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Characterization of the Mediterranean Italian buffaloes melatonin receptor 1A (MTNR1A) gene and its association with reproductive seasonality

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Abstract

The aim of this study was to examine the polymorphism in MTNR1A gene and its relation to reproductive seasonality in Mediterranean Italian buffaloes reared in Sardinia. The mating period and calving of 100 multiparous buffalo-cows were recorded for three years (2005–2008). Genomic DNA was subjected to PCR for the amplification of the exon II, then 40 amplicons were sequenced. The obtained sequence was deposited in GeneBank database (accession number GU817415). PCR products were checked for the presence of HpaI restriction sites and assigned to genotypes "C/C", "C/T" or "T/T". Allelic frequency of C and T alleles was 0.44 and 0.56 and genotypic frequency was 26% for genotype C/C, 40% for C/T and 34% for T/T. In the three observed years the animals with C/C genotype showed the highest number of mating in the semester between August and January and their calving mainly occurred from August to September. On the other hand animals with T/T genotype showed mating mostly in the semester between February and July and calving occurred largely from March to May in all the three years. Heterozygous, in all the three years, showed about the same number of animals mated within each six-month period. The results of the present study provide for the first time a partial sequence as well as one polymorphic site of the MTNR1A receptor gene from buffaloes. Moreover our data showed an association between Single Nucleotide Polymorphism and seasonal reproductive activity in these animals.

Keywords: Buffaloes; MTNR1A gene; Reproductive seasonality

1. Introduction

Buffaloes living under Mediterranean latitudes can be considered to have a tendency to be seasonal breeding animals and their reproductive efficiency is usually negatively affected by increasing day-length which consequently influences productions [1,2]. Photoperiod, through the melatonin secretion, is the main environmental factor affecting the regulation of reproductive seasonality [3,4]. Melatonin is produced by pineal gland at night in direct proportion to the period of darkness [5]. The pattern of melatonin secretion provides photoperiodic information to cells within the brain that possess the relevant receptors and control reproductive function [6]. Melatonin receptors are classified in MTNR1A and MTNR1B subtypes but only the first seems to be involved in the regulation of seasonal reproductive activity [7,8]. The MTNR1A receptor gene in sheep is on chromosome 26, in cattle on chro-

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mosome 27 and in buffalo on chromosome 1 which is a fusion of Bos Taurus chromosome 1 and 27 [9,10]. The melatonin effect is carried out at hypothalamic level, by regulating of GnRH secretion [11]. However the highest concentration of melatonin MTNR1A receptors has been evidenced in the Pars Tuberalis (PT), but this region seems to be particularly involved in the control of Prolactin secretion [12,13]. Inversely, in the Premammillary Hypothalamus (PMH), low density of melatonin binding sites was found (20–100 times lower than in the PT), but functional studies have shown that melatonin micro-implants placed into PMH are able to stimulate the GnRH system [11,14]. In several sheep, goat and cattle breeds, polymorphic sites in MTNR1A receptor gene exon II were found [15]. One G to an A substitution in position 612 in sheep and a G to an A substitution in position 52 in goat of the sequence of MTNR1A receptor gene lead to a less seasonal reproductive activity [16,17]. Polymorphic site was found in cattle but no correlation is known with reproductive activity [15] whereas in buffalo it is still unknown if there are polymorphisms of the melatonin receptor gene and whether there are relationships with seasonality of reproduction. In Italy buffaloes are bred for the main purpose of producing and marketing milk and its derivatives of which mozzarella cheese is probably the best known worldwide [18]. Mediterranean Italian buffalo-cows show a decline in reproductive activity from mid-winter to spring in response to increasing day-length [19]. The seasonal decline in reproductive activity is manifested by a reduced incidence of estrous behaviour, a decrease in the proportion of females that undergo regular estrous cycles and a generally lower conception rate [19]. In the Mediterranean region it is necessary to plan the mating of buffaloes during the seasonal trough in reproduction so that calving coincides with the annual peak for buffalo milk demand [20]. Subsequently, a strategy has been developed in Italy in order to reverse the calving season in buffalo-cows and it has been termed the Out-of-Breeding-Season-Mating (OBSM) technique [21]. This technique is applied by removing bulls from the herd in October and reintroducing them between March and the end of September so that most calving occurs between the end of January and the beginning of August. Over the years the implementation of the OBSM technique has selected animals less sensitive to daylight variation and explained the differences found with respect to the nocturnal and seasonal variation of melatonin [22]. Therefore it could be of great interest to identify a suitable method to recognize buffaloes less sensitive to photoperiod. Thus, the aim of the present research was firstly to study the polymorphism in MTNR1A gene and then to emphasize its association with seasonal reproduction of the Mediterranean Italian buffaloes reared in Sardinia.

2. Materials and methods

2.1. Experimental design

This study was undertaken in a homogeneous herd of about 300 Mediterranean Italian buffaloes, located in the South of Sardinia (39° 36' N). All buffaloes were under natural photoperiod and housed in large open yards with sheltered areas. The daily feed allocation consisted of 5 kg ryegrass Italian hay, 18 kg corn silage (30% dry matter), 2 kg soybean meal (44% crude protein), 4 kg grain mix (22% crude protein), 2 kg corn meal and 0.1 kg hydrolyzed fats. The study was conducted using 100 multiparous buffalo-cows which were 6.24 ± 1.20 years old (range: 4–8). Considering the age influence on reproductive activity, the primiparous and the old cows were excluded from the study. Reproductive activity of the last three years (2005–2008) was recorded for each animal. All the buffaloes included in the study were in good general health and without reproductive disorders. Bulls (1:25 male/female ratio) were kept always within the herd. Ear-mark numbers of the estrous and mated females were recorded by trained technicians. Estrous 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 Piemedical Tringa linear equipment (Esaote Europe B.V., Maastricht, The Netherlands) provided with a 5.0–7.5 MHz multiple frequency linear probe.

2.2. Genomic DNA preparation

10 mL of blood was collected from the caudal vein of each buffalos 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.

2.3. Primer sequences

100–150 ng of genomic DNA were used for PCR reaction using primers by Messer et al. [15], sense