

Reproductive performance in three Slovenian sheep breeds with different alleles for the MTNR1A gene

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1 Reproductive performance in three Slovenian sheep breeds with different genotypes of the  
2 *MTNRIA* gene

3

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14

15 **ABSTRACT**

16 The aim of the research was to highlight the polymorphisms of the *MTNR1A* gene in three  
17 Slovenian sheep breeds, and if these polymorphisms influence the reproductive performance. A  
18 total of 100 Bovška, 110 Istrian Pramenka, and 108 Jezersko-Solčavska sheep, were used. The rams  
19 were introduced into the flock on October 10<sup>th</sup> in the Bovška sheep, on September 20<sup>th</sup> in the Istrian  
20 Pramenka sheep. In each farm, the lambing date and number of newborn lambs were recorded, in  
21 order to evaluate the fertility rate, the distance in days from ram introduction to lambing (DRIL),  
22 and the litter size. In the Jezersko-Solčavska breed rams were kept with ewes all year round and  
23 lambings for a whole year were registered. The obtained sequences showed 8 nucleotide variations  
24 and that in the position g.17355358 was always associated with that in the position g.17355452. In  
25 the Bovška sheep, the statistical analysis showed an association between the G/G genotype in the  
26 position g.17355452, and a higher fertility ( $P<0.05$ ) and shorter DURL ( $P<0.05$ ). In the Istrian  
27 Pramenka sheep T/T genotype in the position g.17355458 was positively associated with a greater  
28 fertility ( $P<0.05$ ) and shorter DRIL ( $P<0.05$ ). In the Jezersko-Solčavska breed the ewes carrying  
29 G/G genotype, in the position g.17355452, showed a greater number of lambing during decreasing  
30 photoperiod whereas the animals carrying A/A genotype exhibited the higher number of lambing  
31 ( $P<0.05$ ) during the increasing photoperiods. The different polymorphisms studied showed an  
32 association with reproductive performances in the three Slovenian breeds.

33

34 **Key words:** Seasonality; Fertility; *MTNR1A* gene, Slovenian sheep breed

## 37 **1. Introduction**

38 Many sheep breeds show a seasonal reproductive activity that is linked to the photoperiodic  
39 trend (Malpaux et al., 2001). In general, these animals exhibit sexual activity during the decreasing  
40 photoperiods and a reproductive rest periods during increasing photoperiods. However, in the sheep  
41 breeds of the Mediterranean basin significant differences occur both in the length of the mating  
42 period and in the moment in which mating occurs (Chemineau et al., 2008). This trend of  
43 reproductive activity is essential for the species conservation but in turn is a limiting factor that  
44 affects the animals' productive potential (Abecia et al., 2012). The main environmental factor that  
45 controls reproductive seasonality is the annual photoperiod variation that regulates the epiphyseal  
46 secretion of melatonin (Carcangiu et al., 2013). In fact, during the long days (i.e. when daylight is  
47 longer than night time) melatonin secretion is low, while during the short days (i.e. when daylight is  
48 shorter than night time) melatonin levels are high. Therefore, this hormone, with its characteristic  
49 trend, is the organic informer of the photoperiodic trend (Arendt 1998). High nocturnal melatonin  
50 levels, typical during autumn, have a positive influence on the beginning of reproductive activity in  
51 small ruminants (Bittman and Karsch, 1984). Melatonin acts through specific receptors located in  
52 different organs including the nuclei of the central nervous system that control reproductive activity  
53 (Sliwowska et al., 2004). In mammals two high-affinity melatonin receptors have been identified,  
54 named MT1 and MT2, but only the first is involved in regulation of reproductive activity  
55 (Dubocovich et al., 2003; Weaver et al., 1996). A high number of receptors was found in Pars  
56 Tuberalis (PT), but this structure is marginally involved in the regulation of reproduction, certainly  
57 playing an important role in the photoperiodic control of prolactin secretion (Lincoln and Clarke,  
58 1994). On the other hand, in the premammillary hypothalamus, where melatonin exerts its action on  
59 reproduction, a low number of these receptors has been found (Migaud et al., 2005). The MT1  
60 receptor gene (*MTNR1A*) is part of the G protein-coupled receptor family and has been discovered  
61 in several species (Messer et al., 1997; Reppert et al., 1994). In particular the exon II of the

62 *MTNR1A* gene exhibits different polymorphisms that influence the seasonal reproductive activity of  
63 different species (Carcangiu et al., 2011, 2009a; Luridiana et al., 2016; Pelletier et al., 2000).  
64 However, in some sheep breeds this effect has not been found suggesting that it might be related to  
65 breed or environmental conditions (Hernandez et al., 2005). Therefore, considering the importance  
66 that sheep have in the Mediterranean basin, it is fundamental to investigate if also other breeds  
67 exhibit *MTNR1A* gene polymorphisms and if these can influence reproductive performances. The  
68 aim of the present research was to investigate three Slovenian sheep breeds in order to highlight  
69 whether the known polymorphisms are present also in these breeds and whether they can affect  
70 reproductive activity. In order to evaluate the reproductive performances, the fertility rate (number  
71 of lambed ewes per ewe exposed to the ram), the distance in days from ram introduction to lambing  
72 (DRIL), and the litter size (number of newborn lambs per lambed ewes) were recorded.

73

## 74 **2. Materials and Methods**

### 75 *2.1. Animals and management*

76 All the animals in this research had veterinary care by the Veterinary Service in accordance  
77 with the Animal Welfare Act. Blood samples collected by veterinarians during routine health  
78 assessments.

79 A total of 100 Bovška, 110 Istrian Pramenka, and 108 Jezersko-Solčavska sheep, regularly  
80 registered in Slovenian flock book of domestic sheep breeds, were used for the present research.  
81 The animals used came from four farms located in the areas where these Slovenian autochthonous  
82 sheep breeds originated from and were the most typical purebred representatives of these breeds.

83 The Bovška sheep has been selected for use in extensive production systems that include  
84 grazing in the hills and mountains of the Soča river valley (for more informations visit:  
85 <https://www.drobnica.si/strokovno-delo/rejski-programi/za-ovce/>). These animals have generally  
86 white wool and are medium-small sized breed, with an average adult weight of 40 kg for ewes and  
87 50 kg for rams. Ewes normally have one lambing per year, and show an average litter size of

88 approximately 1.2 lambs per birth. These sheep when kept in high level of technology farming  
89 system (i.e. mechanized milking, use of supplemental feed, housing facilities and regular veterinary  
90 controls), may produce up to 270 kg (average milk production per lactation is 196 kg) in 180 days  
91 of lactation, with a fat value of around 7.1% and a protein value of 5.4%. The lambs excluded from  
92 replacement are usually slaughtered at the age of 50-80 days with a live weight of 20 kg.

93 The Istrian Pramenka sheep is a dual-purpose milk-meat breed, but its dairy capacity is more  
94 developed and selected. It is a medium-sized sheep, with an adult weight of 95 kg for rams and 60-  
95 75 kg for ewes. The wool is mostly white and sometimes has dark spots on the head and on the  
96 body. The udder has a regular shape, well proportioned, suitable for manual and mechanical  
97 milking. Milk produced in this breed is mainly destined for cheese production. The average milk  
98 production is 172 kg in 210 days of lactation with a fat value of 7.3% and protein of 5.7%. Istrian  
99 Pramenka has an average litter size of 1.13 live lambs per birth.

100 The Jezersko-Solčava sheep is the largest native sheep breed reared in Slovenia. Its wool has  
101 a white color, with the possible presence of black spots on the withers. It is a medium-sized sheep  
102 with an average weight of 70 kg for ewes and about 100 kg for rams. It is a dual-purpose breed for  
103 meat and wool. The meat production is derived mainly from slaughtered lambs. Ewes can lamb  
104 even twice a year (non-seasonal breeding), and quite frequently have twin lambs (average litter size  
105 1.2). The wool produced, 4-5 kg per year from a single animal, is of good quality and used for the  
106 production of typical woolen products.

107 The Bovška sheep used for this research were chosen from a farm, rearing about 150 sheep  
108 located near the town of Bovec. Istrian Pramenka ewes were chosen from a farm of 500 sheep  
109 located on the Vremščica mountain, which contains more than half of all the animals of this breed  
110 in Slovenia. Finally, Jezersko-Solčavska sheep were selected from two farms, one rearing 120 and  
111 the other 30 sheep located in the Jezersko area. In all the farms the ewes chosen for the study were 3  
112 to 7 years old, because in this range of age the animals show their best reproductive efficiency.

113 Moreover, the ewes were clinically examined by a veterinarian and those too thin or with clinical  
114 signs of disease were excluded from the study.

115 The Bovška and Istrian Pramenka ewes lambled in March/April, and in February/March,  
116 respectively. These breeds grazed on a mixture of grass and legume pastures during the day, and  
117 received a supplement of 200 g of corn grain during the two daily milkings. The animals were  
118 penned in a barn during the night where they had water and hay “ad libitum”. The Jezersko-  
119 Solčavska sheep were fed on only pasture, with food supplementation if necessary, eg. adverse  
120 climatic conditions.

121 The rams were introduced into the flock on October 10<sup>th</sup>, 2017 in the Bovška ewes, on  
122 September 20<sup>th</sup>, 2017 in the flock of the Istrian Pramenka breed in the ratio of 1 ram to 25 ewes and  
123 were kept inside the flock for about 60 days. In the Jezersko-Solčavska breed rams were kept with  
124 ewes all year round.

125 In Bovška and Istrian Pramenka sheep farms, the lambing date and number of newborn  
126 lambs were recorded for 60 days after the starting of the lambing in order to evaluate the fertility  
127 rate, litter size and DRIL. The first two reproductive parameters allowed evaluating the  
128 reproductive performance of the studied sheep, while the third allowed an evaluation of the  
129 beginning of the reproductive recovery. For the Jezersko-Solčavska breed, the lambings of a whole  
130 year were registered from June 22<sup>nd</sup>, 2017 to June 21<sup>st</sup>, 2018. Consequently, from the lambing dates  
131 we can go back to the mating period of each animal. Since the rams were always kept with the  
132 ewes, there was no control of the reproductive activity by the farmer. Therefore, each animal could  
133 freely express its reproductive activity at any time of the year. In order to evaluate the influence of  
134 the photoperiod on reproductive activity we considered the partition of the months of the year  
135 which is usually adopted to define the beginning and end of the increasing or decreasing  
136 photoperiods.

137

138 *2.2. DNA extraction and amplification*

139 From each ewe a sample of 10 mL of blood was taken from the jugular vein with sterile  
140 vacuum tubes (BD Vacuntainer System, Belliver Industrial Estate, Plymouth, UK) containing  
141 EDTA as an anticoagulant; subsequently from each sample 5 aliquots of 200  $\mu$ L each were obtained  
142 and stored at -20°C until analyses.

143 Genomic DNA was extracted from an aliquot of 200  $\mu$ L of whole blood with an extraction  
144 kit (Genomic DNA from blood, Macherey – Nagel, Germany), after evaluation of concentration and  
145 purity by spectrophotometric readings the samples were stored at -80°C. The primers used to  
146 amplify exon II of the *MTNR1A* gene were those reported by Mura et al. (2019): (Forward: 5'-  
147 CCAACCTGGCGGTCACGGA-3'; Reverse: 5'-CGCACCTGCTGGATGTAGTT-3'). The PCR  
148 reaction was conducted according to the method reported by Luridiana et al., (2015a) with the use  
149 of the MAXYGENE II Thermal Cycler (Axygen® Tewksbury, MA, USA).

150 The PCR products obtained were confirmed by a 1.5% (w/v) agarose gel in parallel with a  
151 100 bp marker (Invitrogen, Carlsbad, CA, USA) in 1% TAE Buffer, at a constant voltage of 100 V  
152 for 30 minutes. After electrophoretic run, the PCR products were stained in a 1X ethidium bromide  
153 solution for 20-30 minutes and then displayed under UV light at the trans illuminator (UVItec,  
154 Cambridge, UK). All samples were purified and then sequenced in a forward and reverse direction  
155 by a commercial sequencing service. The obtained sequences were compared with the latest  
156 genome version Oar\_rambouillet\_v1.0 - GenBank assembly accession number: GCA\_002742125.1  
157 through the BLAST program ([www.ncbi.nlm.nih.gov/blast/](http://www.ncbi.nlm.nih.gov/blast/)). The analysis and alignment of the  
158 sequences was performed with the BioEdit Sequence Alignment Editor software  
159 (<http://www.mbio.ncsu.edu/BioEdit/BioEdit.html>).

160

### 161 2.3. Statistical analysis

162 Allelic frequencies were determined by direct counting of the observed genotypes. R statistical  
163 software (Version 3.6.1 R Core Team 2019 R: A language and environment for statistical  
164 computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R->

165 project.org/) was used to analyse the associations between genotype and reproductive traits of each  
166 breed, measured as fertility rate (number of lambing ewes per ewe exposed to the ram), litter size  
167 (number of newborn lambs per lambing ewes) and DRIL. To analyse the Bovška and Istrian  
168 Pramenka ewes was used the following linear model:

$$169 \quad Y_{jik} = \mu + G_j + A_i + (G_j A_i) + e_{jik}$$

170 Where  $Y_{jk}$  is the trait measured for each animal,  $\mu$  is the overall mean,  $G_j$  is the fixed effect of the  
171 genotype (3 levels),  $A_i$  is the effect of the age (4 levels),  $G_j A_i$  is the interaction between genotype  
172 and age, and  $e_{jik}$  is the random residual effect of each observation. If the model showed significance,  
173 multiple comparisons of the means were performed using Tukey's method (library *Agricolae*, R  
174 package version 1.3-1. Felipe de Mendiburu (2019): *Statistical Procedures for Agricultural*  
175 *Research*. <https://CRAN.R-project.org/package=agricolae>).

176 To analyse the number of lambing in each semester of Jezersko-Solčavska breed was used a logistic  
177 regression where  $G_j$  was the fixed effect of the genotype (3 levels) and  $F_i$  was the farm effect (2  
178 levels),  $G_j A_i$  is the interaction between genotype and age, and  $e_{jik}$  is the random residual effect of  
179 each observation was used the following model:

$$180 \quad Y_{jik} = \mu + G_j + F_i + (G_j F_i) + e_{jik}$$

181 Statistical significance was set at  $P < 0.05$ .

182

### 183 **3. Results**

184 The PCR analysis resulted in a single fragment of 824-bp in length, corresponding to the  
185 major part of the *MTNR1A* gene exon II. The obtained sequences showed 8 nucleotide variations  
186 (Table 1), some of which had already been recognized in different breeds, and now reported here  
187 according to the latest genome version (Oar\_ambouillet\_v1.0 - GenBank assembly accession  
188 number: GCA\_002742125.1) except for g.17355517, that was found only in two Bovška sheep  
189 ewes. By sequences alignment and analysis, it was found that the variation in the position  
190 g17355358 was always associated with that in the position g.17355452. Instead, the polymorphisms

191 in the positions g.17355458 and g.17355452, although very close, were not associated. We focused  
192 our attention to the g.17355458C>T and g.17355452G>A loci, that are considered those mostly  
193 involving reproductive seasonality. The polymorphisms found in the Bovška sheep were in Hardy-  
194 Weinberg equilibrium for the locus at position g.17355458 and in disequilibrium for that in the  
195 position g.17355452 for an excess of homozygotes. Instead, the Jezersko-Solčavska sheep  
196 population was in Hardy-Weinberg equilibrium for both loci. These two SNPs in Istrian Pramenka  
197 sheep were both in Hardy-Weinberg disequilibrium due to an excess of homozygotes. The allele  
198 and genotype frequencies of the focused SNPs were different in the examined breeds and are shown  
199 in Table 2. The most frequent allele was the G for the locus in the position g.17355452 and  
200 consequently the G/G genotype. Concerning the g.17355458 locus, in the Bovška and Istrian  
201 Pramenka sheep, C was the most frequent allele and consequently C/C genotype, whereas in the  
202 Jezersko-Solčavska sheep the most frequent allele was the T and consequently the T/T genotype.

203 In the Bovška sheep, the statistical analysis showed an association between the G/G  
204 genotype in the position g.17355452, and a higher fertility ( $P<0.05$ ). Moreover G/G and A/G  
205 genotypes showed a shorter DRIL than A/A ( $P<0.05$ ). No association was found with litter size  
206 (Table 3). Furthermore, no association was found in this breed, between the genotype in the position  
207 g.17355458 and reproductive performances. However, in the Istrian Pramenka sheep SNP in the  
208 position g.17355452 did not show to influence any of the analyzed reproductive parameters (Table  
209 4), while the T/T genotype, in the position g.17355458, was positively associated with a greater  
210 fertility ( $P<0.05$ ) and a shorter DRIL ( $P<0.05$ ) compared to the other two genotypes. The data  
211 about lambing in the Jezersko-Solčavska sheep were reported and registered monthly all year-round  
212 (Table 5). Furthermore, we subdivided the year in two semesters based on photoperiod. It is well  
213 known that increasing photoperiod is from 22<sup>nd</sup> December to 21<sup>st</sup> June and consequently the  
214 decreasing photoperiod is from 22<sup>nd</sup> June to 21<sup>st</sup> December. This is the conventional way to evaluate  
215 the effect of photoperiod on the reproductive activity. Under natural or uncontrolled mating  
216 conditions, we evaluated for each genotype when lambing occurred in each semester. Thus in the

217 Jezersko-Solčavska sheep the animals with G/G genotype, in the position g.17355452, showed a  
218 greater number of lambing ( $P<0.05$ ) during decreasing photoperiod, and consequently a higher  
219 number of mating occurred during increasing photoperiod (Figure 1). Instead the animals carrying  
220 A/A genotype, in the position g.17355452, exhibited the higher number of mating during the  
221 decreasing photoperiods and consequently a higher number of lambing during the increase  
222 photoperiod ( $P<0.05$ ). The litter size in the examined sheep was not influenced by the different  
223 polymorphisms.

224

#### 225 **4. Discussion**

226 Sequencing revealed several SNPs within nucleotide sequences of the *MTNRIA* gene that  
227 are the same in all the three studied Slovenian domestic sheep breeds. Only in two Bovška sheep a  
228 further variation in the position g.17355517C>T was found. The rest of the identified SNPs are the  
229 same as already recognized in other sheep breeds (Notter and Cockett, 2005; Pelletier et al., 2000;  
230 Saxena et al., 2014). The allelic and genotypic frequencies found in the three Slovenian breeds in  
231 the position g.17355452 are similar to those observed in the Sarda breed (Carcangiu et al., 2009b).  
232 They are also similar to those found in many other European, Sub temperate, and Sub tropical sheep  
233 breeds (Calvo et al., 2018; Mateescu et al., 2009; Messer et al., 1997; Mura et al., 2014; Saxena et  
234 al., 2014, 2015b).

235 However, for the mutation in the position g.17355458C>T we found different allele and  
236 genotype frequencies, and in particular the Bovška and Istrian Pramenka sheep showed a high  
237 frequency of the C allele while the Jezersko-Solčavska breed has a prevalence of the T allele. These  
238 differences in allele distribution have also been observed in other studied sheep breeds and are  
239 attributed to the different selective pressures that produced the different breeds (Martínez-Royo et  
240 al., 2012; Notter and Cockett, 2005).

241 In many sheep breeds the SNP in the position g.17355452G>A showed an association with  
242 seasonal reproductive activity and in particular the G/G genotype or even the G allele alone,

243 improved out-of-season reproductive activity (Giantsis et al., 2017; Luridiana et al., 2016; Mateescu  
244 et al., 2009; Mura et al., 2014). Furthermore, in the Sarda breed the animals carrying the  
245 aforementioned genotype showed a higher fertility rate and an advanced recovery of reproductive  
246 activity in spring after treatment with melatonin (Mura et al., 2019). In Sarda breed ewes carrying  
247 the G/G genotype showed a better response to the male effect, regardless of factors like age and  
248 body weight (Luridiana et al., 2015b).

249 In the three studied Slovenian sheep breeds, the effect of the g.17355452G>A  
250 polymorphism is manifested in the Bovška and Jezersko-Solčavska sheep, even though these two  
251 breeds have different management conditions, but both reside in the Alps. In fact, in the Bovška  
252 sheep, where the animals were subjected to the male effect, a higher fertility rate and a shorter  
253 DRIL was observed in the ewes carrying G/G genotype compared to the others. This result agrees  
254 with results in other sheep breeds, where the G/G genotype positively influenced reproductive  
255 activity (Luridiana et al., 2015a). It is important to note that in the Bovška sheep the animals  
256 carrying the G allele respond earlier to the male effect, as evidenced by the shorter DRIL, and  
257 therefore are less influenced by the photoperiod changes.

258 We found in Jezersko-Solcavaska sheep, where rams were always kept together with ewes, a  
259 high number of sheep carrying the G/G and A/G genotype lambing during the decreasing compared  
260 to the increasing photoperiods. Consequently, this indicates that these sheep mated mainly during  
261 the increasing photoperiod. On the other hand, in sheep carrying the A/A genotype we observed that  
262 the greatest number of lambing occurred during the increasing photoperiod indicating that they  
263 were mated mainly during the decreasing photoperiod. Therefore, it can be hypothesized that G/G  
264 and A/G animals show a less deep and shorter anoestrus than those carrying A/A genotype, which  
265 resulted in their easier reproductive recovery.

266 The g.17355458C>T SNP polymorphism showed association with reproductive activity only  
267 in the Istrian Pramenka breed. Indeed T/T genotype positively influenced the reproductive recovery  
268 with a reduction of DRIL of approximately ten days. Furthermore, the animals carrying this

269 genotype also showed a better fertility than the others. This data agrees with what was observed in  
270 the Aragonesa breed which has many similarities regarding the allele and genotype frequencies with  
271 the Istrian Pramenka sheep (Calvo et al., 2018; Martínez-Royo et al., 2012). In the Aragonesa breed  
272 a greater percentage of oestrous cyclic ewes between January and August carried the T allele  
273 (Martínez-Royo et al., 2012). In the same breed and in the same months of observation Calvo et al.  
274 (2018) found a linkage disequilibrium between polymorphisms at position g.17355458C>T and  
275 g.17354971G> A (corresponding to g.15099004G>A in the Calvo's study) and both were  
276 associated with lower total days of anoestrus and higher progesterone cycling months which are two  
277 seasonality traits for ovarian function. Although the present study in Istrian Pramenka was carried  
278 out during decreasing photoperiods, the ewes carrying T/T genotype showed the advance of  
279 reproductive recovery.

280         Considering the data obtained from this research and what was already found in researches  
281 of other sheep breeds, it could be assumed that there is a difference among breeds. These  
282 differences not only are linked to allele or genotype frequencies, but also to the association between  
283 genotype and reproductive performances. This was hypothesized also by Hernandez et al. (2005)  
284 who concluded that the effect of these genotypes on reproductive activity might depend on the  
285 purpose of the breed and / or the selection that these animals have undergone.

286         However, it remains to be clarified how these polymorphisms can influence reproductive  
287 activity in sheep. Considering that even the mutations that did not produce amino acid changes can  
288 influence gene functioning is difficult to explain. The g.17355452G>A SNP, which is always  
289 associated with g.17355358G>A SNP, an effect on the mechanism of melatonin action was  
290 hypothesized in different sheep breeds, including the Slovenian breeds analyzed in this study (Mura  
291 et al., 2019; Saxena et al., 2015b). The g.17355358G>A polymorphism determines an amino acid  
292 change (Val>Ile) of the protein chain that does not directly affect the transmembrane domains but is  
293 located between the two domains at positions 195 and 211 (Barrett et al., 2003; Conway et al.,  
294 1997; Kokkola et al., 1998). Trecherel et al. (2010) showed that this amino acid change causes an

295 alteration of signal transduction. Therefore, it is reasonable to think that this could modify the  
296 melatonin effects on different organic systems and in particular at the ovary level, determining  
297 important effects on reproductive activity.

298 In contrast, it is very difficult to explain the effect of the g.17355458C>T SNP, as this does  
299 not determine an amino acid change and no links have been registered with the other detected  
300 mutations. However, as recorded by Calvo et al. (2018) in the Aragonesa breed, there may be other  
301 mutations that can be linked with these already highlighted, which can cause an alteration in the  
302 transmission of the melatonin signal. For all these considerations, new studies are needed in order to  
303 expand the knowledge and to highlight whether these described polymorphisms can determine  
304 changes in the actions of melatonin at the ovarian or another organ level.

305

## 306 **5. Conclusions**

307 The sequence of the *MTNR1A* gene exon II showed 8 SNPs in three Slovenian domestic  
308 sheep breeds. The mutation at the position g.17355452G>A was always associated with that at the  
309 position g.17355358G>A, which causes the change Val→Ile in the amino acid chain that is certainly  
310 implicated in the transmission of the melatonin signal. In Bovška sheep, the G/G genotype in the  
311 position g.17355452 is associated with a higher fertility rate and a shorter DRIL. In the Istrian  
312 Pramenka breed, however, the T/T genotype of the g.17355458C>T locus is associated with a  
313 higher fertility rate and a shorter DRIL. While in the Jezersko-Solčavska sheep, G/G genotype in  
314 the position g.17355452 is associated with a higher number of lambing during decreasing  
315 photoperiod. Therefore, it can be assumed that these ewes mated mainly during the increasing  
316 photoperiods. Although it is evident that g.17355452G>A and g.17355458C>T SNPs are involved  
317 in the regulation of reproductive performances, further studies are needed to understand the  
318 mechanism of action of these mutations.

319

## 320 **Conflict of interest**

321 None of the authors has any conflicts of interest to declare.

322

### 323 **Author contribution**

324 I certify on behalf of all co-authors that this article has not been presented in any other place  
325 for publication. All co-authors have contributed equally to the research (conception, design of  
326 study, acquisition and interpretation of data) as well as to article preparation. All co-authors have  
327 approved the final draft of this article.

328

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332

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Fig. 1

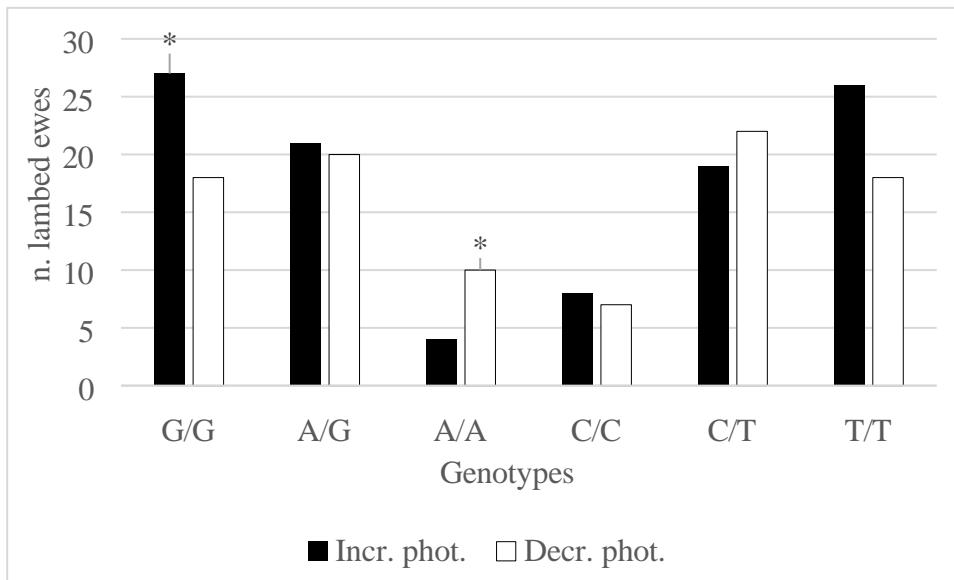


Figure 1

Lambd ewes in increasing and decreasing photoperiods according to genotypes in Jezersko-Solčavska sheep breed (n=108); Increasing photoperiod = from 22<sup>nd</sup> December to 21<sup>st</sup> June; Decreasing photoperiod = from 22<sup>nd</sup> June to 21<sup>st</sup> December; \*= $P < 0.05$

**Table 1**

SNPs position and amino acid change in the *MTNRIA* gene according to the latest genome version Oar\_rambouillet\_v1.0 (GenBank assembly accession number: GCA\_002742125.1) in the three studied Slovenian sheep breeds

SNP position	Nucleotide change	Codons	Amino acid change
g.17355611	G→T	ACG→ACT	None
g.17355458	C→T	TAC→TAT	None
g.17355452	G→A	CCG→CCA	None
g.17355358	G→A	GTC→ATC	Val→Ile
g.17355281	G→A	CTG→CTA	None
g.17355263	G→A	AGG→AGA	None
g.17355173	C→T	CCC→CCT	None
g.17355171	C→A	GCC→GAC	Ala→Asp

**Table 2**

Allele and genotype frequency of the focused *MTNR1A* gene SNPs in the three observed sheep breeds, according to the latest genome version Oar\_rambouillet\_v1.0 (GenBank assembly accession number: GCA\_002742125.1)

Position	Allele frequency				Genotype frequency					
	g.17355452		g.17355458		g.17355452			g.17355458		
Breed	G	A	C	T	G/G	G/A	A/A	C/C	C/T	T/T
Bovška	0.68	0.32	0.59	0.41	0.50	0.36	0.14	0.42	0.34	0.24
Jezersko-Solčavska	0.64	0.36	0.36	0.64	0.45	0.41	0.14	0.15	0.42	0.43
Istrian Pramenka	0.64	0.36	0.59	0.41	0.64	0.26	0.10	0.54	0.25	0.21

**Table 3**

Fertility rate, distance in days from male introduction to lambing and litter size according to genotypes in Bovška sheep breed ( $n=100$ )

SNP	g.17355452A>G			<i>P</i>	g.17355458C>T		
Genotype	G/G	G/A	A/A		C/C	C/T	T/T
Fertility	93.9	79.1	81.8	<0.05	85.7	86.3	93.7
DRIL	155±10	156±6	174±8	<0.05	154±8	154±7	155±12
Litter size	1.08±0.03	1.09±0.03	1.07±0.01		1.08±0.02	1.09± 0.03	1.04±0.01

DRIL: Distance in days from Ram Introduction to Lambing; Reproductive data refers to a single

year

**Table 4**

Fertility rate, distance in days from male introduction to lambing and litter size according to genotypes in Istrian Pramenka sheep breed ( $n=110$ )

SNP	g.17355452A>G			g.17355458C>T			<i>P</i>
	Genotype	G/G	G/A	A/A	C/C	C/T	
Fertility	80.9	53.3	80.0	78.7	46.6	92.8	<0.05
DRIL	165 ± 7	174 ± 5	178 ± 11	187 ± 10	174 ± 5	169 ± 11	<0.05
Litter size	1.1 ± 0.03	1.0 ± 0.01	1.0 ± 0.02	1.12 ± 0.04	1.09 ± 0.01	1.07 ± 0.01	

DRIL: Distance in days from Ram Introduction to Lambing; Reproductive data refers to a single

year

**Table 5**

Lambing distribution during the year of observation in the Jezersko-Solčavska breed ( $n=108$ ) according to genotypes

Genotypes	Lambing distribution per months											
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
G/G	4	4	3	3	2	2	1	3	4	7	7	5
A/G	3	4	5	3	2	3	1	3	3	5	5	4
A/A	0	1	4	3	1	1	1	1	1	1	0	0
C/C	0	2	2	1	1	1	0	1	1	2	2	1
C/T	4	4	5	4	4	1	1	3	3	4	4	5
T/T	3	4	4	3	3	1	1	3	5	5	6	6

**Conflict of interest statement**

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.

### Author statement

I certify on behalf of all coauthors that this article has not been presented in any other place for publication. All coauthors have contributed equally to the research (conception, design of study, acquisition and interpretation of data) as well as to article preparation. All coauthors have approved the final draft of this article.