

# Relationship between MTNR1A melatonin receptor gene polymorphism and seasonal reproduction in different goat breeds

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## Abstract

The reproductive activity of goats bred in temperate latitude follows a seasonal pattern, influenced by annual variation in day length. Daily variation in pineal melatonin secretion is the neuroendocrine signal recognized by animals through the link between this hormone and melatonin receptor 1a (MTNR1A). A total of 345 goats of different breeds (225 Sarda, 30 Saanen, 30 Chamois Coloured, 30 Maltese and 30 Nubian) with a kidding period in October–December or January–March were analysed to verify if a link exists between the structure of the receptor gene and reproductive activity. The main part of exon II of MTNR1A gene was amplified by PCR and then digested with MnlI and RsaI to prove the presence of restriction sites. Sequencing of 20 cloned samples and 20 purified samples permitted comparison with previously published sequences. No polymorphism was found using MnlI enzyme, as all 345 samples showed the cleavage site in position 605 and all the goats were MM genotype. However, using RsaI enzyme, some Sarda goats, showed a polymorphic site in position 53. Nine Sarda goats were R/r genotype, lacking this cleavage site only in one allele, while the other animals, both Sarda and the other breeds, presented the cleavage site in both the alleles and were thus R/R genotype. No r/r genotype was found in any of the breeds. In Sarda goats the allelic frequency was 0.98 for R allele and 0.02 for r allele; genotypic frequency was 96.00% for R/R genotype and 4.00% for R/r genotype. A strong link emerged from statistical analysis ( $P < 0.001$ ) between R/r genotype and reproductive activity, which was strongly influenced by photoperiod. Sequencing indicated six nucleotide changes that did not induce any amino acid change. Data showed that polymorphism was present and that it influences reproductive activity only in the Sarda breed.

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## 1. Introduction

Reproduction activity in small ruminants reared in temperate latitudes is characterized by a seasonal trend with a period of sexual inactivity that can last for several months, usually from late winter to early spring (Chemineau et al., 1995). Photoperiod is the principal environmental factor which affects the succession of reproductive periods (Karsch et al., 1984). Light signal perceived by the retina is translated into hormonal message by the pineal gland through melatonin secretion (Goldman, 2001). Melatonin shows a low blood-concentration during daylight and a high concentration during darkness, thus it can be considered as an organic informer of the annual photoperiodic trend (Lincoln et al., 2005). High melatonin levels, typical during autumn, have a positive influence on reproduction in small ruminants (Bittman et al., 1985; Carcangiu et al., 2005). Melatonin functions through specific receptors located in different areas of the central nervous system (CNS), including nuclei which regulate reproduction (Sliwowska et al., 2004). In mammals two type of melatonin receptor have been identified and characterized, named MT1 and MT2, but only the first seems to be involved in regulation of reproductive activity (Weaver et al., 1996; Dubocovich et al., 1998). Although a high number of receptors have been found in the Pars Tuberalis (PT), this structure does not seem to be involved in the regulation of reproduction, but is instead involved in photoperiodic control of prolactin secretion (Lincoln and Clarke, 1994). However, a low number of these receptors were found in preammillary hypothalamus, where an effect of melatonin on reproduction can be seen (Migaud et al., 2005). MT1 receptor belongs to the G protein-coupled receptor family and its gene was cloned (Reppert et al., 1994) and mapped in several animal species (Messer et al., 1997). The ovine exon II of MT1 receptor gene presents two RFLP polymorphic sites, one for MnlI enzyme and one for RsaI enzyme (Messer et al., 1997). The presence of a cleavage site in position 605 of the coding sequence (GenBank AF045219) using MnlI enzyme causes a slight sensibility to photoperiod in reproductive activity (Pelletier et al., 2000; Notter et al., 2003). This same site, in different goat breeds with seasonal or year-round estrous was not polymorphic (Migaud et al., 2002). This study was recently confirmed by Chu et al. (2006), in Asian goat breeds. Using the RsaI enzyme these authors also noticed the presence of a polymorphic site in position 53 (GenBank AF419334) which seems to have a correlation with seasonality in goats reproduction. The aims of the present study were firstly to detect the presence of polymorphisms in the exon II of the melatonin receptor gene in Sarda, Saanen, Chamois Coloured, Maltese, and Nubian breed, and secondly, to correlate reproductive activity of these animals with their found genotypes.

## 2. Materials and methods

### 2.1. Animals

Three hundred and forty-five goats of five different breeds reared in Sardinia were selected. The animals were distributed as follows: 225 Sarda breed in eight farms, 30 Saanen in two farms, 30 Chamois Coloured in two farms, 30 Maltese in three farms and 30 Nubian in one farm. The reproductive activity of all the goats was recorded during the two years preceding the study. In each farm, half the goats chosen showed reproductive activity in spring and the others in autumn. Goats were kept under natural photoperiod since birth and were fed on natural pasture, integrated with concentrated food.

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