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RELATIONSHIP OF MTRN1A GENE POLYMORPHISM AND SOME REPRODUCTIVE AND MESURMENTS OF BODY AND UDDER IN IRAQI BUFFALOES (*BUBALUS BUBALI*)

AL-Sarai T.Q.* and Al-Anabri N.N.

Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.

Abstract

This study was conducted in two location for breeding Buffaloes in Baghdad (White Gold Village/Abu Ghhriub AL-Fadhalya) and Laboratories for Biotechnology and Molecular Genetics Analysis for the period from 1/11/2017 to 1/12/2018. The objective in this study to the effect of genotypes for MTRN1A gene on some reproductive traits and mesurments of body and udder for a sample from 50 female buffalo. Genotype differed for the MTRN1A gene, which were three genotype TT (Wild) TC (Heterozygous) and CC (Mutant) and their distribution ratios were 34.00, 28.00 and 38.00% respectively. The effect of MTRN1A genotypes in the age at first calving was highly significant ($P<0.01$) while the female buffaloes with TC and CC genotype gave the early of age at first calving, while was late in TT genotype. There is a significant variance ($P<0.05$) in the heart girth of female buffaloes with the different genotype of MTRN1A gene, while the other body measurements were not influenced. The results of this study showed that the udder measurements represented by front left teat length and hind left teat length were influenced significantly ($P<0.05$) by the differences between the genotype of MTRN1AN gene, and front right teat length and hind right teat length were not significant. Was can concluded from the study of the MTRN1A genotype that the use of these markers to put a strategically method of genetically improvement for the buffaloes in middle of Iraq to increased of economical income from these buffaloes farms by selection and genotypes crossing which gave a good reproductive performance. The application of this study in bigger samples of animals for many productivity seasons may give more accurate results for applying replacement and culling strategy.

Key words: mesurments of body, MTRN1A gene, Iraqi Buffaloes

Introduction

Many studies have dealt with Breeding Value of Buffalo and applied it for breeding programs however studies still lack many genetic information, especially the buffalo genome is still highly shortage of alot information for many of the quantitative characteristics associated with the traits (Hayes, 2014). In particular, the study of single-nucleotide polymorphisms of cattle does not provide ideal coverage for the buffalo genome. That is the researchers used the information resources of cattle genome because of the close touch development between them (De Camargo *et al.,* 2015). Recently many studies have been searching about several genes related production traits and to identify genetic mutations and their association with the overall performance of the animal, thus contributing to increased efficiency in the selection of individuals with distinct genetic structures and best performance (Coizet *et al.,* 2018). Melatonin is associated with many physiological functions including anti-inflammatory, insomnia, daily rhythm regulation and endocrine activity, and its antioxidant activity, as well as the role of melatonin in regenerating cells or tissue after partial loss (Riaz *et al.,* 2018). The melatonin receptor MTRN1A plays a large role in regulating many productive and reproductive traits so it's very important to study the receptor gene and its genetic polymorphism (Majidinia *et al*., 2018). MTRN1A concedered the key to work of the hormone melatonin, which enters into many animal functions such as regulating the growth of ovarian follicles and pregnancy rate and fertility (Ramadan *et al*., 2014). It was found that the process of regulating seasonal differences is controlled by both the pineal gland (where

***Author for correspondence :** E-mail : alsaraitamara@gmail.com

the melatonin secretion is located) and PT (where the receptors are located) and the Tanycytes cells, which are special neurons in the third ventricle of the brain and have operations extending deep into the hypothalamus, Its function is to transport chemical signals within the nervous system (Wen *et al*., 2016). The MTNR1A receptor gene is located on chromosome 1 in the Buffalo and contains 2 exons, encodes the receptor that achieves the effect of melatonin stimulation in periods of darkness and thus has a close relationship to reproductive characteristics. Therefore, the present study aimed at studying the relationship of the genetic polymorphism of MTRN1A and the age at the first birth and a number of body and udder measurements in a sample of the Iraqi buffalo in Baghdad.

Materials and Methods

The research was conducted in Baghdad (White Gold Village/Abu Ghraib-20 km west of Baghdad City and Al-Fadhiliya location-20 km northeast of Baghdad) for the period from 1/12/2017 until 1/2/2018 on a sample of 50 female Buffalo. This study aimed at determining the genotypes of the melatonin receptor gene on the buffalo sample (the extraction of the distribution ratios of these manifestations and their allelic frequencies) by the means of RFLP-PCR technique, to study the genotypes of this gene and its relateationship to some of reproductive traits and number of body and udder measurements in a sample of the Iraqi buffalo in Baghdad.

Blood collected by a medical syringe from the jugular vein in a 1.5 ml sterile polypropylene tubes containing 0.5 ml of EDTA (0.5 M) as an anticoagulant by the phenol chloroform extraction by the veterinarian at the station, The blood samples were then transferred by a cool box then stored in freezer at-200^{0}C temperature till transferred to the lab to extracting DNA , for the calves blood also collected by medical syringe from the jugular vein in a 10 ml tubes, the DNA samples were checked for their quality, purity and concentration, the quality of the genomic DNA was checked by using agarose gel electrophoresis, DNA samples of good quality, purity and concentration were used for further analysis. The polymerase chain reaction (PCR) technique for MTRN1A typing is based upon the extensive polymorphism that is present in Exon 2 of the MTRN1A gene under consideration depending on the size of the pieces and type of primers used, the 825 bp fragment consisting (Barbosa *et al*., 2016) present in the genomic DNA of cattle was amplified by employing the corresponding primer pairs (forward and reverse). The details of the primer sequences are as follows:

F : 5'TGTGTTTGTGGTGAGCCTGG3'

R : 5'ATG GAG AGG GTT TGC GTTTA3'

After the polymerase reaction was completed, the polymorphism of MTRN1A gene were identified in blood samples from the cows by used sequence technique through the program Blast software from NCBI (National Center For Biotechnology Information) and information program. And the genotype's of MTRN1A identified by the different between the sequences in nitrogen bases for the studied cows and compared it with the wild sequence of the gene in NCBI.

The data was analyzied by used Statistical Analysis System (SAS, 2012) to study the polymorphism of MTRN1A gene according the mathematical model, significant differences was compared by used least square means method.

$Yijk = \mu + Gi + Lj + eijk$

Yijk: observed value k, which belong to phenotype i and Location j.

μ : Overall means.

Gi : Effect of MTRN1A polymorphism (TT, TC, CC).

Lj : Effect of Location (White Gold Village/Abu Ghhriub AL-Fadhalya).

eijk: Random error which distributed normally with mean = 0 and variation σ^2e.

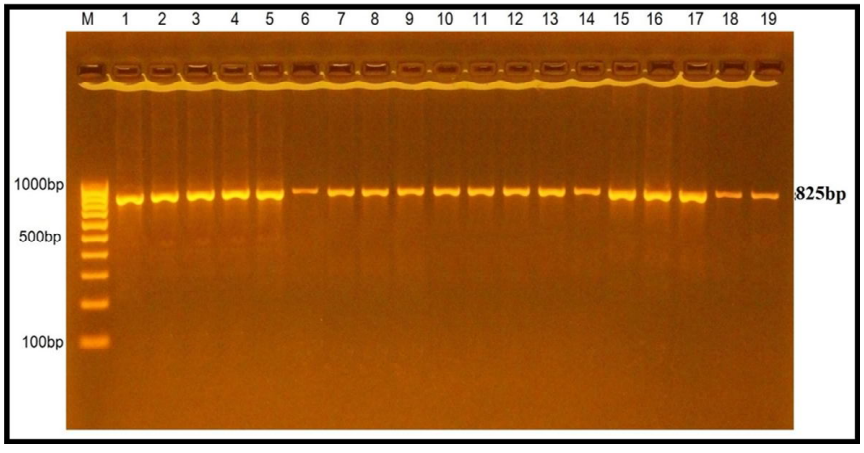

Fig. 1: Extraction fragment (1000bp) of the MTNR1A gene by PCR technique

Results and Discussion

The genotypes of the animals which have been studied are determined by the application of RFLP-PCR and the HpaI restriction enzyme to identify the distribution of the studied animal genotypes (Table 1) according to the number and the size of the formed bands. The size of the DNA fragments

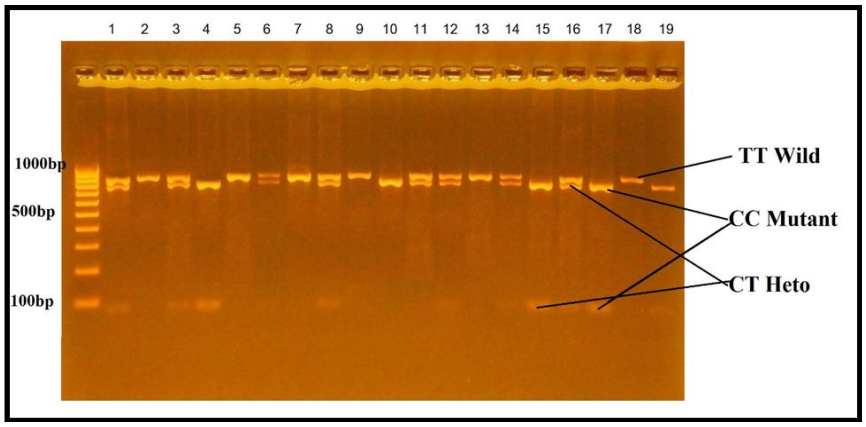

Fig. 2: Genotypes of the MTRN1A gene studied using the restriction enzyme-*HpaI.*

was used as a marker 1500-100 bp DNA ladder. , as it appears in Fig. 2.

The HpaI was cut off after identifying the sensitive position within the specific sequence (GTT / AAC) of the target gene segment, thus forming the cutting process forms one band, two or three bands which can be compared with the ladder. The enzyme cuts in the site 824bp base pair sequence of the target gene. The genotype of MTRN1A has been identified as three genetic structures (TT, TC and CC).

The genotypes of the MTRN1A gene in buffalo sample

Table 1: Number and percentages of genotypes and allele frequency MTRN1A.

Genotype	Numbers	Percentages%
TT: Wild	17	34
TC: Hetro	14	28
CC: Mutant	19	38
Total	50	100
Chi-square(χ2)	----	**9.840
Allele		Frequency
T		0.51
C		0.49
** (P<0.01).		

Table 2: Relationship between the polymorphism of the MTRN1A gene and the first birth of the Iraqi buffalo sample

Genotype	Numbers	Age at first birth ± Standard error (years)
TT: Wild	17	30.10±0.35 a
TC: Hetro	14	27.29±0.36 b
CC: Mutant	19	27.29±0.29 b
Significant	----	
The different letters within a row indicate a significant difference; *(P<0.01).		

Table 1 shows the number and percentage of MTRN1A genotypes in the studied sample. There are significant differences (P<0.01) between different buffalo genotypes, which reached 34.00, 28.00 and 38.00% for the TT, TC and CC sequences respectively, That is, the ratio of individuals with a mutant type is higher than those of the TC hybrid with a low rate of the same wild genotype in the MTRN1 gene. The law for the allele frequency was applied according to the Hardy and Weinberg equilibrium rule. The frequency of the allele T was 0.51% while allele C was 0.49%. In previous studies, of the water buffalo indicated that allelic frequency of C and T was 0.41 and 0.59%, respectively. The distribution genotype for TT, TC and CC was 16.58, 34.76 and 48.66% respectively (Gunwant *et al.,* 2018). In another study, there were three genotypes of MTRN1A, CC, AA and CA the frequencies of them were 0.45, 0.41 and 0.14 and their allele frequency were 0.65 and 0.35% for C and A respectively, (Kianpoor *et al*., 2018).

Genotypes of MTRN1A in Age at First Birth

Table 2 Shows that there is a significant variation (P < 0.01) in age at first calving according to the genetic genotypes of MTRN1A. the lest age this age was in females of buffalo which have TC and CC, with the average of 27.29±0.36 and 27.29±0.29 months, respectively, while the age at the first birth was the longest in the wild type TT with 30.10±0.35 month The age at the first birth is an important indicator of reproductive traits in the buffalo, as it is positively associated with the length of productive life, the number of births for each female, the number of seasons of milk and the length of the milk season. Thus, the selection of the distinguished genetic type may achieve an economic return. Gunwant *et al.,* (2018), in a study of multiple genotypes of MTRN1A in Exon2, shows that the polymorphism in this part of MTRN1A gene for has higher activity in hybrid and mutant type females than the wild type, this result matches the results of the current study. Other studies have indicated the genotypes of this gene is of breeds. The multiple genotypes of MTRN1A gene are considered to be an important molecular marker to selection sheep for age at the first birth, the results varied according to different breeds (Fathy *et al.,* 2018). MTRN1A has an important role in the formation of a corpus luteum that support its active effect on age at the first birth (He and others, 2016).

Table 3: Relationship of the MTRN1A gene genotypes with the milk composition.

Genotype	Numbers	mean ± standard error(cm)				
		Hip heigh	wither heigh	Length body	Belly girth	Heart grith
TT: Wild	11	135.71±1.95 a	140.07±1.79 a	155.59±3.51c	246.89±5.46a	184.45±10.71 b
TC: Hetro	10	137.31±1.96 a	140.18±1.72 a	153.89±3.65 a	246.89±5.41 a	201.36±10.62 a
CC: Mutant	16	138.53±1.4 a	140.51±1.33 a	154.72±2.70 a	245.02±4.19 a	203.12±8.38 a
Significant	----	NS	NS	NS	NS	NS
The different letters within a row indicate a significant difference; *(P<0.05).						

Table 4: Relationship of the MTNR1A gene to the measure of the teats of the Iraqi buffalo sample.

Genotype	Numbers	means± Standard error(cm)			
		Front left teat	Front right teat	Rear left teat	Rear right teat
TT: Wild	3	6.41 ± 0.52 a	5.71 ± 0.26 a	6.43 ± 0.36 b	5.53 ± 0.39 b
TC: Hetro	6	6.03 ± 0.12 a	7.29 ± 0.16 ab	6.02 ± 0.18 ab	6.65 ± 0.23 a
CC: Mutant	14	6.97 ± 0.18 a	6.07 ± 0.09 a	7.65 ± 0.12 a	6.25 ± 0.14 a
Significant	-----	NS	NS	*	*
The different letters within a row indicate a significant difference; *(P<0.05).					

Relationship genotypes of MTRN1A in body dimensions

Table 3 shows that the genotypes of MTRN1A gene has a significant effect (P<0.05) in the heart girth buffalo of females, with a mean of 201.36 ± 10.62 and 203.12 ± 8.38 cm in females with TC and CC respectively, while the rate was lower in the buffaloes of the TT genotype with mean 184.45 ± 10.71 cm, while it was no effect on the other dimensions of the body, which are belly girth, the length of the body and wither and hip height. There are not sufficient studies to indicate the effect of genetic polymorphism of MTRN1A on the studied traits, but the increase in the heart girth may indirectly be affected by the MTRN1A. thus the animals which have an increase in the heart girth own the genotypes TC and CC, and at the same time they also have the highest rate of the milk production, that is the measurement of the heart girth factor correlates with milk production (Yanar *et al.,* 2000).

Relationship of the MTNR1A gene to the measure of the teats

It is clear from table 4 that there is a significant difference (P<0.05) in the frontal teat length of the buffalo females according to the genetic polymorphism of the MTRN1A gene. The highest mean of this trait was in CC (6.25±0.14 cm) while the lowest mean was in the genotype TT (5.53±0.39 cm), and the female buffalo with hybrid genotype has a middle average between the two previous genotypes (6.02±0.18 cm). The differences were significant (P<0.05) in the length of the rear left teat and the result of the front left teat length was the same which were 6.43±0.36, 7.29±0.16 and 7.65±0.12 cm sequentially for TT, TC and CC. The differences in the length of the right frontal teat and the right rear teat length were not significant according to the genetic polymorphisms of the MTRN1A gene in this study. Suarez-Trujillo and Casey (2016) reffer that the polymorphism of melatonin receptor plays a key role in the process of forming the lactic glands and their secretory channels so that the length and diameter of the teat are associated with milk production.

Also, the females of buffalo with the genetic structure CT and CC of the MTRN1A gene were the youngest in their first birth compared with their TT structure. Therefore, selective programs to improve reproductive performance may be developed based on the results of the MTRN1A gene compositions. It is also necessary to conduct more studies about all coding regions of the MTRN1A gene and gene expression of them for more numbers of sample and for several seasons.

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