-protein-coupled receptors (Dubocovich and Takahashi, 1987; Reppert et al., 1994). In mammals, two high-affinity melatonin receptors have been identified, termed MT1 and MT2, but only MT1 is associated with modulation of reproductive functions (Dubocovich et al., 2003; Weaver et al., 1996). The largest concentration of melatonin MT1 receptors has been detected in the Pars tuberalis (PT) of the hypothalamus, while there is a lesser concentration of MT1 receptors in the PMH (Chabot et al., 1998; Malpaux et al., 1998). Melatonin actions in the PT are associated both with the control of prolactin secretion and in sensitivity to photoperiodic changes (Dardente, 2007). It, however, remains to be precisely established as to how the photoperiodic signal perceived in the PT is transmitted to the medial basal hypothalamus (MBH), where the nuclei regulating GnRH secretion are located, however, this signal is likely modulated by thyroid hormones and Kisspeptin (Dardente, 2007). The MT1 receptor is encoded by the *MTNR1A* gene, in which several polymorphic sites have been recognized in different mammalian species, including sheep, goats and buffalo (Luridiana et al., 2012; Messer et al., 1997). In sheep, two single nucleotide polymorphisms (SNPs) at position g.17355458G>A (rs406779174, ex 606/RsaI) and g.17355452C>T (rs430181568, ex 612/MnlI) have been associated with different seasonal reproductive traits (Carcangiu et al., 2009; Chu et al., 2006; Mateescu et al., 2009; Notter and Cockett, 2005). In the Ile de France sheep breed, however, there is no association between these SNPs and reproductive functions. There was an association of those SNPs with reproductive seasonality that might be breed-specific or might be affected by environmental conditions (Hernandez et al., 2005). Different aspects of reproductive seasonality have been examined in several studies where there was a focus on the effects of *MTNR1A* gene

polymorphisms. Results in many of the studies were inconsistent due to the different methodologies utilized or the different periods of the year when the studies were conducted. Pelletier et al. (2000), reported that there were reproductive functions as indicated by fluctuating progesterone concentrations only in April, in Merino d'Arles, and reported that the rs430181568 SNP (previously 612/MnlI) is associated with seasonal patterns of reproduction. By evaluating lambing dates, Carcangiu et al., 2009; and Chu et al., 2006, reported that ewes with the C/C genotype at rs430181568 and the A/A at rs406779174 loci had reproductive functions during periods of the year when ewes of the breed evaluated were anoestrus. In Rasa Aragonesa breed, when there was evaluation of ram-ewe mating patterns, Martínez-Royo et al. (2012) determined that ewes with the A/A genotype at the rs406779174 locus had greater fertility between January and August compared to those with the G/A or G/G genotypes. In Indian breeds, Saxena et al. (2014) reported that both of these loci were associated with ewes breeding during periods of the year when ewes of these breeds were typically anoestrus. In Slovenian dairy and meat sheep breeds, Starič et al. (2020) reported that there was an association of these two SNPs with the re-initiation of reproductive functions in the autumn, after seasonal anoestrus (reproductive recovery). The results for all these studies indicated there was an association of *MTNR1A* gene in modulating seasonal effects on reproduction of ewes in the breeds evaluated, but the determination of the effects in the various studies on reproductive variables were evaluated using different methods. To obtain comparable data, therefore, it is necessary to conduct studies using the same methods for determination of effects on reproductive functions.

Based on the findings of previous studies, the hypothesis was tested that the *MTNR1A* gene polymorphism affects reproductive functions in sheep. Consequently, the aims of this study were 1) to investigate the effect of *MTNR1A* gene polymorphisms on reproductive performance, in the same year, after placement of rams with ewes, of two Slovenian and one Italian dairy sheep breed (Istrian Premenka and Boska, and Sarda, respectively) that were located at different latitudes, and 2) to determine if the season of male placement with ewes was associated in the different breeding

92 systems utilized in Sardinia (Italy) and Slovenia had effects on how these polymorphisms affected
93 reproductive functions.
94
95 **2. Material and methods**
96 Ewes (*n* = 300) of dairy-type breeding were utilized in the present study. The ewes were of
97 three different breeds, Bovska and Istrian Pramenka – two Slovenian autochthonous dairy breeds -
98 and Sarda, the main Italian autochthonous dairy breed. The Sarda ewes were produced and located
99 in North West of Sardinia - Italy - while the Bovska and Istrian Pramenka ewes were produced and
100 located in the Soča valley and in the Vremščica mountain area, respectively, which are Slovenian
101 regions. For the characteristics of these breeds, see the report of Starič et al. (2020). For each breed,
102 100 reproductively mature ewes were used, with an average age of 4.3 ± 0.8 years for the Bovska
103 breed, 4.5 ± 0.7 years for the Istrian Pramenka and 4.1 ± 0.6 years for the Sarda breed. Bovska and
104 Istrian Pramenka ewes grazed during the day in a paddock where there were gramineous and
105 leguminous grasses, and were provided a supplement of 200 g of corn grain during the periods
106 when the ewes were milked twice daily. The animals were penned in a barn during the night where
107 there were water and hay provided *ad libitum*. The Sarda ewes, grazed leguminous and gramineous
108 grasses during the daylight hours and were provided 300 g per ewe daily concentrated commercial
109 food (crude protein 20.4% and 12.5 MJ ME/kg DM) during the period of the afternoon milking. The
110 ewes were penned during the night hours, and had free access to hay and water during this period.
111 Lambing generally occurs in March/April for the Bovška ewes, in February/March in the Istrian
112 Pramenka, and from September to January in the Sarda breed. Rams of the three breeds were placed
113 with ewes of the flocks at different times of the year, depending on the different farming systems.
114 For the two Slovenian breeds, rams were placed with ewes in the autumn so that in May the ewes
115 could be moved to the mountain area to graze, when lambs had developed to the extent they could
116 easily stay with their mothers during the transit to the mountains in the region used for summer
117 grazing. In Sardinia, however, the rams are commonly placed with ewes in May so that lambing can

occur mainly in October, when there is natural lush grass which ensures adequate milk production for optimal lamb development. Furthermore, autumn lambing ensures that there can be a lactation period from October to July because in Sardinia winters are generally mild from a climatic perspective, thus favouring a long lactation period in a semi-extensive management system. For the present study, rams were placed with ewes on 1 October, 25 September and 15 May in the Bovska, Istrian Pramenka, and Sarda flocks, respectively, with a male/female ratio of one ram to 25 ewes. Rams were removed from the ewe flocks after 60 days from the time of placement with ewes. The lambing dates and the number of lambs born were recorded for each ewe. From the recorded data, the data for the following reproductive variables were collected: fertility (number of ewes that lambed per number of ewes with which there was ram placement), litter size (number of lambs born per ewe that lambed) and the duration in days from ram placement with ewes to lambing (DRPEL).

2.1. Sampling and genotyping

An individual 10 ml blood sample was collected using jugular venepuncture procedures, utilising a vacuum tube with EDTA (ethylenediaminetetraacetic acid) as anticoagulant (BD Vacutainer Systems, Plymouth, UK). A total of 300 blood samples were collected, 100 from each of the three breeds.

The nucleic acid extraction was performed using a commercial genomic DNA extraction kit (NucleoSpin® Blood, Catalogue No., Macherey-Nagel, Germany). The concentration and purity values of the extracted DNA were evaluated using spectrophotometric procedures. The average concentration was 132 ng/µl, and a 260/280 ratio of 1,80 was considered suitable for the analysis.

Polymerase chain reaction (PCR) was performed utilising 150 ng of genomic DNA using MAXYGENE II Thermal Cycler (Axygen® Tewksbury, MA, USA). The primers used were the following: Forward: 5'-TGTGTTTGTGGTGAGCCTGG-3'; Reverse: 5'-ATGGAGAGGGTTTGCGTTTA-3', as reported by Reppert et al. (1994). The PCR reaction was performed as reported by Luridiana et al. (2015). The PCR products were separated using

electrophoresis procedures on a 1.5% agarose gel added with 9 µl of RedSafe Dna Stain 20.000X (iNtROn Biotechnology, Inc., Sangdaewon-dong, Seongnam-si, Gyeonggi-do, Korea), processed in parallel with 100 bp DNA marker (Invitrogen, Carlsbad, USA) in TAE 1% Buffer, at steady voltage of 110 V for 30 minutes. The gel was then visualized using a UV light transilluminator (UVItec, Cambridge, UK).

The PCR products were purified (multiscreen filter plates - Millipore) and then sequenced in forward and reverse direction, by a commercial service. Before the Sanger sequencing (ABI PRISM 3730 DNA Analyzer, Applied Biosystems), the samples were prepared using the Big Dye Terminator sequencing kit v3.1 (Applied Biosystems). The alignment of the resulting sequences and the comparison with the latest version of the sheep genome - Oar_rambouillet_v1.0 - GenBank Assembly Accession Number: GCF_002742125.1 - was performed using the BLAST program (www.ncbi.nlm.nih.gov/blast/). To perform nucleotide sequence alignments, the ClustalW, within "Accessory Application" of BioEdit Sequence Alignment Editor software (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html) was used, selecting the IUB weight matrix (for DNA) scoring matrix.

2.2. Statistical analysis

All the statistical analyses were conducted using R statistical software (Version 4.0.4 R Core Team 2021 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). To analyse the association between each genotype and fertility rate or number of ewes lambed in 10-day periods (from 150 to 200 days after ram placement with ewes) a Chi-square test was used. The differences among breeds in fertility rate were analysed using a Chi-square test. To assess for normal distribution and homogeneity of variances of the duration in days from ram placement with ewes to lambing (DRPEL) and the litter size, the Shapiro and Lavene tests were used, respectively. The ranges in

169 DRPEL and in litter size were 60 and 1, respectively. The duration in days from ram placement
170 with ewes to lambing (DRPEL) and litter size were analysed using the following linear model:

171 $$Y_{jkm} = \mu + G_j + R_{kj} + e_{jkm}$$

172 where Y_{jkm} is the trait measured for each animal (DRPEL or litter size), μ is the overall
173 mean of each trait in considered ewes, G_j is the fixed effect of each genotype (three levels), R_{kj} is
174 the random effect of the ram (four levels within genotype), and e_{jkm} is the random residual effect of
175 each observation. Data of DRPEL and litter size are expressed as least-square mean ± SEM. There
176 were considered to be mean differences when there was a $P < 0.05$.
177
178 **3. Results**
179 The PCR product resulted in a single fragment of 824 bp in length, corresponding to the
180 major part of the *MTNR1A* gene exon II. From the sequence analysis of the amplified fragment in
181 the Bovska, Istrian Pramenka and Sarda breeds, there were eight nucleotide variations detected
182 (Table 1), additionally, in two Bovska ewes a single nucleotide variant (SNV), at position
183 g.17355517G>A, was identified. All the nucleotide variations were reported according to the latest
184 sheep genome version (Oar_rambouillet_v1.0 - GenBank assembly accession number:
185 GCA_002742125.1). Three of the SNPs (g.17355458G>A, g.17355452C>T and g.17355358C>T),
186 which are the most frequently evaluated in many breeds for the association with seasonal
187 reproduction, did not result in a Hardy-Weinberg equilibrium in all the three breeds ($P<0.05$). In
188 addition, two of these SNPs (g.17355452C>T and g.17355358C>T) were always associated so that
189 these were considered to be unique SNP. The SNP at the position g.17355358C>T (rs407388227)
190 resulted in an amino acid change (Ile/Val). The SNPs g.17355452C>T and g.17355458G>A were
191 of different allelic and genotype frequencies in the three breeds evaluated, as reported in Table 2.
192 In the three breeds, the C allele was the most frequent both in the g.17355452 and
193 g.17355358 loci, and, consequently C/C resulted the most frequent genotype. Instead, for the locus

194 at position g.17355458, in the three breeds evaluated, G was the most frequent allele and, therefore,
195 was also the genotype (Table 2).
196 In Bovška and Sarda ewes, the results of the statistical analysis indicated there were C/C and
197 C/T genotypes, at the positions g.17355452 and g.17355358 that were associated with greater
198 fertility (P<0.05), compared with the T/T genotype. Furthermore, the Bovška and Sarda ewes with
199 the C/C genotype at the previously described positions, had a shorter DRPEL than ewes with the
200 T/T genotype (P<0.05). Comparison of results for fertility rate between these two breeds as related
201 to the C/C and C/T genotypes indicated the Bovska ewes were more fertile than Sarda ewes (P
202 <0.05). There was no association between genotypes and litter size neither in ewes of the Bovška
203 nor Sarda breed (Tables 3 and 4). There was no association between the SNP at position
204 g.17355458 and the values for reproductive variables both in ewes of the Bovska and Sarda breed
205 (Table 3 and 4). Otherwise, in the Istrian Pramenka ewes the A/A genotype, at g.17355458 position,
206 was positively associated with a greater fertility (P<0.05) and a shorter DRPEL (P<0.05) compared
207 to the ewes with the other two genotypes (Table 5).
208 In the Istrian Pramenka ewes, litter size was also not affected by the different
209 polymorphisms (Table 5). The distribution in percentage of lambing, every 10 days, in the Bovska
210 and Sarda breed indicated the ewes with the C/C or C/T genotype at g.17355452 and g.17355358
211 positions, initiated reproductive functions earlier after a period of anoestrus, and consequently
212 lambed earlier in the lambing season, compared to the ewes with the T/T genotype (Table 6 and 7).
213 Furthermore, the Bovska ewes with the T/T genotype had the greatest occurrence of lambing
214 approximately 10 days earlier than ewes of the Sarda breed. In the Istrian Pramenka breed, the
215 percentage distribution of lambing every 10 days indicated ewes with the A/A genotype at position
216 g.17355458 initiated lambing earlier in the lambing season compared to the ewes of the other two
217 genotypes (Table 8).
218
219 **4. Discussion**

The three breeds of dairy sheep evaluated had the same eight SNPs within the exon II of the *MTNR1A* gene; additionally, only in two Bovska ewes there was an additional nucleotide variation detected, but due to its extremely small frequency, it was considered a SNV rather than a polymorphism. These results are consistent with those reported in previous studies by Starič et al. (2020) and Luridiana et al. (2020). Furthermore, these SNPs were also reported to be present in other sheep breeds, with some differences among the breeds which may be related to the analysed sequence length of the *MTNR1A* gene (Cosso et al., 2021; Pelletier et al., 2000; Saxena et al., 2014). The results of the present study, also confirmed that the SNPs at positions g.17355452 and g.1755358 are fully linked and these data are consistent with the findings of Starič et al. (2020) and Mura et al. (2019). In the present study, the reproductive functions in the Istrian Pramenka breed were affected by the SNP at position g.17355458 of the *MTNR1A* gene, while in the Bovska and Sarda breeds there were no effects on reproductive functions of the polymorphisms at positions g.1755452 and g.1755358. In the Bovska and Sarda breed, ewes with C/C or C/T genotype at locus g.17355452 and g.1755358 were more fertile and had a shorter DRPEL than ewes with T/T genotype. These findings are consistent with those in previous studies with Slovenian and other European sheep breeds (Luridiana et al., 2016; Starič et al., 2020). Considering the different dates of ram placement with ewes in the present study, however, it was expected that there would be considerable differences between the two sheep breeds. In the Bovska breed, the rams were placed with the ewes on 1 October (complete breeding season), while in the Sarda breed this placement of rams with ewes was on 15 May (breeding during the season when breeding does not typically occur). Surprisingly, the differences in reproductive response to ram placement with ewes in the two breeds were very few, therefore, effects of ram placement with ewes was similar at both seasons of the year. In the Bovska breed there was a greater fertility of ewes with the C/C and C/T genotypes, than in Sarda ewes (for C/C genotype 92% compared with 80%, and for C/T 88% compared with 82%, in Bovska and Sarda ewes, respectively), however, it is important to consider that the rams were placed with the Bovska ewes during their natural breeding season, while rams

were placed with Sarda ewes during their seasonal anoestrous period. It, therefore, is expected that the Bovska ewes were already having oestrous cyclic functions at the initiation of the breeding season, leading to the earlier response to the rams compared with the Sarda ewes. Likely, this could be the reason for the difference in fertility in the ewes of the two breeds. It was expected that Sarda ewes would be in the transitional phase from being anoestrus to initiation of oestrous cyclic patterns during which the reproductive axis gradually regains full functionality (Fabre-Nys et al., 2015; Mura et al., 2019). The lesser fertility detected in Sarda compared to Bovska ewes, therefore, probably depended on the lesser capacity of these ewes to promptly respond to the ram stimuli during the initial period when rams were placed with ewes, maybe because the "male effect" alone was not adequate to induce onset of oestrous cyclic patterns. When all of the findings in the present study are considered, the Sarda and Bovska ewes with the C/C and C/T genotype had a greater reproductive response when placed with rams even when the evaluations occurred during different periods of the year when ewe reproductive functions were markedly different. Wang et al. (2017) reported that the *MTNR1A* gene is involved in the development and maturation of the ovarian follicle. It could be hypothesized that the two previously described genotypes are involved in regulating ovarian functions, explaining why ewes with these two genotypes are more fertile. The ovary, however, produces or has an uptake in the follicle of melatonin which is thought to sustain ovarian follicle functions and inhibit apoptosis (Tamura et al., 2008). Furthermore, there are melatonin receptors in different portions of the ovarian follicle, as reported by Wang et al. (2014), and it is well known that the MT1 receptor is involved in modulation of physiological processes in the ovarian follicle (Wang et al., 2017). The "silencing" of this receptor results in a lesser expression of anti-apoptotic genes and a decrease in the antioxidant effect of melatonin. Melatonin also modulates the expression of genes involved in steroidogenesis, in the conversion of progesterone to androgens, and in luteinisation of granulosa cells (He et al., 2016; Lima et al., 2015). Hence, it is reasonable to hypothesize that the different genotypes at the *MTNR1A* gene can influence the transmission of the melatonin signal, as reported by Trecherel et al. (2010), and thus

can affect the growth and sustenance of the follicle functions. The greater fertility of Bovska and Sarda ewes with the C/C and C/T genotype is certainly due to an enhanced ovarian sensitivity to the melatonin signal.

Ewes of the two breeds with the T/T genotype at loci g.1755452 and g.1755358 have some differences in fertility and peak time of lambing during the lambing season. The Bovska ewes with the T/T genotype were more fertile than Sarda T/T ewes (73% compared with 66%, respectively; Table 3 and 4). In addition, for Bovska T/T ewes there was the peak occurrence of lambing peak at day 181 to 190, while for Sarda T/T ewes that was at day 191 to 200 after ram placement with the ewes (Table 6 and 7). This genotype is most prevalent in non-domesticated sheep (*Ovis gmelini musimon*) which are very sensitive to the photoperiod in regulation of reproductive functions (Carcangiu et al., 2010). In these non-domesticated sheep, the spring lambing period ensures the survival of the species, enhancing the probability for optimal conditions for offspring development. It, therefore, can be hypothesized that in both the Sarda and Bovska breeds, the ewes with the T/T genotype are less sensitive to the stimuli resulting when there is placement with rams, compared to the ewes with the other two genotypes. This was more evident in the Sarda breed because the period when rams were placed with ewes was a period when ewes were expected to be anoestrus. In the present study, the greater fertility rate in the ewes with the C/C or C/T genotype confirmed the hypothesis about these ewes being lesser sensitive to photoperiod than ewes with the other genotype. Presumably, there was a shorter period of anoestrus which led to a greater response when rams were placed with the ewes as compared with ewes having the T/T genotype, which may have longer periods of seasonal anoestrus. The different hypothalamic sensitivity to photoperiodic signals could be the reason for the different reproductive responses among the ewes with the three genotypes.

In the Istrian Pramenka breed, however, only the polymorphism at position g.17355458 had an effect on reproductive functions, which is consistent with findings in the same breed by Starič et al. (2020) and Martínez-Royo et al. (2012) for the Aragonesa breed. In particular, ewes with the

A/A genotype were more fertile and had a shorter DRPEL than ewes with the A/G and G/G genotypes (Table 5). This association is difficult to explain because this polymorphism does not result in an amino acid change and, therefore, there is not a change in the conformation of the protein which could affect signal transmission as occurs when there is the polymorphism at position g.17355358 (Trecherel et al., 2010). It, however, could also be that this polymorphism is linked to another polymorphism not yet discovered within the same gene, or within other genes involved in the regulation of reproductive activity. It is reasonable to consider that in a system as complex as that modulating reproductive functions, several genes are involved such as the Fec, or Kiss-1 genes that could have combined functions with the protein encoded by the *MTNR1A* gene in regulating reproductive efficiency. In future research, therefore, it would be interesting to investigate the associations among the proteins encoded for by the previously described genes and how these proteins may interact to modulate reproductive functions in ewes of different sheep breeds.

5. Conclusion

In conclusion, for the *MTNR1A* gene polymorphisms there is an association with initiation of reproductive functions following seasonally induced anoestrus in ewes of all the three breeds evaluated. In addition, although the period of ram placement with ewes of the Bovska breed was different compared with the ewes of the Sarda breed, the effect of the polymorphism on initiation of reproductive cyclic functions after a period of seasonal anoestrus was evident in ewes of both of these breeds. Bovska ewes were more fertile than Sarda ewes, but the effect of the polymorphism occurred in both of breeds. In both breeds, the ewes with the C/C or C/T genotype at position g.17355452 and g.17355358 of the *MTNR1A* gene were more fertile and had a shorter DRPEL than the ewes with the T/T genotype. Instead, the SNP that was associated with having effects on initiation of oestrous cycles after a period of seasonal anoestrus in the Istrian Pramenka breed was that at position g.17355458. In this breed ewes with the A/A genotype were more fertile and had a shorter DRPEL than ewes with the A/G and G/G genotypes. The findings of the present study could

be used in animal breeding programs for reproductive genetic improvement in dairy sheep. To more precisely elucidate the mechanism through which there is the effect of *MTNR1A* gene polymorphisms on reproductive functions, other studies need to be conducted. In particular, there should be studies focused on ovarian functions ascertaining whether the polymorphisms at this gene can have effects on the maturation of the ovarian follicle. These types of studies would be useful for clarification of the effect of these polymorphisms on initiation of reproductive functions in ewes during the transition period of ewes from being seasonally anoestrus to the time when ewes are oestrous cyclic.

Author contribution

All co-authors have contributed equally to the research (Conceptualization, Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing) as well as to article preparation. All co-authors have approved the final draft of this article.

Declaration of Competing Interest

None of the authors of this manuscript has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of this paper.

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Table 1

SNPs in the three breeds evaluated (Bovska, Istrian Pramenka and Sarda) and resulting amino acid changes in the MTNR1A gene according to the latest genome version Oar_rambouillet_v1.0 (GenBank assembly accession number: GCA_002742125.1)

SNP position	Nucleotide change	Amino acid change
g.17355611	C->A	none
g.17355458	G->A	None
g.17355452	C->T	None
g.17355358	C->T	Val→Ile
g.17355281	C->T	None
g.17355263	C->T	None
g.17355173	G->A	None
g.17355171	G->T	Ala→Asp

Table 2

Allele and genotype frequency of the *MTNR1A* gene SNPs in the three sheep breeds evaluated in the present study, according to the latest genome version Oar_rambouillet_v1.0 (GenBank assembly accession number: GCA_002742125.1)

	Allele frequency				Genotype frequency					
Position	g.17355452		g.17355458		g.17355452			g.17355458		
Breed	C	T	G	A	C/C	C/T	T/T	G/G	G/A	A/A
Bovška	0.67	0.33	0.60	0.40	0.50	0.35	0.15	0.43	0.34	0.23
Istrian Pramenka	0.75	0.25	0.64	0.36	0.63	0.25	0.12	0.52	0.25	0.23
Sarda	0.78	0.22	0.66	0.34	0.68	0.20	0.12	0.53	0.26	0.21

Table 3

Fertility rate, number of days from date of ram placement with ewes to lambing (DRPEL) and litter size for ewes of the different genotypes of the Bovška breed (n = 100); [a, b] within the row, values without a common superscript differ (P<0.05).

SNP	g.17355452C>T			g.17355458G>A		
Genotype	C/C	C/T	T/T	G/G	G/A	A/A
Fertility rate	92.0[a]	88.0[a]	73.0[b]	87.0	83.0	85.0
DRPEL	172.3±11.6[a]	17.4±11.9[a]	185.9±11.6[b]	173.4±13.1	176.4±13.2	180.5±13.5
Litter size	1.07±0.03	1.08±0.03	1.09±0.01	1.09±0.02	1.08± 0.03	1.07±0.01

Table 4

Fertility rate, number of days from date of ram placement with ewes to lambing (DRPEL) and litter size for ewes of the different genotypes of the Sarda breed (n = 100); [a, b] within the row, values without a common superscript differ(P<0.05).

SNP	g.17355452C>T			*P*	g.17355458G>A			*P*
Genotype	C/C	C/T	T/T		G/G	G/A	A/A	
Fertility rate	80[a]	81.0[a]	66.0[b]	*	76.0	74.0	78.0	ns
DRPEL	170.7±11.6[a]	172.1±12.6[a]	190.7±12.8[b]	*	177.512.7	176.1±12.3	179.8±12.3	ns
Litter size	1.08±0.03	1.09±0.03	1.07±0.01	ns	1.08±0.02	1.09± 0.03	1.04±0.01	ns

Table 5

Fertility rate, number of days from date of ram placement with ewes to lambing (DRPEL) and litter size for the different genotypes in the ewes of the Istrian Pramenka breed (n = 100); [a, b] within the row, values without a common superscript differ (P<0.05).

SNP	g.17355452C>T			*P*	g.17355458G>A			*P*
Genotype	C/C	C/T	T/T		G/G	G/A	A/A	

Fertility rate	82.0	79.0	84.0	ns	77.0 [a]	76.0 [a]	91.0 [b]	*
DRPEL	178.5±12.6	180.3±13.0	181.4±11.1	ns	186.2 ± 12.7[a]	180.5± 11.6[a]	172.6±11.8[b]	*
Litter size	1.11±0.03	1.09 ±0.01	1.10 ±0.02	ns	1.12±0.04	1.09±0.01	1.07± 0.01	ns

Table 6

Percentage of lambing distribution each 10 days during the observation period in the Boska breed (*n* = 100) based on the ewe genotype at position g.17355452. [a, b] within the column, values without a common superscript differ ($P<0.05$).

	150-160	161-170	171-180	181-190	191-200	201-210
C/C	10.9[a]	45.7[a]	21.7	13.0[a]	6.5	2.2[a]
C/T	6.5[a]	48.4[a]	25.8	9.7[a]	3.2	6.5[a]
T/T	0.0[b]	9.1[b]	18.2	45.5[b]	9.1	18.2[b]

Table 7

Percentage of lambing distribution each 10 days during the observation period in the Sarda breed (*n* = 100) based on the ewe genotype at position g.17355452. [a, b] within the column, values without a common superscript differ ($P<0.05$).

	150-160	161-170	171-180	181-190	191-200	201-210
C/C	11.1[a]	50.0[a]	22.2[a]	7.4[a]	7.4[a]	1.9[a]
C/T	6.3[a]	56.3[a]	25.0[a]	6.3[a]	6.3[a]	0.0[a]
T/T	0.0[b]	0.0[b]	12.5[b]	25.0[b]	50.0[b]	12.5[b]

Table 8

Percentage of lambing distribution each 10 days during the observation period in the Istrian Pramenka breed (*n* = 100) based on the ewe genotype at position g.17355458. [a, b] within the column, values without a common superscript differ ($P<0.05$).

	150-160	161-170	171-180	181-190	191-200	201-210
C/C	10.0[a]	15.0[a]	22.5	22.5[a]	20.0[a]	10.0
C/T	10.5[a]	15.8[a]	15.8	42.1[a]	5.3[a]	10.5
T/T	19.0[b]	38.1[b]	23.8	4.8[b]	4.8[a]	9.5