

# Genotype of Melatonin Receptor MT1 (MTNR1A) and Puberty in Mediterranean Italian Buffalo

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## Summary

In adult buffaloes, polymorphism of the MT1 receptor gene has shown to influence the reproductive seasonality. The aim of study was to assess whether the polymorphism of the MTNR1A gene may influence puberty in Mediterranean Italian buffalo. The study was conducted using 50 prepubertal buffalo cows that at the age of 15 months were placed into the group where there was the male. Estrus detection was performed by observing estrous-behaviour and pregnancy checking by palpation per rectum and/or ultrasound between days 40 and 60 post-mating. Also of each animal dates of calving was recorded. From each buffalos a blood sample was collected and used for DNA extraction. PCR analysis was performed using 100-150 ng of DNA to amplify the second exon of the MTNR1A gene. All PCR products were digested with 2U of enzyme HpaI to highlight the polymorphism at position 82 (characterized by a C to a T substitution) of the MTNR1A gene. Frequency of C and T alleles was respectively 0.42 and 0.58 in the analyzed population which resulted in Hardy Weinberg equilibrium. The genotypic frequency was 28% for genotype C/C, 38% for C/T and 34% for T/T. The registration of reproductive data showed that the first heat is around the age of 20 months and the first calving around 32 months. Our data show that the genotype of the MTNR1A does not influence the onset of reproductive activity in prepubertal buffalo cows.

## Key words

buffaloes, melatonin receptor, seasonal reproduction, puberty

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### Aim

The reproductive activity of buffaloes is influenced by several environmental factors that are typical of the area in which they live (Borghese et al., 2005). In temperate areas such as Italy, where buffalo are fed with a balanced diet, a distinct seasonal reproductive pattern is also found, and this seasonality is influenced by photoperiod (Zicarelli, 1997; Presice, 2007). Buffalo heifers generally attain puberty when they reach about 55–60% of their adult body weight, but the age can be highly variable, ranging from 18 to 46 months (Jainudeen and Hafez, 1993). The different factor that influence the puberty are nutrition, social environment, and photoperiod (Perera, 2010). Seasonal effects on the reproductive function are governed by the pineal gland and melatonin which constitute its principal secretory product (Bittman and Karsch, 1984). Melatonin acts through specific receptors, but MT1 seems to be the only one involved in the regulation of seasonal reproduction in several animal species. In adult buffaloes, polymorphism of the second exon of the MT1 receptor gene has shown to influence the reproductive seasonality (Carcangiu et al., 2011). The aim of this research was to assess whether the polymorphism of the melatonin receptor MT1 gene may influence puberty in Mediterranean Italian buffalo.

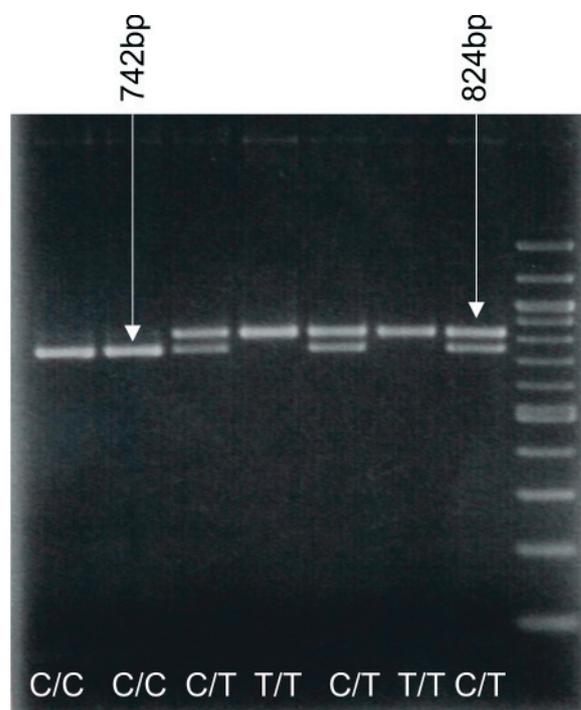
### Materials and methods

The study was carried out in a homogeneous herd of about 300 Mediterranean Italian buffaloes, located in the South Sardinia (39° 36' N). All buffaloes were under natural photoperiod and housed in large open yards with sheltered areas. The daily feed allocation was 8 kg corn silage, 4 kg meadow hay and 1 kg of concentrate pellets. The study was conducted using 50 prepubertal buffalo-cows which at the age of 15 months were placed into the group where there was the male. All the buffaloes included in the study were in good general health. Bulls (1:25 male/female ratio) were kept always within the herd. Ear-mark numbers of the estrus and mated females were recorded by trained technicians. Estrus detection was performed by observing estrous-behaviour (marked by bellowing, homosexual mounting, being sniffed, mounted, or serviced by the male). The pregnancy checking was performed by palpation per rectum and/or ultrasound between days 40 and 60 post-mating using an Esaote Piomedical Tringa linear equipment (Esaote Europe B.V., Maastricht, The Netherlands) provided with a 5.0 – 7.5 MHz multiple frequency linear probe. Each animal date of calving was recorded. A sample of 10 mL of blood was collected from the caudal vein of each buffalo using a tube with EDTA as an anticoagulant (Believer Industrial Estate, Plymouth, UK). Genomic DNA was extracted from whole blood, using a commercial kit (NucleoSpin Blood QuickPure, Macherey-Nagel, Duren, Germany) and then kept at -20°C until use. PCR analysis was performed with template amounts ranging from 100 ng to 150 ng per reaction, using primers by Messer et al. (1997): sense primer 5' – TGT GTT TGT GGT GAG CCT GG – 3' and antisense primer 5' – ATG GAG AGG GTT TGC GTT TA – 3' (Sigma Genosys Ltd, Pampisford, Cambs, UK). Polymerase Chain Reaction (PCR) was performed according to Carcangiu et al. (2011). All the PCR products were digested using 2U of HpaI enzyme (New England Biolabs, Beverly, MA, USA), according to Carcangiu et al. (2011) Resulting frag-

ments were separated by electrophoresis on 4% (wt/vol) agarose gel (GellyPhor, Euroclone, UK), in parallel with a 50 bp DNA marker (Invitrogen, Carlsbad, CA, USA). Data was subjected to Hardy-Weinberg equilibrium analysis and to allelic and genotypic frequency analysis (Rousset, 2008). One way analysis of variance was used to evaluate the distance in days from birth to first mating and from birth to first calving.

### Results and discussion

PCR product of the 50 sample resulted in a 824 bp fragment corresponding to the most part of the MTNR1A melatonin receptor gene exon II. The presence of the cleavage site in position 82, revealed with HpaI, was caused by the presence of a C and after electrophoresis it produced one band of 79bp and one band of 745 bp (Fig. 1). The absence of this cleavage site was caused by the presence of a T that leaves uncut the 824 bp fragment (Fig. 1).



**Figure 1.** Electrophoresis of digestion with HpaI on a 4% agarose gel in the Mediterranean Italian buffalo. Lane 8: 100 bp DNA marker. Lane 1 and 2: cleavage site present genotype C/C; Lane 4 and 6: cleavage site absent genotype T/T; Lane 3, 5 and 7: cleavage site present in only one parental chromosome genotype C/T.

Frequency of C and T alleles was respectively 0.42 and 0.58 in the analyzed population which resulted in Hardy Weinberg equilibrium. The genotypic frequency was 28% for genotype C/C, 38% for C/T and 34% for T/T (Table 1). The allelic and genotypic frequencies resulted nearly equal to those observed in previous reports (Carcangiu et al., 2011).

The registration of mating showed that the first heat is around the age of 20 months (Table 2). This finding was not in-

**Table 1.** Allele and genotype frequency in the Mediterranean Italian buffalo (n=50)

Allele		Genotype		
C	T	C/C	C/T	T/T
0.42	0.58	28%	38%	34%

**Table 2.** Average distance in days from birth to first mating and from birth to first calving, in the three genotype observed.

HpaI genotype	C/C	C/T	T/T
Distance in days from birth to first mating	656.2±42.4	661.7±48.3	664.6±52.6
Distance in days from birth to first calving	978.5±48.4	986.4±45.7	989.2±50.4

fluenced by the melatonin receptor genotype. Our observation about the average age of first mating is in accordance what was observed by Barile (2005) who reported that buffaloes reared in Italy, when well fed, reached puberty at an average age of 21 months. Regardless to genotype, we observed that there are more buffalo cows that come back into heat between the end of January and mid March rather than the other months. In fact at Mediterranean latitudes buffaloes manifested their sexual activity mainly when daylight hours decrease, with a poor reproductive efficiency from mid-winter to spring, when daylight hours increase (Campanile et al., 2005). From births dates recording was found that the most female buffalo calved about 32 months of age. This data is in accordance with what was found by De Franciscis et al. (1988) who observed that buffaloes calved for the first time between 32 and 36 months. Moreover, Borghese et al. (1994) by measuring the progesterone, showed that the buffaloes had a full cyclic ovarian activity approximately at 21 months of age, and calving can occur only after about 300 days. The genotype has not been shown to influence the onset of reproductive activity in the pre-pubertal buffalo cows (Table 2). This is consistent what we observed on Sarda ewes, in which the genotype did not influenced the puberty, while in the Dorset breed lambs has been observed that the genotype of the MT1 melatonin receptor influences the advance of puberty and reduces the time between the first and second lambing (Mateescu et al., 2010; Mura et al., 2010). In adult sheep of different breeds, including the Sarda, the gene above mentioned showed affect the seasonal reproductive (Pelletier et al., 2000; Chu et al., 2006; Carcangiu et al., 2009). Even in adult buffaloes the influence of the MT1 melatonin receptor gene on reproductive seasonality has been observed (Carcangiu et al., 2011) and thus it is like to what happens in the Sarda breed sheep. So it is possible to hypothesize, that at Mediterranean latitudes, for reaching puberty other endogenous signal (eg development body) are more important than the environmental factors.

However, in adult animals the genotype C/C affected the reproductive season. In fact, animals carrying this genotype showed greater reproductive activity during the long photoper-

iod compared to genotype T/T. Evidently in this species other factors, such as the body development, can influence the onset of reproductive activity in relation to photoperiod.

## Conclusion

Our data shows that the genotype of the MT1 melatonin receptor does not influence the onset of reproductive activity in prepubertal buffalo cows. But it would be interesting to investigate whether these genotypes can affect the reproductive seasonality in the next breeding season.

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