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

Reproductive performance in three Slovenian sheep breeds with different genotypes of the *MTNR1A* gene

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ABSTRACT

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

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

1. Introduction

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

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

2. Materials and Methods

2.1. Animals and management

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

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

The Bovška sheep has been selected for use in extensive production systems that include grazing in the hills and mountains of the Soča river valley (for more informations visit: https://www.drobnica.si/strokovno-delo/rejski-programi/za-ovce/). These animals have generally white wool and are medium-small sized breed, with an average adult weight of 40 kg for ewes and 50 kg for rams. Ewes normally have one lambing per year, and show an average litter size of approximately 1.2 lambs per birth. These sheep when kept in high level of technology farming system (i.e. mechanized milking, use of supplemental feed, housing facilities and regular veterinary controls), may produce up to 270 kg (average milk production per lactation is 196 kg) in 180 days of lactation, with a fat value of around 7.1% and a protein value of 5.4%. The lambs excluded from replacement are usually slaughtered at the age of 50-80 days with a live weight of 20 kg.

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

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

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

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

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

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

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

2.2. DNA extraction and amplification

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

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

The PCR products obtained were confirmed by a 1.5% (w/v) agarose gel in parallel with a 100 bp marker (Invitrogen, Carlsbad, CA, USA) in 1% TAE Buffer, at a constant voltage of 100 V for 30 minutes. After electrophoretic run, the PCR products were stained in a 1X ethidium bromide solution for 20-30 minutes and then displayed under UV light at the trans illuminator (UVItec, Cambridge, UK). All samples were purified and then sequenced in a forward and reverse direction by a commercial sequencing service. The obtained sequences were compared with the latest genome version Oar_rambouillet_v1.0 - GenBank assembly accession number: GCA_002742125.1 through the BLAST program (www.ncbi.nlm.nih.gov/blast/). The analysis and alignment of the sequences was performed with the BioEdit Sequence Alignment Editor software (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html).

2.3. Statistical analysis

Allelic frequencies were determined by direct counting of the observed genotypes. R statistical software (Version 3.6.1 R Core Team 2019 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) was used to analyse the associations between genotype and reproductive traits of each breed, measured as fertility rate (number of lambed ewes per ewe exposed to the ram), litter size (number of newborn lambs per lambed ewes) and DRIL. To analyse the Bovška and Istrian Pramenka ewes was used the following linear model:

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

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

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

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

Statistical significance was set at $P<0.05$.

3. Results

The PCR analysis resulted in a single fragment of 824-bp in length, corresponding to the major part of the *MTNR1A* gene exon II. The obtained sequences showed 8 nucleotide variations (Table 1), some of which had already been recognized in different breeds, and now reported here according to the latest genome version (Oar_rambouillet_v1.0 - GenBank assembly accession number: GCA_002742125.1) except for g.17355517, that was found only in two Bovška sheep ewes. By sequences alignment and analysis, it was found that the variation in the position g17355358 was always associated with that in the position g.17355452. Instead, the polymorphisms in the positions g.17355458 and g.17355452, although very close, were not associated. We focused our attention to the g.17355458C>T and g.17355452G>A loci, that are considered those mostly involving reproductive seasonality. The polymorphisms found in the Bovška sheep were in Hardy-Weinberg equilibrium for the locus at position g.17355458 and in disequilibrium for that in the position g.17355452 for an excess of homozygotes. Instead, the Jezersko-Solčavska sheep population was in Hardy-Weinberg equilibrium for both loci. These two SNPs in Istrian Pramenka sheep were both in Hardy-Weinberg disequilibrium due to an excess of homozygotes. The allele and genotype frequencies of the focused SNPs were different in the examined breeds and are shown in Table 2. The most frequent allele was the G for the locus in the position g.17355452 and consequently the G/G genotype. Concerning the g.17355458 locus, in the Bovška and Istrian Pramenka sheep, C was the most frequent allele and consequently C/C genotype, whereas in the Jezersko-Solčavska sheep the most frequent allele was the T and consequently the T/T genotype.

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

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

4. Discussion

Sequencing revealed several SNPs within nucleotide sequences of the *MTNR1A* gene that are the same in all the three studied Slovenian domestic sheep breeds. Only in two Bovška sheep a further variation in the position g.17355517C>T was found. The rest of the identified SNPs are the same as already recognized in other sheep breeds (Notter and Cockett, 2005; Pelletier et al., 2000; Saxena et al., 2014). The allelic and genotypic frequencies found in the three Slovenian breeds in the position g.17355452 are similar to those observed in the Sarda breed (Carcangiu et al., 2009b). They are also similar to those found in many other European, Sub temperate, and Sub tropical sheep breeds (Calvo et al., 2018; Mateescu et al., 2009; Messer et al., 1997; Mura et al., 2014; Saxena et al., 2014, 2015b).

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

In many sheep breeds the SNP in the position g.17355452G>A showed an association with seasonal reproductive activity and in particular the G/G genotype or even the G allele alone, improved out-of-season reproductive activity (Giantsis et al., 2017; Luridiana et al., 2016; Mateescu et al., 2009; Mura et al., 2014). Furthermore, in the Sarda breed the animals carrying the aforementioned genotype showed a higher fertility rate and an advanced recovery of reproductive activity in spring after treatment with melatonin (Mura et al., 2019). In Sarda breed ewes carrying the G/G genotype showed a better response to the male effect, regardless of factors like age and body weight (Luridiana et al., 2015b).

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

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

The g.17355458C>T SNP polymorphism showed association with reproductive activity only in the Istrian Pramenka breed. Indeed T/T genotype positively influenced the reproductive recovery with a reduction of DRIL of approximately ten days. Furthermore, the animals carrying this genotype also showed a better fertility than the others. This data agrees with what was observed in the Aragonesa breed which has many similarities regarding the allele and genotype frequencies with the Istrian Pramenka sheep (Calvo et al., 2018; Martínez-Royo et al., 2012). In the Aragonesa breed a greater percentage of oestrous cyclic ewes between January and August carried the T allele (Martínez-Royo et al., 2012). In the same breed and in the same months of observation Calvo et al. (2018) found a linkage disequilibrium between polymorphisms at position g.17355458C>T and g.17354971G> A (corresponding to g.15099004G>A in the Calvo’s study) and both were associated with lower total days of anoestrus and higher progesterone cycling months which are two seasonality traits for ovarian function. Although the present study in Istrian Pramenka was carried out during decreasing photoperiods, the ewes carrying T/T genotype showed the advance of reproductive recovery.

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

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

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

5. Conclusions

The sequence of the *MTNR1A* gene exon II showed 8 SNPs in three Slovenian domestic sheep breeds. The mutation at the position g.17355452G>A was always associated with that at the position g17355358G>A, which causes the change Val→Ile in the amino acid chain that is certainly implicated in the transmission of the melatonin signal. In Bovška sheep, the G/G genotype in the position g.17355452 is associated with a higher fertility rate and a shorter DRIL. In the Istrian Pramenka breed, however, the T/T genotype of the g.17355458C>T locus is associated with a higher fertility rate and a shorter DRIL. While in the Jezersko-Solčavska sheep, G/G genotype in the position g.17355452 is associated with a higher number of lambing during decreasing photoperiod. Therefore, it can be assumed that these ewes mated mainly during the increasing photoperiods. Although it is evident that g.17355452G>A and g.17355458C>T SNPs are involved in the regulation of reproductive performances, further studies are needed to understand the mechanism of action of these mutations.

Conflict of interest

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

Author contribution

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

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References

Abecia, J.A., Forcada, F., González-Bulnes, A., 2012. Hormonal control of reproduction in small ruminants. Anim. Reprod. Sci. 130, 173–179. https://doi.org/10.1016/j.anireprosci.2012.01.011

Arendt, J., 1998. Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology. Rev. Reprod. 3, 13-22. https://doi.org/10.1530/ror.0.0030013.

Barrett, P., Conway, S., Morgan, P.J., 2003. Digging deep - Structure-function relationships in the melatonin receptor family. J. Pineal Res. 35, 221–230. https://doi.org/10.1034/j.1600-079X.2003.00090.x

Bittman, E.L., Karsch, F.J., 1984. Nightly duration of pineal melatonin secretion determines the reproductive response to inhibitory day length in the ewe. Biol. Reprod. 30, 585–593. https://doi.org/10.1095/biolreprod30.3.585

Calvo, J.H., Serrano, M., Martinez-Royo, A., Lahoz, B., Sarto, P., Ibañez-Deler, A., Folch, J., Alabart, J.L., 2018. SNP rs403212791 in exon 2 of the MTNR1A gene is associated with reproductive seasonality in the Rasa aragonesa sheep breed. Theriogenology 113, 63–72. https://doi.org/10.1016/j.theriogenology.2018.02.013

Carcangiu, V., Luridiana, S., Vacca, G.M., Daga, C., Mura, M.C., 2011. A polymorphism at the melatonin receptor 1A (MTNR1A) gene in Sarda ewes affects fertility after AI in the spring. Reprod. Fertil. Dev. 23, 376–380. https://doi.org/10.1071/RD10014

Carcangiu, V., Mura, M.C., Parmeggiani, A., Piccione, G., Bini, P.P., Cosso, G., Luridiana, S., 2013. Daily rhythm of blood melatonin concentrations in sheep of different ages. Biol. Rhythm Res. 44, 908–915. https://doi.org/10.1080/09291016.2013.781317

Carcangiu, V., Mura, M.C., Vacca, G.M., Pazzola, M., Dettori, M.L., Luridiana, S., Bini, P.P., 2009b. Polymorphism of the melatonin receptor MT1 gene and its relationship with seasonal reproductive activity in the Sarda sheep breed. Anim. Reprod. Sci. 116, 65–72. https://doi.org/10.1016/j.anireprosci.2009.01.005

Carcangiu, V., Vacca, G.M., Mura, M.C., Dettori, M.L., Pazzola, M., Luridiana, S., Bini, P.P., 2009a. Relationship between MTNR1A melatonin receptor gene polymorphism and seasonal reproduction in different goat breeds. Anim. Reprod. Sci. 110, 71–78. https://doi.org/10.1016/j.anireprosci.2007.12.014

Chemineau, P., Guillaume, D., Migaud, M., Thiéry, J.C., Pellicer-Rubio, M.T., Malpaux, B., 2008. Seasonality of Reproduction in Mammals: Intimate Regulatory Mechanisms and Practical Implications. Reprod. Domest. Anim. 43, 40–47. https://doi.org/10.1111/j.1439-0531.2008.01141.x

Conway, S., Canning, S.J., Barrett, P., Guardiola-Lemaitre, B., Delagrange, P., Morgan, P.J., 1997. The roles of valine 208 and histidine 211 in ligand binding and receptor function of the ovine Mel(1αβ) melatonin receptor. Biochem. Biophys. Res. Commun. 239, 418–423. https://doi.org/10.1006/bbrc.1997.7482

Dubocovich, M.L., Rivera-Bermudez, M.A., Gerdin, M.J., Masana, M.I., 2003. Molecular pharmacology, regulation and function of mammalian melatonin receptors. Front. Biosci. 8, d1093-1108. https://doi.org/10.2741/1089

Giantsis, I.A., Laliotis, G.P., Stoupa, O., Avdi, M., 2017. Polymorphism of the melatonin receptor 1A (MNTR1A) gene and association with seasonality of reproductive activity in a local Greek sheep breed. J. Biol. Res. 23. https://doi.org/10.1186/s40709-016-0050-y

Hernandez, X., Bodin, L., Chesneau, D., Guillaume, D., Chemineau, P., Malpaux, B., Migaud, M., 2005. Relationship between MT1 melatonin receptor gene polymorphism and seasonal physiological responses in Île-de-France ewes. Reprod. Nutr. Dev. 45, 151–162. https://doi.org/10.1051/rnd:2005012

Kokkola, T., Watson, M.-A., White, J., Dowell, S., Foord, S.M., Laitinen, J.T., 1998. Mutagenesis of human Mel(1a) melatonin receptor expressed in yeast reveals domains important for receptor function. Biochem. Biophys. Res. Commun. 249, 531–536. https://doi.org/10.1006/bbrc.1998.9182

Lincoln, G.A., Clarke, I.J., 1994. Photoperiodically-lnduced Cycles in the Secretion of Prolactin in Hypothalamo-Pituitary Disconnected Rams: Evidence for Translation of the Melatonin Signal in the Pituitary Gland. J. Neuroendocrinol. 6, 251–260. https://doi.org/10.1111/j.1365-2826.1994.tb00580.x

Luridiana, S., Mura, M.C., Daga, C., Cosso, G., Bodano, S., Farci, F., Zidda, F., Carcangiu, V., 2016. Influences of melatonin treatment, melatonin receptor 1A (MTNR1A) and kisspeptin (KiSS-1) gene polymorphisms on first conception in Sarda ewe lambs. Reprod. Fertil. Dev. 28, 750–756. https://doi.org/10.1071/RD14120

Luridiana, S., Mura, M.C., Daga, C., Diaz, M.L., Bini, P.P., Cosso, G., Carcangiu, V., 2015a. The relationship between melatonin receptor 1A gene (MTNR1A) polymorphism and reproductive performance in Sarda breed sheep. Livest. Sci. 171, 78–83. https://doi.org/10.1016/j.livsci.2014.11.004

Luridiana, S., Mura, M.C., Daga, C., Farci, F., Di Stefano, M. V, Zidda, F., Carcangiu, V., 2015b. Melatonin treatment in spring and reproductive recovery in sheep with different body condition score and age. Anim. Reprod. Sci. 160, 68–73. https://doi.org/10.1016/j.anireprosci.2015.07.004

Malpaux, B., Migaud, M., Tricoire, H., Chemineau, P., 2001. Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. J. Biol. Rhythms 16, 336–347. https://doi.org/10.1177/074873001129002051

Martínez-Royo, A., Lahoz, B., Alabart, J.L., Folch, J., Calvo, J.H., 2012. Characterisation of the Melatonin Receptor 1A (MTNR1A) gene in the Rasa Aragonesa sheep breed: Association with reproductive seasonality. Anim. Reprod. Sci. 133, 169–175. https://doi.org/10.1016/j.anireprosci.2012.06.018

Mateescu, R.G., Lunsford, A.K., Thonney, M.L., 2009. Association between melatonin receptor 1A gene polymorphism and reproductive performance in Dorset ewes. J. Anim. Sci. 87, 2485–2488. https://doi.org/10.2527/jas.2008-1688

Messer, L.A., Wang, L., Tuggle, C.K., Yerle, M., Chardon, P., Pomp, D., Womack, J.E., Barendse, W., Crawford, A.M., Notter, D.R., Rothschild, M.F., 1997. Mapping of the melatonin receptor 1a (MTNR1A) gene in pigs, sheep, and cattle. Mamm. Genome 8, 368–370. https://doi.org/10.1007/s003359900444

Migaud, M., Daveau, A., Malpaux, B., 2005. MTNR1A melatonin receptors in the ovine premammillary hypothalamus: Day-night variation in the expression of the transcripts. Biol. Reprod. 72, 393–398. https://doi.org/10.1095/biolreprod.104.030064

Mura, M.C., Luridiana, S., Bodano, S., Daga, C., Cosso, G., Diaz, M.L., Bini, P.P., Carcangiu, V., 2014. Influence of melatonin receptor 1A gene polymorphisms on seasonal reproduction in Sarda ewes with different body condition scores and ages. Anim. Reprod. Sci. 149, 173–177. https://doi.org/10.1016/j.anireprosci.2014.07.022

Mura, M.C., Luridiana, S., Pulinas, L., Di Stefano, M. V, Carcangiu, V., 2019. Reproductive response to male joining with ewes with different allelic variants of the MTNR1A gene. Anim. Reprod. Sci. 200, 67–74. https://doi.org/10.1016/j.anireprosci.2018.11.012

Notter, D.R., Cockett, N.E., 2005. Opportunities for detection and use of QTL influencing seasonal reproduction in sheep: A review. Genet. Sel. Evol. 37, S39–S53. https://doi.org/10.1051/gse:2004031

Pelletier, J., Bodin, L., Hanocq, E., Malpaux, B., Teyssier, J., Thimonier, J., Chemineau, P., 2000. Association between expression of reproductive seasonality and alleles of the gene for Mel(1a) receptor in the ewe. Biol. Reprod. 62, 1096–1101. https://doi.org/10.1095/biolreprod62.4.1096

Reppert, S.M., Weaver, D.R., Ebisawa, T., 1994. Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron 13, 1177–1185. https://doi.org/10.1016/0896-6273(94)90055-8

Saxena, V.K., Jha BK, Meena AS, Naqvi SM. 2015b. Characterization of MTNR1A gene in terms of genetic variability in a panel of subtemperate and subtropical Indian sheep breeds. J. Genet. 94, 715–721. https://doi.org/10.1007/s12041-015-0587-9

Saxena, V.K., Jha, B.K., Meena, A.S., Naqvi, S.M.K., 2014. Sequence analysis and identification of new variations in the coding sequence of melatonin receptor gene (MTNR1A) of Indian Chokla sheep breed. Meta Gene 2, 450–458. https://doi.org/10.1016/j.mgene.2014.05.005

Saxena, V.K., Jha, B.K., Meena, A.S., Narula, H.K., Kumar, D., Naqvi, S.M.K., 2015a. Assessment of genetic variability in the coding sequence of melatonin receptor gene (MTNR1A) in tropical arid sheep breeds of India. Reprod. Domest. Anim. 50, 517–521. https://doi.org/10.1111/rda.12503

Sliwowska, J.H., Billings, H.J., Goodman, R.L., Coolen, L.M., Lehman, M.N., 2004. The premammillary hypothalamic area of the ewe: Anatomical characterization of a melatonin target area mediating seasonal reproduction. Biol. Reprod. 70, 1768–1775. https://doi.org/10.1095/biolreprod.103.024182

Trecherel, E., Batailler, M., Chesneau, D., Delagrange, P., Malpaux, B., Chemineau, P., Migaud, M., 2010. Functional characterization of polymorphic variants for ovine MT1 melatonin receptors: Possible implication for seasonal reproduction in sheep. Anim. Reprod. Sci. 122, 328–334. https://doi.org/10.1016/j.anireprosci.2010.10.007

Weaver, D.R., Liu, C., Reppert, S.M., 1996. Nature's Knockout: The Mel1b Receptor Is Not Necessary for Reproductive and Circadian Responses to Melatonin in Siberian Hamsters. Mol. Endocrinol. 10, 1478–1487.3

Fig. 1

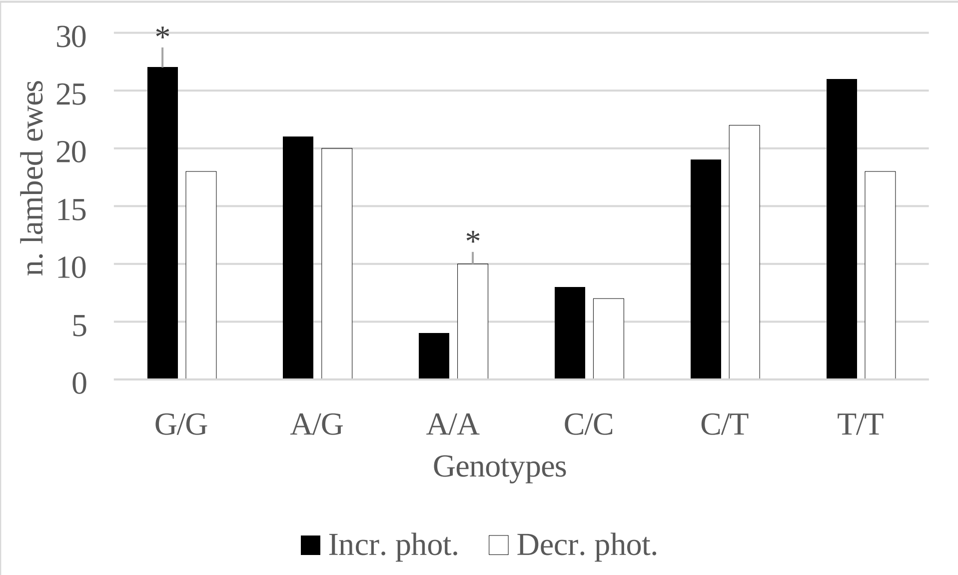

Figure 1

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

Table 1

SNPs position and amino acid change in the *MTNR1A* 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)

	Allele frequency				Genotype frequency					
Position	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			*P*	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			*P*
Genotype	G/G	G/A	A/A	C/C	C/T	T/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.