Abstract

The genotypes of the melatonin receptor 1A (MTNR1A) were determined by PCR-RFLP in the native Iranian Shall and karakul breeds of sheep. Blood samples were collected from 60 karakul and 50 Shall breeds. Genomic DNA was extracted based on the Guanidin Thiocyanate-slica gel method. After PCR reaction, PCR products were digested by the MnlI restriction enzyme. The MTNR1A locus had two genotypes with frequencies of 0.7 (+ +) and 0.3 (+ -) in the Karakul breed, 0.58 (+ +) and 0.42 (+ -) in the shall breed. Heterozygosis value for the MTNR1A locus in Shall and Karakul breeds were 0.42 and 0.3, respectively. This study provided evidence that sheep breeds, Shall and Karakul have variability in MTNR1A locus. Therefore, this locus can be used as a marker for the reproduction trait in selection programs.

Keywords: MTNR 1A; Seasonal reproductive; Melatonin; Polymorphism; Shall and Karakul sheep.

Seasonal reproductive activity is a common feature among various mammalian species of the temperate latitudes (Ortavant et al., 1985). Ewes have a breeding season, characterized by succession of 16-18 days long estrus cycles, which usually appear in summer or at the beginning of autumn and finish in late winter or at the very beginning of spring (Thiery et al., 2002). The seasonality of reproductive activity in sheep breeds in temperate latitudes is controlled by the photoperiod (Thiery et al., 2002). The photoperiodic information is conveyed through several neural relays from the retina to the pineal gland where the light signal is translated into the daily cycle of melatonin secretion (Malpaux et al., 1999). Melatonin level is high at night and low during the day (Ganguly et al., 2002). The length of nocturnal secretion of melatonin reflects the duration of night and it regulates the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus (Malpaux et al., 1999). Changes in GnRH release induce corresponding changes in luteinising hormone (LH) secretion which are responsible for the alternating presence or absence of ovulation in the female and varying sperm production in the male (Malpaux et al., 1999). Three specific melatonin receptor subtypes have been cloned and characterized in mammals: Mel1a, Mel1b and Mel1c (Migaud et al., 2002). Thus so far only Mel1a and Mel1b have been found in mammalian species, but three are found in lower vertebrates. Only the Mel1a receptor is involved in central control of seasonal reproduction (Pelletier et al., 2000).

In sheep two polymorphic RFLP sites within the ovine melatonin receptor 1a gene (MTNR1A) were discovered by Messer et al. (1997). A particular allelic form of the Mel1a receptor gene characterized by the absence of an MnlI restriction site at position 605 of the coding sequence, leading to the homozygous genotypes (- -) when the corresponding mutation occurs on both chromosomes (Migaud et al., 2002). Pelletier et al. (2000) also reported that the MTNR1A genotype was associated with seasonal reproduction in Merino Darles ewes. Notter et al. (2003) reported that in adult ewes (3 years old and older), fertility of ewes with genotype mm for polymorphic MnlI site in MTNR1A gene is 10 to 11.2% less than that in other ewes. They also this researcher reported that ewes litter size is not significantly associated with MTNR1A genotype, but adult ewes with the mm genotype had approximately 11% fewer lambs per ewe than ewes of other genotypes (Notter et al., 2003). The aim of the
The present study was to identify the different genotypes of the MTNR1A gene in Iranian Shall and Karakul sheep by the PCR-RFLP method.

Genomic DNA was extracted from blood samples of 60 Karkul sheep from Sarakhs Karakul Breeding Center, Mashhad and 50 Shall sheep from Smael Abad Shall Breeding Center, Ghazvin, Iran. 100 µl blood samples were used for DNA extraction as described by Boom et al. (1990). 50 ng of genomic DNA was used for PCR employing the primers of Messer et al. (1997), (MTNR1A) 5’ TGT GTT TGT GGT GAG CCT GG 3’; (MTNR2A) 5’ ATG GAG AGG GTT TGC GTT TA 3’. Sense primer corresponding to position 1089-1108 was used for amplification of PCR product from exons 2 of the MTNR1A gene with a length of 824 bp (Fig. 1). The reaction mixture consisted of 1 µl of taq polymerase, 2.5 µl of PCR buffer, 200 µM dNTPs, 3 µl of each primer and 11 µl ddH2O. PCR was performed in 35 cycles (denaturation: 94°C, 1 min; annealing: 59°C, 1 min; extension: 72°C, 2 min) and a final extension step at 72°C for 10 min.

Genotyping samples: 6 µl of each PCR product was digested at 37°C using 2U of MnlI restriction enzyme. The polymorphic site in exon 2 of MTNR1A gene is located at position 605. The presence of the cleavage site for MnlI resulted in 236 bp and 67 bp products (allele -), while the loss of this site led to a single 303 bp (allele +). The (+ +) genotype exhibited both fragments of 236 and 67 bp. The (+ -) genotype exhibited 303, 236, and 67 bp products. The (- -) genotype was absent in this study. The allele and genotype frequencies and the H-W test were calculate using Pogene software version 1.32. The MTNR1A locus was polymorphic in the Iranian Shall and Karakul sheep breeds. the genotype (+ +) and (+ -) for the MTNR1A locus were observed but the genotype (- -) was absent in both breeds (Fig. 2).

In this study two genetic variants (+ and –) with allele frequencies of 0.79 and 0.21 and 0.85 and 0.15 were observed in the Shall and Karakul breeds, respectively. Similar results were observed in Merino d’Arles ewes (Pelletier et al., 2000). The most frequent genotype in the Shall and Karakul breeds was the (+ +) genotype with frequencies of 0.58 and 0.7, respectively. Mean observed heterozygosis was higher than mean expected heterozygosis. The deviation from the Hardy-Weinberg equilibrium was not detected in the Shall and Karakul breeds. The χ² test confirms the H-W equilibrium in both populations (Table 1). Polymorphism in the MTNR1A locus has been studied by many researchers. Pelletier et al. (2000). resulted that there is association between (- -) genotype for site MnlI at position 605 and seasonal anovulation activity in Merino Darles. It was shown that the effect of MTNR1A genotypes on fertility and litter size was not significant for mating involving ewes of all ages.

**Table 1.** Allele frequencies, observed heterozygosity and expected heterozygosity.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Allele (+)</th>
<th>Allele (-)</th>
<th>Genotype frequencies</th>
<th>Obs-Het</th>
<th>Exp-Het</th>
<th>Nei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shall</td>
<td>0.79</td>
<td>0.21</td>
<td>29</td>
<td>0.42</td>
<td>0.3352</td>
<td>1.4966</td>
</tr>
<tr>
<td>Karakul</td>
<td>0.85</td>
<td>0.15</td>
<td>42</td>
<td>0.3</td>
<td>0.2571</td>
<td>1.7928</td>
</tr>
</tbody>
</table>

Obs-Het: observed heterozygosity.  
Exp-Het: expected heterozygosity.
However in adult ewes (3 yr old and older), fertility of ewes of mm genotype was 10 to 11% less than that of other ewes. Also adult ewes with the mm genotype had approximately 0.11 fewer lambs per ewe lambing than ewes of other genotypes (Notter et al., 2003). It has been shown by Pelletier et al. (2000) that there is association between the (- -) genotype for site Mnl1 at position 605 and seasonal anovulation activity. Based on the experimental results of Pelletier et al. (2000), the present study indicates that the Shall and Karakul ewes, genetically have the potential to show estrus during short and long days.

References