INTRODUCTION

Melatonin, the pineal hormone, plays an important role in regulation of seasonal reproduction and circadian rhythms. Its effects are mediated via high affinity melatonin receptors, located on cells of the pituitary pars tuberalis (PT) and suprachiasmatic nucleus (SCN), respectively.

There are two subtypes of mammalian melatonin receptors, the MT1 (Mel1a) and the MT2 (Mel1b) melatonin receptor subtypes. Both subtypes are members of the seven-transmembrane G protein-coupled receptor family (Gall et al. 2002).

The melatonin is secreted from the pineal gland during the hours of darkness and acts as a hormonal message of the photoperiod in vertebrates. In mammals, melatonin has two major physiological functions:

1. Melatonin is critical for the regulation of seasonal changes in various aspects of physiology and neuroendocrine function (Bartness et al. 1993; Malpaux et al. 2001).
2. Melatonin affects the phase of circadian rhythms by a direct action on the biological clock that resides within the hypothalamic suprachiasmatic nucleus (Gall et al. 2002).

The MTNR1A gene has been mapped on chromosome 26 of sheep (Messer et al. 1997) consists of two exons divided by a large intron (Reppert et al. 1994). The exon one shows low degree of polymorphism (Trecherel et al. 2010). The exon 2 of MTNR1A gene coding for the ovine MT1 receptors is known to be highly polymorphic (Barrett et al. 1997;
MATERIALS AND METHODS

Genomic DNA preparation, amplification and digestion

One hundred Zandi sheep were randomly selected from Khojir Zandi sheep breeding station. All the ewes had seasonal reproduction records and the nutrition status was similar for all of sampled individuals. 10 mL of blood were collected from the jugular vein in EDTA coated tubes. Genomic DNA was extracted by modified salting out method with the size of 824bp from exon 2 of ovine MTNR1A gene was amplified with a quality which could be directly analyzed by RFLP (Figure 1). Digestion of amplified fragment with MnlI produced nine fragments of 303, 236, 218, 135, 82, 67, 36, 28 and 22 bp from exon 2 of ovine MTNR1A gene was amplified with following primer pair:

Forward: 5'-TGT TGT GGT GAG CCT GG-3'
Reverse: 5'-ATG GAG AGG GTT TGC GTT TA-3'

The amplification reactions were carried out in 25 µL tube containing 2.5 µL 10X PCR buffer (50 mMol/L KCl, 10 mMol/L Tris-HCl (pH 8.0), 0.1% Triton X-100), 2.5 mMol/L MgCl₂, 0.5 mMol/L each dNTP, 2 µmol/L each primer, 50 ng ovine genomic DNA, and 1U Taq DNA polymerase. The PCR conditions were carried out by an early denaturation at 95 °C for 5 minutes, followed by 45 PCR cycles with Touchdown program (Table 1), extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR products were separated by electrophoresis on 1.5% agarose gel. After amplification, the 7µL of PCR product was digested with 2 unites MnlI endonuclease at 37 °C for 4 hours, followed by a deactivation process at 65 °C for 20 minutes. For genotyping of studied samples, the digested fragments were electrophoresed on 3% agarose gel and stained with ethidium bromide. The allelic and genotype frequencies and test of Hardy Weinberg (HW) equilibrium were done using Pop Gene software version 1.32.

Table 1 Cycle numbers and temperature for Touchdown PCR

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Cycle number</th>
<th>Time</th>
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<tbody>
<tr>
<td>63</td>
<td>6</td>
<td>45 s</td>
</tr>
<tr>
<td>62</td>
<td>5</td>
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<td>61</td>
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<td>60</td>
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<td>59</td>
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<tr>
<td>58</td>
<td>5</td>
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Marker trait association study

The GLM procedure of the SAS package (SAS, 2000) was used to estimate the effect of MTNR1A/MnlI genotypes on production (body weight at birth, 1, 3, 6, 9 and 12 months of age) and reproduction trait (litter size) of Zandi ewes with following statistical model:

Yijk = µ + Gi + eij

Where:
Yijk: phenotypic value of interested traits.
µ: overall population mean.
Gi: the effect of ith genotype.
eij: residual error.
respectively (Figure 2 and table 2). The mm genotype was not detected in Zandi sheep.

The allelic frequencies of 0.9109 and 0.0891 were observed for M and m alleles of MTNR1A locus. The X² test confirms that studied Zandi sheep population was in HW equilibrium.

**Marker trait association study**

Association analysis showed that MTNR1A/MnlI marker site had significant effect (P>0.05) on BW1, as ewes with Mm genotypes had higher BW1 than MM individuals (Table 3). No significant association was observed between MTNR1A/MnlI marker site and other studied traits (Table 3).

According to our results, the M allele in Small Tailed Han sheep (Chu et al. 2003), Shall and Karakul sheep (Ghiasi et al. 2006), Sarda sheep (Mura et al. 2010), and Merinos d’Arles sheep (Teyssier et al. 2010) had higher frequency than m allele. The m allele may lead to a reproductive activity less linked to photoperiod. Kaczor et al. (2006) investigated the polymorphism at the MTNR1A locus in prolific Olkuska sheep, Polish mountain sheep, Suffolk and in sheep F1 crosses (Merino-Romanov). A high frequency of the M allele was found in sheep with seasonal sexual activity: prolific Olkuska sheep (0.643), Polish mountain sheep (0.684) and Suffolk (0.60). In aseasonal F1 (Merino-Romanov) sheep, a higher proportion of the m allele was found (0.795). The frequencies of MM genotype was 0.529, 0.474, 0.6 and 0.205 in prolific Olkuska sheep, Polish mountain sheep, Suffolk and F1 (Merino-Romanov) crosses, respectively.

The RFLP analysis showed nine cleavage sites of MnlI enzyme were presented in exon 2 of MTNR1A marker site. However, only one of those was shown to be polymorphic. The polymorphic site was at fragment 303 bp. Results of variance analysis indicated that there was no association between the MTNR1A/MnlI marker site and litter size in Zandi sheep (Table 3). Chu et al. (2003) showed that the marker site for MnlI endonuclease on MTNR1A gene had no significant effect on litter size in both the first parity and the second parity in Small Tail Han sheep (P>0.05). Reports on the effect of genotype at the MTNR1A loci on the
sheep litter size indicated that genetic factors had no significant effect on this productive trait despite the fact that slightly larger litters were observed in sheep with one copy of the m allele (Notter et al. 2003; Chu et al. 2003).

The effect of MTNR1A/MnlI genotypes on production traits was found in Zandi sheep. The MTNR1A/MnlI genotypes were significantly associated with BW1 (P<0.05). Least square analysis indicated that Mm genotypes had higher BW1 than MM genotype (Table 3). No significant differences (P<0.05) were detected between MTNR1A/MnlI genotypes and other studied traits (Table 3).

CONCLUSION

Two genotypes of MM and Mm were found in MTNR1A/MnlI marker site in Zandi sheep. No mm genotype was detected in the present study. Marker trait association analysis showed the significant effects of the MTNR1A/MnlI polymorphism on BW1. In the subsequent studies using a large number of samples along with the other important parameters such as sire effect and the lamb health status which may influence lamb body weight is recommended.

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REFERENCES


