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Polymorphisms in the melatonin receptor gene promoter and their associations with fertility characteristics in buffalo herd in Eastern Amazon

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ABSTRACT. Buffalo production is spreading globally because of its economic advantage. Then, it has become necessary to improve the reproductive and productive efficiency of these animals, as well as to look for genetic factors that increase this efficiency. The objectives of this study were to characterize the promoter region of the melatonin 1A receptor gene (MTRN1A), to detect possible SNPs and associate them with fertility characteristics, and identify binding sites of transcription factors involved in the regulation of genetic expression

in buffaloes in the Amazon. The conventional PCR method was carried out using the two primers designed from the reference sequence deposited in the GenBank AY52466.1. The products of the PCRs were purified, sequenced, and subsequently edited and aligned. Twenty-six SNPs were found, where 73% presented allele frequencies of wild nucleotides above 0.5, and 73% presented deviations from the Hardy-Weinberg equilibrium ($P < 0.05$) and F_{IS} varying between 0.06 and 1.00, characterizing high degrees of inbreeding within the population. A block of ACAA deletion (position -1483) was observed in 25% of samples. The associations between these SNPs and reproductive characteristics were observed for calving interval and 5 SNPs: -1289, -1139, -911, -724, and -656 ($P < 0.05$), and three other SNPs: -1395, -724, and -94 ($P < 0.05$) were associated significantly with age at first calving, and were not associated with calving concentration. The promoter region was characterized by the different types of binding factors, where only 11 sites are significantly strong enough for transcription factor bindings. The ACAA deletion also exhibited a strong association with transcription factors. As a result, it would be necessary to test the SNPs above with other reproductive characteristics of economic relevance to approve the gene as a strong candidate for the selection of buffaloes in the Amazon.

Key words: MTRN1A; SNP; First calving; Calving interval

INTRODUCTION

The production of buffaloes has been increasing throughout the world because of the economic advantages buffalo rearing offers to other domestic ruminants, mainly for the fact that buffaloes display characteristics such as resilience and ability to adapt to varied climatic conditions and forms of rearing (Camargo Júnior et al., 2012). Brazil has an effective of 1.3 million buffaloes, with the Northern Region being the biggest producer of the animal possessing 64.1% of the national effective while the State of Pará has an effective constituting 36% of the national population and 56.1% of the regional population. As such the State is the biggest producer of buffalo in Brazil (IBGE - Instituto Brasileiro de Geografia e Estatística, 2012).

To consolidate this practice in Brazil, it is necessary to improve the efficiency of these animals. Therefore, it is vital to have an understanding of the productive and reproductive potential, as well as genetic and environmental factors that influence the relative characteristics (Snel-Oliveira et al., 2013). The importance of reproductive characteristics in genetic improvement programs is mainly related to rates of annual genetic gain. Younger ages at first calving allow a reduction in the interval between generations, while smaller intervals between calvings make available larger numbers of female calves of high genetic potential, which would be able to substitute adult females as they are phasing out (Cassiano et al., 2004).

The photoperiod demonstrates a strong influence on the reproductive pattern of the species, through the secretion from the pineal gland, which controls the change between light periods and dark (Borghese et al., 1995). However, in regions near to the Equator, female buffaloes are animals of continuous polyestral behavior (Vale and Ribeiro, 2005). In the

Brazilian Amazon, depending on the geographical location of rearing, the pattern of calving changes. In the State of Pará, in breeds reared in Terra Firme region, the highest concentration of calving take place in the first half of the year with larger indexes between the months of April and August (Brunetta et al., 2003).

A search is being performed, therefore, for a solution to increase the population of buffalo in the Amazon Region via the applicability of molecular markers in a selection program (Garcia and Porto-Neto, 2006). It is known that the melatonin receptor (MTRN1A) appears to exert a stimulatory effect on the reproductive axis of short-day species. Studies on various breeds of goats and sheep have demonstrated that a polymorphism in this gene results in animals with less seasonality (Rocha et al., 2011; Zetouni et al., 2014). As such, it is important to analyze the promoter region of MTRN1A, given that this region regulates the synthesis of mRNA by binding to regulatory proteins and transcription factors. Therefore, little research exists that addresses the importance of the promoter region of the most different genes (Smale and Kadonaga, 2003; Thomas and Chiang, 2006; Heintzman and Ren, 2007; Juven-Gershon et al., 2008).

The promoter region of MTRN1A in sheep was first described by Johnston et al. (2007), who characterized it as having a size of about 1.5 kb. Two transcription factors were detected as being involved in the development of the pituitary gland (Pitx-1 and Egr-1) and multiple boxes of bonds, which are both involved in the regulation of developmental genes and the circadian rhythm. In buffaloes, the studies into the MTRN1A gene are more orientated towards a polymorphism in exon II at position 318, which is a synonymous mutation and does not change the sequence of amino acids of the receptor (Carcangiu et al., 2011; Luridiana et al., 2012; Zetouni et al., 2014; Barbosa et al., 2016a). Barbosa et al. (2016b) also found a novel polymorphism in exon 1 with nonsynonymous mutation, but there was no association with reproduction characters in female buffaloes from Amazon.

The objective of this study was to characterize the promoter region of the MTRN1A gene in buffaloes and locate possible SNPs, associate them with fertility characteristics in buffaloes in the Amazon, and identify binding sites of transcription factors involved in the regulation of genetic expression in a herd of buffaloes in the Brazilian Amazon.

MATERIAL AND METHODS

The study complied with the Code of Ethics Protocol Nos. 003/2015 (Ethics Committee on Animal Use) and 23084.006321/2015-25 (Universidade Federal Rural da Amazônia).

Samples and laboratory procedures

Information on age at first calving, calving period, and calving concentration was collected for over 180 female Murrah buffaloes reared in the Terra Firme region of the State of Pará in the Brazilian Amazon. For DNA extraction, approximately 60 hairs with their capillary bulbs were collected and preserved at 4°C until laboratory processing. Next, DNA extraction was carried out using the phenol:chloroform:isoamyl alcohol method (25:24:1) according to the protocol of Sambrook et al. (1989).

The promoter region of the MTRN1A gene in buffaloes was based on the sequence of the same gene in sheep deposited in Genbank under reference number AY52466.1. Two pairs of primers were designed using the Primer3 program (http://simgene.com/Primer3).

The primer sequences of fragment 1 were forward - 5'-GCACAAAAAGAAGCCAAGG-3' and reverse - 5'-CCAGGTTCCTCATCTGTAAAATG-3' (position of the amplified fragment from -1545 to -659 and a size of 887 bp). The sequences of fragment 2 were forward - 5'-TTTTTCATCTCTTACCATCTAG-3' and reverse - 5'-GCGAGACGTTGAGCAGC-3' (position of the amplified fragment from -734 to 76 and a size of 810 bp). Next, the polymerase chain reactions (PCRs) of both pairs of primers were carried out for a final reaction volume of 15 µL under the following conditions: 1X reaction buffer 10X, 1 mM $MgCl_2$, 10 mM of each of the dNTPs, 5 µM of both primers (forward and reverse), 2 U *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA), and 20% Solution-Q (Quiagen, Chatsworth, CA, USA) diluted in ultrapure water. The reactions were carried out in the 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under denaturation temperature conditions starting at 95°C for 5 min, followed by 30 cycles at a denaturation temperature of 94°C for 1 min, annealing temperatures of 58° and 54°C for 1 min, for the fragments I and II, respectively, and an extension temperature of 72°C for 1 min, completing the process with a final extension temperature of 72°C for 10 min. The PCR products were submitted to electrophoresis on 1.5% agarose gel stained with Gelred (Biotium, Fremont, CA, USA) at 90 V for 30 min, and then viewed using a UV Transilluminator imaging system (Applied Biosystems).

The PCR products of the two fragments were purified with enzyme Illustra ExoProStar 1-Step (GE Healthcare, UK), following the manufacturer's recommendations. Once purified, the products were sequenced in an automatic DNA sequencer ABI 3500XL (Applied Biosystems) using the BIG DYE kit (Life Technologies). The sequences obtained were edited using the Finch TV Version 1.4.0 computer program (Geospiza Research Team, USA), while always using as a reference for comparison the sequences contained in the GenBank via the BLAST system and, then, aligned in the Bioedit (Hall, 1999) to be compared with the same sequences among the animals to detect possible polymorphisms.

Statistical analyses

The polymorphisms detected in the promoter region of MTRN1A in buffaloes were tabulated and submitted to the GENEPOP v.5 program (Raymond and Rousset, 1995), which determined the allele and genotype frequencies, the inbreeding coefficients (F_{IS}), and the probabilities for the Hardy-Weinberg equilibrium. The SNPs that were detected were tested for associations with calving concentration using the chi-squared test as well as for associations with calving interval and age at first calving characteristics using ANOVA test from the SAS 9.2 statistical program (SAS Institute, Inc., 2000) obeying the mathematical model:

$$\gamma_{ijk} = \mu + G_i + F_j + \left(GxF\right)_{ij} + \varepsilon_{ijk}$$

where γ_{ijk} represents the phenotype characteristics; µ represents the average of phenotype characteristics; G_i represents the fixed effect of genotypes; F_j represents the fixed effect of farms; $(GxF)_{ij}$ represents the interaction effect between genotypes and farms; ε_{ijk} represents the random error.

The deletion was analyzed using the Student *t*-test for calving interval and age at first calving characteristics, while the characteristics for calving concentration was analyzed using the chi-squared test. The level of significance adopted was 0.05.

The binding domains of the transcription factors in the promoter region of the MTRN1A gene were identified by comparing them with those of different species with the help of the TRANSFAC 4.0 program (Wingender et al., 2001) for all the SNPs and the block of deletion.

RESULTS

After all of the laboratory processing and analysis, 26 SNPs were found between the transition- and transversion-type point mutations. All allele and genotype frequencies, the F_{IS}, and the Hardy-Weinberg probability were estimated and are described in Table 1. It was observed that 73% of the 26 SNPs demonstrated allele frequencies of wild nucleotides above 0.5, and 73% demonstrated a deviation from the Hardy-Weinberg equilibrium. A block of deletion with the sequence ACAA was observed between the positions -1483 and -1480 in 25% of the samples.

Table 1. Positions of SNPs and respective statistical descriptions in the promoter region of the MTNR1A gene in buffaloes.

Positions (SNP)	Alleles (frequency)		Genotypes (frequency)			F_{IS}	HWP
-1511 (C→T)	C (0.350)	T (0.650)	CC (0.350)	CT (0.000)	TT (0.650)	1.00	0.000
-1465 (G→T)	G (0.933)	T (0.077)	GG (0.900)	GT (0.067)	TT (0.033)	0.47	0.015
-1422 (A→G)	A (0.792)	G (0.208)	AA (0.683)	AG (0.217)	GG (0.100)	0.35	0.014
-1411 (G→A)	G (0.292)	A (0.708)	GG (0.183)	GA (0.217)	AA (0.600)	0.48	0.000
-1395 (G→T)	G (0.842)	T (0.158)	GG (0.783)	GT (0.117)	TT (0.100)	0.57	0.000
-1298 (A→G)	A (0.867)	G (0.133)	AA (0.767)	AG (0.200)	GG (0.033)	0.14	0.267
-1295(G→A)	G (0.892)	A (0.108)	GG (0.800)	GA (0.183)	AA (0.017)	0.06	0.516
-1242 (A→C)	A (0.883)	C (0.117)	AA (0.800)	AC (0.167)	CC (0.033)	0.20	0.165
-1150 (C→T)	C (0.775)	T (0.225)	CC (0.617)	CT (0.317)	TT (0.067)	0.10	0.464
-1147 (G→C)	G (0.908)	C (0.092)	GG (0.833)	GC (0.150)	CC (0.017)	0.11	0.398
-1136(A→G)	A (0.450)	G (0.550)	AA (0.267)	AG (0.367)	GG (0.367)	0.27	0.069
-911 (G→A)	G (0.842)	A (0.158)	GG (0.750)	GA (0.183)	AA (0.067)	0.32	0.029
-909 (A→G)	A (0.767)	G (0.233)	AA (0.617)	AG (0.300)	GG (0.083)	0.17	0.265
-724 (C→G)	C (0.908)	G (0.092)	CC (0.867)	CG (0.083)	GG (0.050)	0.50	0.004
-656 (A→C)	A (0.650)	C (0.350)	AA (0.587)	AC (0.167)	CC (0.267)	0.64	0.000
-649 (T→C)	T (0.725)	C (0.275)	TT (0.583)	TC (0.283)	CC (0.133)	0.30	0.046
-644 (G→A)	G (0.442)	A (0.558)	GG (0.333)	GA (0.217)	AA (0.450)	0.57	0.000
-511 (A→C)	A (0.150)	C (0.850)	AA (0.083)	AC (0.133)	CC (0.783)	0.48	0.002
-481 (G→A)	G (0.925)	A (0.075)	GG (0.900)	GA (0.050)	AA (0.050)	0.65	0.001
-425 (C→T)	C (0.975)	T (0.025)	CC (0.967)	CT (0.017)	TT (0.017)	0.66	0.026
-395 (G→A)	G (0.975)	A (0.025)	GG (0.967)	GA (0.017)	AA (0.017)	0.66	0.027
-383 (G→T)	G (0.550)	T (0.450)	GG (0.433)	GT (0.233)	TT (0.333)	0.54	0.000
-254 (C→T)	C (0.942)	T (0.058)	CC (0.917)	CT (0.050)	TT (0.033)	0.55	0.007
-206 (T→C)	T (0.383)	C (0.617)	TT (0.250)	TC (0.267)	CC (0.483)	0.44	0.001
-133 (T→G)	T (0.358)	G (0.642)	TT (0.283)	TG(0.150)	GG (0.567)	0.68	0.000
-94 (C→T)	C (0.525)	T (0.475)	CC (0.383)	CT(0.283)	TT (0.333)	0.44	0.001

SNPs = single nucleotide polymorphism; F_{IS} = inbreeding coefficient; HWP = Hardy-Weinberg probability.

The associations between the SNPs and the reproductive characteristics in buffaloes evaluated were described in Table 2. The calving concentration was characterized by high concentration between the months of April and August and low concentration between the months of September and March ($P < 0.05$). No associations were detected between the genotypes of the individual SNPs and the periods of calving concentration ($P > 0.05$). Only five SNPs (-1298, -1136, -911, -724, and -656) had significant association with the calving interval characteristic ($P < 0.005$) and only three other SNPs (-1395, -724, and -94) had significant association with age at first calving.

Table 2. Associations between polymorphisms and reproductive characteristics in buffaloes.

SNPs	Genotypes	AFC (months) (means ± SD)	CI (months) (means ± SD)	CC (Abril to August and September to March)
-1511	CC	17.48 ± 4.74	33.94 ± 3.77	$\chi^2 = 0.140$
	CT	0	0	d.f. = 2
	TT	16.31 ± 4.79	34.39 ± 3.68	
	Probability	0.268	0.661	0.708
-1465	GG	16.36 ± 4.60	34.04 ± 3.68	$\chi^2 = 0.186$
	GT	18.17 ± 3.73	36.92 ± 3.86	d.f. = 2
	TT	22.21 ± 10.00	34.72 ± 2.74	
	Probability	0.091	0.312	0.911
- 1422	AA	17.41 ± 5.32	34.69 ± 3.55	$\chi^2 = 0.195$
	AG	14.40 ± 1.68	32.66 ± 2.64	d.f. = 2
	GG	16.30 ± 3.87	34.43 ± 5.53	
	Probability	0.062	0.236	0.907
- 1411	AA	16.86 ± 4.13	33.56 ± 3.85	$\chi^2 = 0.522$
	AG	15.26 ± 4.49	34.95 ± 3.15	d.f. = 2
	GG	17.69 ± 6.58	35.50 ± 3.55	
	Probability	0.268	0.203	0.770
- 1395	**GG**	17.15 ± 5.13	**34.97^{a} ± 3.65**	$\chi^2 = 0.339$
	GT	14.22 ± 1.32	**31.93^{ab} ± 2.53**	d.f. = 2
	TT	15.82 ± 3.51	**31.35^{b} ± 2.72**	
	Probability	0.155	**0.009**	0.844
- 1298	**AA**	**15.66^{b} ± 3.78**	33.96 ± 3.53	$\chi^2 = 2.075$
	AG	**19.09^{a} ± 4.30**	35.63 ± 4.00	d.f. = 2
	GG	**25.57^{a} ± 14.75**	32.68 ± 5.62	
	Probability	**0.0002**	0.301	0.354
- 1295	AA	13.34 ± 0.00	34.22 ± 0.00	$\chi^2 = 0.224$
	AG	14.79 ± 2.63	33.82 ± 4.88	d.f. = 2
	GG	17.13 ± 5.04	34.34 ± 3.49	
	Probability	0.158	0.918	0.894
- 1242	AA	16.97 ± 5.00	34.39 ± 3.45	$\chi^2 = 1.011$
	AG	15.90 ± 3.59	32.95 ± 4.86	d.f. = 2
	GG	12.95 ± 0.55	36.75 ± 3.57	
	Probability	0.293	0.345	0.603
- 1150	CC	17.27 ± 5.16	33.46 ± 3.83	$\chi^2 = 0.392$
	CT	15.83 ± 4.27	35.13 ± 2.62	d.f. = 2
	TT	15.30 ± 2.54	37.39 ± 4.97	
	Probability	0.305	0.053	0.822
- 1147	CC	13.34 ± 0.00	34.22 ± 0.00	$\chi^2 = 1.499$
	CG	14.24 ± 1.74	32.46 ± 3.45	d.f. = 2
	GG	17.18 ± 5.03	34.57 ± 3.70	
	Probability	0.081	0.276	0.473
- 1136	**AA**	**17.90^{a} ± 5.00**	34.08 ± 3.98	$\chi^2 = 2.122$
	AG	**14.84^{b} ± 2.51**	33.87 ± 3.60	d.f. = 2
	GG	**17.63^{a} ± 5.83**	34.76 ± 3.67	
	Probability	**0.018**	0.712	0.346
- 911	**AA**	**23.83^{a} ± 10.53**	32.45 ± 3.28	$\chi^2 = 4.128$
	AG	**17.35^{b} ± 3.40**	35.70 ± 3.22	d.f. = 2
	GG	**15.88^{b} ± 3.85**	34.06 ± 3.78	
	Probability	**0,0007**	0.242	0.127
- 909	AA	17.30 ± 5.13	33.65 ± 4.06	$\chi^2 = 0.226$
	AG	15.68 ± 4.35	35.09 ± 2.69	d.f. = 2
	GG	15.66 ± 2.87	35.65 ± 3.52	
	Probability	0.268	0.262	0.893
- 724	**CC**	**16.24^{b} ± 4.20**	**34.04^{b} ± 3.34**	$\chi^2 = 2.168$
	CG	**20.95^{a} ± 8.74**	**32.33^{b} ± 3.40**	d.f. = 2
	GG	**17.11^{ab} ± 3.97**	**41.14^{a} ± 3.52**	
	Probability	**0.039**	**0.002**	0.338
- 656	**AA**	**17. 86^{a} ± 5.63**	34.59 ± 4.13	$\chi^2 = 2.350$
	AC	**13.61^{b} ± 1.11**	33.53 ± 3.44	d.f. = 2
	CC	**16.08^{ab} ± 3.01**	33.99 ± 2.87	
	Probability	**0.007**	0.690	0.309
- 649	CC	16.65 ± 3.21	34.32 ± 1.88	$\chi^2 = 2.861$
	CT	15.20 ± 4.03	34.78 ± 3.57	d.f. = 2
	TT	17.38 ± 5.24	33.99 ± 4.03	
	Probability	0.145	0.763	0.239
- 644	AA	17.09 ± 4.54	33.59 ± 3.70	$\chi^2 = 0.575$
	AG	15.68 ± 4.47	33.17 ± 2.41	d.f. = 2
	GG	16.77 ± 5.36	35.84 ± 3.96	
	Probability	0.553	0.051	0.750

Continued on next page

Table 2. Continued.

SNPs	Genotypes	AFC (months) (means ± SD)	CI (months) (means ± SD)	CC (Abril to August and September to March)
- 511	AA	15.71 ± 3.38	34.05 ± 5.93	$\chi^2 = 0.756$
	AC	15.49 ± 3.26	33.66 ± 3.77	d.f. = 2
	CC	16.98 ± 5.10	34.37 ± 3.48	
	Probability	0.517	0.872	0.685
- 481	AA	15. 97 ± 4.00	37.29 ± 5.54	$\chi^2 = 0.711$
	AG	17.69 ± 4.48	31.39 ± 4.27	d.f. = 2
	GG	16.66 ± 4.88	34.24 ± 3.51	
	Probability	0.857	0.148	0.701
- 425	CC	16.78 ± 4.81	34.21 ± 3.74	$\chi^2 = 0.284$
	CT	12.63 ± 0.00	34.02 ± 0.00	d.f. = 2
	TT	15.14 ± 0.00	36.66 ± 0.00	
	Probability	0.529	0.805	0.868
- 395	AA	15.14 ± 0.00	36.66 ± 0.00	$\chi^2 = 0.284$
	AG	12.63 ± 0.00	34.02 ± 0.00	d.f. = 2
	GG	16.78 ± 4.81	34.21 ± 3.74	
	Probability	0.529	0.805	0.868
- 383	GG	16.54 ± 3.84	34.61 ± 3.39	$\chi^2 = 0.680$
	GT	15.28 ± 6.00	33.52 ± 4.26	d.f. = 2
	TT	17.91 ± 4.89	34.29 ± 3.76	
	Probability	0.150	0.669	0.712
- 254	CC	16.74 ± 4.88	34.46 ± 3.69	$\chi^2 = 0.434$
	CT	16.65 ± 4.78	29.78 ± 1.45	d.f. = 2
	TT	15.02 ± 0.75	35.14 ± 0.007	
	Probability	0.828	0.087	0.805
- 206	CC	16.91 ± 4.40	33.92 ± 3.98	$\chi^2 = 0.204$
	CT	16.36± 5.86	34.02 ± 3.53	d.f. = 2
	TT	16.57 ± 4.45	35.14 ± 3.32	
	Probability	0.890	0.555	0.903
- 133	GG	16.78 ± 4.25	33.63 ± 3.89	$\chi^2 = 0.985$
	GT	16.79 ± 7.50	34.44 ± 3.03	d.f. = 2
	TT	16. 42 ± 4.27	35.40 ± 3.47	
	Probability	0.947	0.256	0.611
- 94	**CC**	16.39 ± 4.22	**35.39^{a} ± 3.56**	$\chi^2 = 1.273$
	CT	15.93 ± 5.37	**32.51^{b} ± 3.33**	d.f. = 2
	TT	17.65 ± 4.90	**34.43^{ab} ± 3.72**	
	Probability	0.362	**0.045**	0.529
ACAA Deletion	Presence	18.07 ± 5.04	33.71 ± 3.69	T = -1.305
	Absence	16.22 ± 4.64	34.44 ± 3.71	d.f. = 58 (IEP)
	Probability	0.100	0.260	T= -0.662
				d.f. = 58 (IPP)

SNPs = single nucleotide polymorphisms; CI = calving interval; AFC = age at first calving; CC = calving concentration. The significance levels are represented in bold and superscript lower-case letters indicate significant difference between genotypes ($P < 0.05$).

In the SNP -1298 (G→A), the homozygote AA had a lower average (15.66 ± 3.78 months), and in the SNP -911 (G→A), the genotypes AG (17.35 ± 3.40 months) and GG (15.88 ± 3.85 months) demonstrated the lowest averages in the calving interval. In the SNP -724 (C→G), the homozygote CC (34.04 ± 3.34 months) and the heterozygote CG (32.33 ± 3.40 months) showed the lowest averages in the age at first calving. In the SNP -94 (C→T), the homozygote TT (34.43 ± 3.72 months) showed an intermediary value between the other genotypes.

The mutant SNPs, with the allele being in homozygosis or heterozygosis, provided a better effect in these characteristics, leaving them at small or intermediate values and thus exhibiting a positive effect on calving interval and age at first calving.

An ACAA deletion in position -1482 was detected in 25% of the herd and was also associated with calving interval and age at first calving using the Student *t*-test. The results were not significant ($P > 0.05$) and the chi-squared test for calving concentration was not significant either ($P > 0.05$), i.e., the deletion did not exhibit any influence on reproductive characteristics in the buffaloes analyzed.

In an analysis of the characterization of the promoter region of the MTRN1A gene in buffaloes, the different binding sites of transcription factors were tested using the TRANSFAC 4.0 program (Wingender et al., 2001). Of the total 26 SNPs detected, only 11 were significantly strong for transcription factor binding sites, when compared with the other promoter regions of other genes in other species. In the same way, the block of deletion ACAA also exhibited a strong association with various binding factors, which influence the transcription of the gene (Table 3).

Table 3. Associations of SNPs with binding factors that regulate the transcription of the gene in comparison with other promoters in different species of eukaryotes.

Position	Binding factors
-1465	NF-1, NF-ATp
-1422	TGIF
-1298	TGIF, PBF-1, BPF2
-1295	AREB6
-1150	T-Ag, GCBP-1, SP1
-1147	Cad
-1136	HNF-3alpha, HNF-3beta
-909	ADR1
-383	LIP15, GBF1
-254	GT-1, GT-1a, SBF-1, GT-2
-133	OSBZ8, TRAB1, GBF-1, GT-2
ACAA deletion	GR, AR, RAR-alpha1, RAR-beta2, RAR-gamma1, RXR-alpha, T3R-alpha, CAR

DISCUSSION

Point mutations can be found in the entire genome; however, when detected in the gene (exon), the same mutations can influence the modification of the amino acid sequence (Hunt et al., 2009). When the mutations occur in the promoter region of the genes, alterations in gene expression can occur (Kininis and Kraus, 2008), which could result in different productive or reproductive roles in the buffaloes.

The allele and genotype frequencies were high for the wild alleles and genotypes in the majority of SNPs, which explains the high degree of consanguinity, as such the genotype frequencies observed and expected exhibited significant differences ($P < 0.05$), resulting in deviations from the Hardy-Weinberg equilibrium as observed in the same manner for microsatellite markers in bovines (Silva Filho et al., 2014).

As for reproductive parameters, in the calving concentration, there was not any association observed with the SNPs found in the promoter region of the MTRN1A gene, although we can observe a superior calving concentration that occurs between the moths of April and August corroborating with Rolim Filho et al. (2009). As a result, we can justify this calving concentration because of the presence of other factors such as rainfall, air humidity, and temperature that have considerable effects on the reproductive behaviour of this species. These variations are affected by the type of rearing and level of technology employed in this type of breeding, as well as the possibility of being associated with the type of nutrition administered and diseases (Rolim Filho et al., 2009).

An example of this interaction between rearing and environmental conditions, Cassiano et al. (2003) in their experiment in Embrapa (Monte Alegre-PA), demonstrated that approximately 78% of births occur between the months of July and December. At this ranch, the herd is maintained in a system of integration between Várzea and Terra Firme. The

seasonality of births reveals that the largest number of conceptions occurs between the months of September and February, exactly when animals occupy the areas of lowland where water levels start to lower during this period given the slack flow of the Amazon River. According to the authors of this study, during this season the native lowland plains exhibit excellent conditions for cattle rearing, where there is a clear abundance of quality and nutritious grazing that the animals can have access.

The calving interval is one of the most important parameters to measure the reproductive efficiency, as this is reflected directly in the cost of production where it is acceptable for a buffalo to give birth to two calves every 3 years (Pereira et al., 2008). The calving interval in the present study showed higher association with SNPs ($P < 0.05$) when compared to the results found by Marques (1991) who observed average calving intervals of 13.29 ± 2.77 months, Camargo Júnior et al. (2012) with 13.14 ± 0.78 months, and Bezerra Junior et al. (2014) with 13.89 ± 2.99 months.

The results presented by Rolim Filho et al. (2009) analyzing 2115 registers from 1974 to 2005, showed an average interval between the birth of 16.54 ± 3.74 months, which on overall average corroborate with those found and associated with SNPs with $P < 0.05$. The results found by Oliveira Neta et al. (2012), with 70 lactating buffaloes in the municipality of Moju, PA, showed that they obtained superior interval between births to the authors above, with an average of 18.6 months.

The age at first calving birth is another parameter of greatest importance in the relationship between productive and reproductive characteristics; herd buffaloes that reach puberty early will produce more young and milk in their productive life than those that enter puberty later (productivity of the herd) (Pereira et al., 2008).

These results presented an association ($P < 0.05$) with SNPs inferior or similar to those found by Camargo Júnior et al. (2012) who observed in their studies with 6 different breeds of buffalo a value of 34.58 ± 3.96 months. However, Marques (1991) observed an average of 37.94 ± 5.53 months and Rolim Filho et al. (2009) observed an average of 39.52 ± 7.54 months or in other words they demonstrated superior results to those found in our study.

The promoter region was characterized and associated with binding factors according to the positions and are exhibited in Table 3. We observed in the SNPs that demonstrated such a degree of statistical representativeness two important binding sites: the SNP -1298 with binding factors TGIF, PBF-1, and BPF2, and the SNP -1136 with the HNF-3alpha and HNF-3beta. The TGIF, a protein with a binding homeodomain, which plays a role in the transformation in the growth factor B, acts in repressive multifunctional transcription and also in the recruitment of HDAC activity (histone deacetylase) (Wotton et al., 1999). The PBF-1 was identified in the extracts of potato tubers, and connects a fragment of PR-7º, as well as being involved in the eliciting induction of transcription accumulation of PR-7º (Chasan, 1995). The regulation of transcription factors HNF-3alpha and HNF-3beta was studied during a mediated differentiation using retinoic acid of P19 cells in mice. Treatment with retinoic acid converts P19 stem cells in neurons and astrocytes, and genetic expression of both HNF-3alpha and HNF-3beta are activated during this process. By analogy with HNF-alpha, the activation of HNF-3beta also occurs at the level of transcription initiation. Studies conducted by Jacob et al. (1997) state that HNF-3alpha and HNF-3beta act at the beginning mammal neurogenesis.

The promoter region of the melatonin receptor gene 1A (MTRN1A) was characterized for the first time in a buffalo species, and 26 SNPs and ACAA deletion were observed. Some SNPs were associated with reproductive characteristics such as calving interval and age at

first calving and were not associated with the calving concentration. In total 26 SNPs were detected, 11 of them demonstrated strong associations with various binding factors, which influence the transcription of genetic regulation. However, it would be necessary to test the individual SNPs with other reproductive characteristics in buffaloes regarding economy to validate the gene as a strong candidate for selection.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Barbosa EM, Souza BB, Guimarães RC, Azevedo JS, et al. (2016a). Corrigendum Polymorphism in the melatonin receptor gene in buffalo populations of the Brazilian Amazon. *Genet. Mol. Res.* 15: https://doi.org/10.4238/gmr.150279601.

Barbosa EM, Souza BB, Guimarães RC, Azevedo JS, et al. (2016b). Novel polymorphism in exon 1 of the melatonin receptor gene unassociated with reproductive characteristics of buffaloes in the Amazon Region. *Genet. Mol. Res.* 15: https://doi.org/10.4238/gmr.15028309.

Bezerra Junior JS, Fraga AB, Couto AG, Barros CC, et al. (2014). Produção de leite, duração da lactação e intervalo entre partos em búfalas mestiças Murrah. *Rev. Caatinga* 27: 184-191.

Borghese A, Barile VL, Terzano GM, Pilla AM, et al. (1995). Melatonin trend during season in heifers and buffalo cows. *Bubalus bubalis* 1: 61-64.

Brunetta GAM, Ribeiro HFL, Camelo ASA, Vale GW, et al. (2003). Distribuição de partos de búfalas criadas em sistema produtivo de pastagem artificial e várzea na região Amazônica. *Rev. Bras. Reprod. Anim.* 27: 245-247.

Camargo Júnior RNC, Marques JRF, Marcondes CR, Araújo CV, et al. (2012). Índices de eficiência reprodutiva de búfalos da Amazônia Oriental do Brasil. *Arq. Bras. Med. Vet. Zootec.* 64: 796-803. https://doi.org/10.1590/S0102-09352012000400002

Carcangiu V, Mura MC, Pazzola M, Vacca GM, et al. (2011). Characterization of the Mediterranean Italian buffaloes melatonin receptor 1A (MTNR1A) gene and its association with reproductive seasonality. *Theriogenology* 76: 419-426. https://doi.org/10.1016/j.theriogenology.2011.02.018

Cassiano LAP, Mariante AS, McManus C, Marques JRF, et al. (2003). Caracterização fenotípica de raças bubalinas nacionais e do tipo Baio. *Pesqui. Agropecu. Bras.* 38: 1337-1342. https://doi.org/10.1590/S0100-204X2003001100013

Cassiano LAP, Mariante AS, McManus C, Marques JRF, et al. (2004). Parâmetros genéticos das características produtivas e reprodutivas de búfalos na Amazônia brasileira. *Pesqui. Agropecu. Bras.* 39: 451-457. https://doi.org/10.1590/S0100-204X2004000500007

Chasan R (1995). Eliciting Phosphorylation. *Plant Cell* 7: 495-497. https://doi.org/10.1105/tpc.7.5.495

Garcia JF and Porto-Neto LP (2006). Uso de marcadores moleculares em programas de transferência de embriões. *Acta Sci. Vet.* 34 (Suppl 1): 197-203.

Hall TA (1999). Bioedit: a user friendly biological sequence alignment editor and analysis for Windows 95/98/NT. *Nucl. Ac. Symp. Ser* 41: 95-98.

Heintzman ND and Ren B (2007). The gateway to transcription: identifying, characterizing and understanding promoters in the eukaryotic genome. *Cell. Mol. Life Sci.* 64: 386-400. https://doi.org/10.1007/s00018-006-6295-0

Hunt R, Sauna ZE, Ambudkar SV, Gottesman MM, et al. (2009). Silent (synonymous) SNPs: should we care about them? *Methods Mol. Biol.* 578: 23-39. https://doi.org/10.1007/978-1-60327-411-1_2

IBGE - Instituto Brasileiro de Geografia e Estatística (2012). Produção da Pecuária Municipal, Rio de Janeiro, 40: 1-71.

Jacob A, Budhiraja S and Reichel RR (1997). Differential induction of HNF-3 transcription factors during neuronal differentiation. *Exp. Cell Res.* 234: 277-284. https://doi.org/10.1006/excr.1997.3622

Johnston JD, Schuster C, Barrett P and Hazlerigg DG (2007). Regulation of the ovine MT1 melatonin receptor promoter: interaction between multiple pituitary transcription factors at different phases of development. *Mol. Cell. Endocrinol.* 268: 59-66. https://doi.org/10.1016/j.mce.2007.01.015

Juven-Gershon T, Hsu JY, Theisen JW and Kadonaga JT (2008). The RNA polymerase II core promoter - the gateway to transcription. *Curr. Opin. Cell Biol.* 20: 253-259. https://doi.org/10.1016/j.ceb.2008.03.003

Kininis M and Kraus WL (2008). A global view of transcriptional regulatory by nuclear receptors: gene expression, factor localization, and DNA sequence analysis. *Nucl. Rec. Sig.* 6: 1-11.

Luridiana S, Mura MC, Pazzola M, Paludo M, et al. (2012). Association between melatonin receptor 1A (MTNR1A) gene polymorphism and the reproductive performance of Mediterranean Italian buffaloes. *Reprod. Fertil. Dev.* 24: 983-987. https://doi.org/10.1071/RD11297

Marques JRF (1991). Avaliação genético-quantitativa de algumas características do desempenho produtivo de grupos genéticos de búfalos (*Bubalus bubalis*). Doctoral dissertation, Universidade Estadual Paulista, Botucatu. Available at [http://www.bdpa.cnptia.embrapa.br/consulta].

Oliveira Neta AC, Ribeiro HFL, Rolim Filho ST, Chaves LCS, et al. (2012). Intervalo entre partos e produção leitera de búfalos criados no Estado do Pará. *Ciênc. Anim.* 22: 212-215.

Pereira RGA, Barbosa SBP, Lopes CRA, Santoro KR, et al. (2008). Intervalo entre partos em rebanho bubalino no Estado de Rondônia. *Boletim de Pesquisa e Desenvolvimento/Embrapa Rondônia* 53: 1-12.

Raymond M and Rousset F (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* 86: 248-249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Rocha RMP, Matos MHTM, Lima LF, Saraiva MJA, et al. (2011). Melatonina e Reprodução Animal: Implicações na Fisiologia Ovariana. *Act. Vet. Bras.* 5: 147-157.

Rolim Filho ST, Ribeiro HFL, Vale WG, Picanço NS, et al. (2009). Desempenho reprodutivo de fêmeas bubalinas criadas em sistema misto (várzea e pastagem artificial) no Estado do Pará. I. Idade a primeira cria, intervalo entre partos, época de parição, eficiência reprodutiva e taxa de prenhez. *Cienc. Anim. Bras.* 10: 754-763.

Sambrook J, Fritsch EF and Maniatis T (1989). Molecular Cloning, A Laboratory Manual. 2nd edn. Cold Spring Harbor Laboratory Press, New York.

SAS Institute, Inc. (2000). SAS/STAT® 8.0 User's Guide. SAS Institute Inc., Cary.

Silva Filho E, Silva MH, Campelo JEG, Derosia MR, et al. (2014). Genetic characterization of Curraleiro Pé-Duro bovine breed from a conservation herd of Brazilian semiarid. *Genet. Mol. Res.* 13: 2149-2154. https://doi.org/10.4238/2014.March.24.22

Smale ST and Kadonaga JT (2003). The RNA polymerase II core promoter. *Annu. Rev. Biochem.* 72: 449-479. https://doi.org/10.1146/annurev.biochem.72.121801.161520

Snel-Oliveira MV, Wetzel-Gastal D, Sartori R, Neves JP, et al. (2013). Procedimentos ginecológicos frequentes no desempenho produtivo e reprodutivo de fêmeas bubalinas Murrah. *Cienc. Anim. Bras.* 14: 468-472. https://doi.org/10.5216/cab.v14i4.16377

Thomas MC and Chiang CM (2006). The general transcription machinery and general cofactors. *Crit. Rev. Biochem. Mol. Biol.* 41: 105-178. https://doi.org/10.1080/10409230600648736

Vale WG and Ribeiro HFL (2005). Características reprodutivas dos bubalinos: puberdade, ciclo estral, involução uterina e atividade ovariana no pós-parto. *Rev. Bras. Reprod. Anim.* 29: 63-73.

Wingender E, Chen X, Fricke E, Geffers R, et al. (2001). The TRANSFAC system on gene expression regulation. *Nucleic Acids Res.* 29: 281-283. https://doi.org/10.1093/nar/29.1.281

Wotton D, Lo RS, Swaby LA and Massagué J (1999). Multiple modes of repression by the Smad transcriptional corepressor TGIF. *J. Biol. Chem.* 274: 37105-37110. https://doi.org/10.1074/jbc.274.52.37105

Zetouni L, de Camargo GM, da Silva Fonseca PD, Cardoso DF, et al. (2014). Polymorphisms in the MTRN1A gene and their effects on the productive and reproductive traits in buffaloes. *Trop. Anim. Health Prod.* 46: 337-340. https://doi.org/10.1007/s11250-013-0493-1