Study the Relationship Between Polymorphism of Melatonin Receptor Type 1A (MTNR1A) Gene with Some Reproductive Performance, in Local Iraqi Goats

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ABSTRACT

Melatonin is the main molecule that transmits the signal of seasonal change to the neuroendocrine system in seasonal breeding species, through specific melatonin receptors, e.g. Melatonin Receptor Type 1A (*MTNR1A*). In the present study, a total of 40 local Iraqi non pregnant goats were used to study the relationship between Polymorphism of Melatonin Receptor Type 1A (*MTNR1A*) gene with some reproductive performance. Animals were reared in a farm located in Al-Yusufiya/Baghdad. Animals were divided based on breading season into two groups with 20 animals each as follows: goats which gave birth in expected breading season (January, February, and March), and goats which gave birth in unexpected breading season (October, November, and December). The number of kids (single or twins), weight and sex of kids were record. Five milliliters of blood were collected from each goat through jugular vein puncture with EDTA coated tubes, this was used for DNA extraction. The main part of exon II of *MTNR1A* gene was amplified by PCR using specific primers, and then the PCR product was digested with *Bsa*I restriction enzyme. The restriction fragment length polymorphism (RFLP) analysis was used to determine the presence of the cleavage site that produces two fragments, 279 and 577 bp (C allele), while the absence of this site produces only one fragment of 856 bp (A allele). The results suggested the presence of three genotypes, including the homozygote CC (279 bp, 577 bp), homozygote AA (856 bp) and the heterozygote CA (279 bp, 577 bp, 856 bp). In all of the analyzed 40 local goats, the genotypic frequency was 35.0% for CC genotype, 52.5% for CA genotype and 12.5 for AA genotype. The allele frequency of C and A was 61.25% and 38.75%, respectively, this was in Hardy–Weinberg equilibrium. Allele C associated with out of season breeding ability of goats, and allele A associated with season breeding. No relationship was found between kids weights, sex and type of parturition (single or twin) with the genetic analysis model of *MTNR1A* gene in local Iraqi goats.

Keywords: MTNR1A, PCR-RFLP, Polymorphism, Iraqi goat

Introduction

Domestic goats (*Caprashircus*) are one distinct species in the family Bovidae. It was first domesticated for milk, meat, and fiber (1).

Goats are generally classified as seasonal poly estrous and short-day breeders in temperate regions, the degree of seasonality varies among breeds and their location. In tropical region near the equator, goat breeds show less seasonality and breed all year-round (2). The season in tropical region is determined by several factors, including physical environment, nutrition, management and genetics (3).

Photoperiod is the factor that affects the succession of reproductive periods (4). Light signal received by the retina is translated into hormonal message by the pineal gland through melatonin secretion

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(5). High melatonin levels, typical during autumn, have a positive influence on reproduction in small ruminants (6, 7). The breeding season begins as the day light length becomes shorter. Melatonin functions act through specific receptors located in different areas of the central nervous system (CNS), including nuclei which regulate the reproduction (8). In small ruminants, polymorphisms at the *MTNR1A* gene are associated with non-seasonal breeding (9). The pre-mammillary area in the hypothalamus is an important target for melatonin to regulate reproductive activity (10). Melatonin is thought to mediate its circadian rhythm and reproductive effects through specific high affinity receptors located on cells of the pituitary pars tuberalis and suprachiasmatic nucleus, respectively. The mammalian melatonin receptors MT1 and MT2 have been cloned and characterized to be members of the G protein coupled receptor superfamily (11, 12). The structure of exon 2 of MT1 gene was associated with reproductive seasonality in goat (13).

Several DNA polymorphisms have been considered as potential tools for the selection programs in domestic animals. DNA based molecular methods have made a more efficient and flexible selection tools for genotyping of animals at any age and sex for milk, reproduction and meat genes. Selection efficiency, however, depends on allele frequencies, the effect on these polymorphisms on dairy, reproduction and meat traits and their technological properties (14 and 15). In seasonal breeding, it has been reported that the polymorphisms of restriction enzymes *Mnl* I and *Rsa*I in the *MTNR1A* gene are strictly bound with seasons in Sarda goats (16).

Another research used *MTNR1A* PCR products, which were digested separately with *Eco31*I, found that the polymorphism sites of the enzymes in the *MTNR1A* gene were significantly related to seasonal reproduction in goat (17). In Iraq, many clinical studies on local female and male goats confirmed but not strictly seasonal animals so that the reproduction in similar and they reproduce throughout the year, but optimal reproductive efficiency is seen in autumn and summer (18, 19). Because of little available literature about the relationship between genetic criteria and seasonality rate in local Iraqi goat, this study was designed.

Materials and Methods

Forty multiparous local goats were used in this study. All animal were reared with breeding bucks for all time in one flock in Baghdad /Al-Yusufiya, that locate 25 Km south of Baghdad in latitude 33.17 and longitude 44.33. The experiment was conducted in the period extended from January 2018 to March 2019. The study reported some reproductive performance in local goats in different breeding months, including number of kids (single or twins), weight and sex of kids (male, female). The goats were divided into 2 main groups depending on the season of breeding. The first group included goats which gave birth at the breeding season (begins from January, February till March). The second group included goats which gave birth out of the breeding season (starts from October, November till December). The flock has been managed under the same health program and nutritional conditions. All open goats were placed with breeding buck for all time.

Five ml of blood were collected from the jugular vein in EDTA coated tubes, blood samples transported to ACSO. Learning center laboratory/Baghdad for DNA extraction. Genomic DNA was isolated from the blood samples according to the protocol of ReliaPrep™ Blood gDNA Miniprep System (Promega / USA) and stored at -20°C. Polymorphism was identified using the PCR-RFLP method as described by (17). A fragment with the size of 856 bp from exon II of *MTNR1A* gene was amplified with a specific primers:

(forward: 5' GCCTGGCAGTTGCAGACCTG -3', and reverse: 5'- CATTTTTAAACGGAGTCCACC -3'). The total volume of PCR reaction was 25 µl, and included the use of PCR Pre Mix Kit. The amplification reactions were started according to (17). The amplification was conducted in thermal cycler (Bio-Rad, USA) with the following temperatures profile, consisting of an initial denaturation at 95°C for 5 min, followed by 30 cycles program with denaturation at 95 °C for 30 second, annealing at 58 °C for 30 second, elongation at 72 °C for 30 second and final elongation at 72 °C for 7 min. Agarose gel was prepared and stained with ethidium bromide. DNA bands were visualized by electrophoresis and captured by gel documentation system.

The PCR products were separated by electrophoresis on 1% agarose gel in 1X TBE buffer alongside with a 100 bp DNA size marker (Promega, USA). Variation in melatonin receptor 1A gene was examined after enzymatic digestion for the PCR product with Smart restriction enzyme *Bsa*I (Biolabs/USA). The digestion reaction was conducted in 10 µL final volume at 37°C for 1 hr. In *Bas*I, the restriction site is 5' GGTCTC 3' that cause blunt cut. In the studied DNA samples, there were three cleavage sites (279 bp, 577 bp, and 856 bp) created by *Bsa*I within the amplification fragments. The digested fragments were electrophoresed on 2% agarose gel and stained with ethidium bromide. Three genotypes were found, including: CC (279 bp/577 bp), CA (279 bp/577/856) bp) and AA (856 bp) (17). The allelic and genotypic frequencies using Hardy-Weinberg (HW) equilibrium were done.

Results and Discussion

Reproductive traits are the most important economic characters in farm animal breeding. There are a number of genes that affect reproduction and that can be employed in selection programs. One of these genes is melatonin receptor 1A (*MTNR1A*), which plays a significant role in the reproductive process in animals (20, 21).

Therefore, knowledge of the genes involved in the control of the reproductive seasonality linked to genetic markers, allows implementing intensive and effective selection programs out of reproductive seasons and include this advantage in commercial flocks (20). Moreover, if the genetic potential of the flock is to be improved, both males and females should be selected (22). The result showed that the local goats which were used in this study had breeding months 4, 11, 5, 11, 3, 6 in January, February, March, October, November and December, respectively. In the first three months, the suspected goats gave birth in breeding season, while in the other three months the suspected goats gave birth out of breeding season. The present study found a significant difference between months in the number of kids, but the animals were similar during February, March and October (11, 5, 11) kid numbers, respectively when the light hours decreased 9.5, 8.5, and 9.5, respectively, with moderate temperature.

This result is in agreement with many clinical studies (18, 19).

These studies indicate that the local female and male (middle part of country) are not strictly seasonal animals in their reproduction, in which they reproduce throughout the year, but optimal reproductive efficiency is seen in autumn and summer. This result explains the role of melatonin in seasonal reproductive activity for species living in temperate latitudes, it is significantly influenced by melatonin (23). Melatonin can affect reproduction through activation of receptor sites within the hypothalamic pituitary gonadal axis (24).

The type of kidding in the present study showed significant difference at $P<0.01$ between single kid than twins , in which the twins kidding was high in February (54.55%), October (54.55%), January (50%), and December (50%) than that occurred in November (33.34%) and March (20%). However, no significant difference was noticed between different breeding seasons, this is in a agreement with (25). Zuniga and his colleagues suggested that ovulation rates are higher earlier in the natural breeding season than later, but factors such as body size, body weight and condition, and genotype may also contribute to an increase in ovulation rate. This demonstrates the role played by melatonin to induce estrous cycles, and is associated with an improvement of the ovulation rate (26), litter size (27), enhanced luteal function (28) and greater embryo viability (29). The weight of kids in the present study was not significantly different throughout various breeding months, and the weight of kids ranged between 3.32-3.50 kg (Table 1).

The effect of allele frequency of *MTNR1A* gene in local goats. After amplifing a fragment of 856 bp from exon II of *MTNR1A* gene with a specific primer, the PCR products were separated by electrophoresis on 1% agarose gel (Figure 1).

Variations in the *MTNR1A* gene were examined after enzymatic treatment of the PCR products with the restriction enzyme BsaI (Figure2). The *MTNR1A* gene frequencies for (C) and (A) variants seen in this study were 61.25% and 38.75% for the BsaI polymorphism (alleles C and A, respectively). Among the 40 does, the genotypic frequencies were 35.0%, 52.5 %, and 12.5% for CC, CA, and AA genotypes, respectively at the BsaI polymorphism (Tables 2 and 3). This result was in accordance with the findings of (17). In six breeds in China, Boer breeds gene frequencies were 0.554 and 0.446 when using the restriction endonuclease

*Eco31*I, which showed polymorphism in alleles C and D, respectively. Also similar data have BEEN reported in Duzu Black goats, in which the allele frequencies were 0.558 and 0.412 for the *Eco31*I. Polymorphism genotypic frequencies were 0.353, 0.471 and 0.176 for CC, CD, and DD genotypes at the *Eco31*I polymorphism. Types of parturition (single or twin) were significantly different at P<0.05 between the genotype frequencies CC, CA and AA, respectively. The sex of kid was significantly different at P<0.01 in male kid than female in the genotype frequency (Table 2), this result of allele distribution attributed to genetic and non-genetic factors. So fertility and fecundity in small ruminants varies by breed, season, age, nutritional status, health and breeding management (30). Body mass and Body Condition Score (BCS) have a significant effect on fertility of goats during the breeding season and the necessary of using higher energy feeding in goats with lower BCS and weight before the breeding season (31).

The effect of season on the distribution of allele frequency of *MTNR1A* gene in local goats in the present study. There was no significant effect of season on allele distribution of *MTNR1A* gene in local goats (Table 4), this result agrees with (19) who conducted his experiment in Iraqi goats, and found no season in the strict sense of breeding but the breeding level increases in autumn and starts in June. Another result confirmed that genotyping of alleles had no significant effect on birth weight, this result agrees with (32) in local Iraqi sheep. There was no relationship between individual weights with the genetic analysis model of *MTNR1A* gene in local ewes. In goats, variations in birth weight of kids born in different seasons reflected variations in the level of management, some environmental effects like temperature and humidity, and availability of good quality feed in sufficient quantity.These results were in accordance with the findings of (33), who reported that kids born during rainy season were significantly heavier than those born in winter and/or summer. In conclusion, there was no relationship between kids weights, sex of the kid, type of parturition (single or twin) with the genetic analysis model of *MTNR1A* gene in local Iraqi goats. PCR-RFLP analysis of the caprine *MTNR1A* gene detected allele frequencies of 61.25% and 38.75% for the *Bsa*I polymorphism of alleles C and A, respectively, and the genotype frequency of CC homozygote was 35% compared with CA heterozygote 52.5% and AA homozygote 12.5% in local goats. Moreover, allele C can be associated with breeding around year, but allele A can be associated with breeding season.

Table 1. Breeding months with number of offspring single or twins, weight of kid in local goats

Parameter	**In breeding months**		**Out- breeding months**				**Chi-Square**
	January	**February**	**March**	**October**	**November**	**December**	
Number of goats	4	11	5	11	3	6	---
Type of birth (%) single	(2) 50 %	(5) 45.45%	(4) 80%	(5) 45.45 %	(2 66.66%	(3) 50.00%	9.37 **
twins	(2) 50 %	(6) 54.55%	(1) 20 %	(6) 54.55 %	(1) 33.34%	(3) 50.00%	9.37 **
Chi-Square	0.00 NS	4.39 *	13.25 **	4.29 *	9.52 **	0.00 NS	---
Weight of kid (Kg)(mean)	3.47 ± 0.14	3.50 ± 0.17	3.31 ± 0.09	3.32 ± 0.11	3.40 ± 0.11	3.44 ± 0.14	NS
Estrus Months	August	September	October	May	Jun	July	---
Light (hrs.)	11.8	9.5	8.5	9.2	12.4	12.6	---
Temp C° Max/Min	47.4/28.3	43.5/23.7	33.7/17.0	35.7/20.8	42.0/25.1	44.1/26.7	---

* (P<0.05), ** (P<0.01)

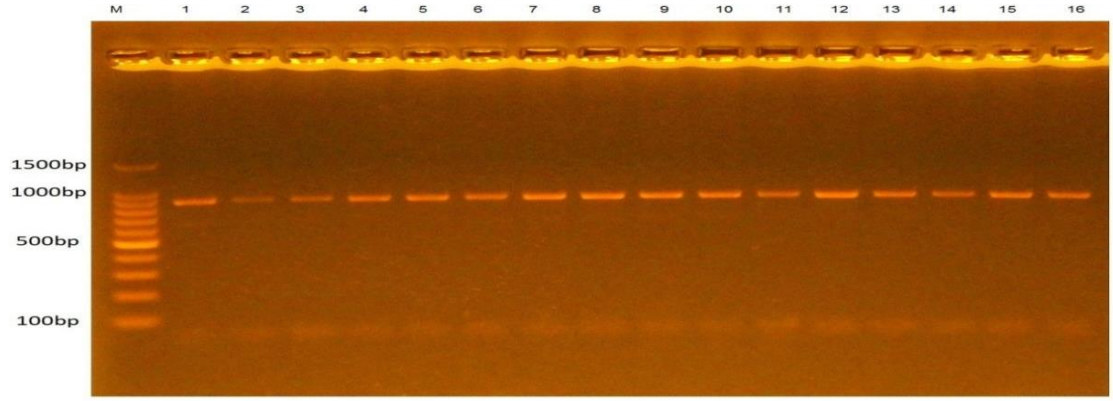

Figure 1. Electrophoresis pattern of PCR product of exon II of *MTNR1*A gene with 856 bp size in local goats. Lane M:DNA molecular marker 100 bp size. Ethidium Bromide stained bands in the 1% agarose gel

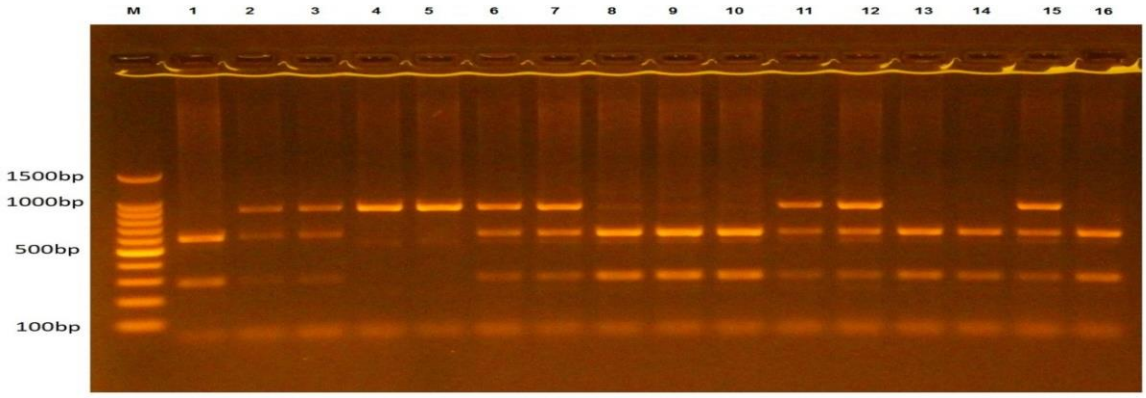

Figure 2. Electrophoresis pattern of PCR product digested with *Bsa*I restriction enzyme, 2% agarose gel in local goats. Lanes 1,8,9,10,13,14,16 show homozygous CC genotype; Lanes 2,3,6,7,11,12,15 are heterozygous AC genotype. Lanes 4,5 are homozygous AA genotype. M: DNA molecular marker 100 bp size. Ethidium Bromide stained bands in the gel

Table 2. Distribution of *MTNR1A* gene polymorphism in male and female kids of local goats

Polymorphism	No. goats	(%)	Single	Twins	No .kids	Male kid	Female kid
CC	14	35.0	7 (50.00%)	7 (50.00%)	21	9 (42.86%)	12 (57.14%)
CA	21	52.5	10 (47.62%)	11 (52.38%)	33	18 (54.55%)	15 (45.45%)
AA	5	12.5	4 (80.00%)	1 (20.00%)	6	3 (50.00%)	3 (50.00%)
Total	40	100 %	21 (52.5%)	19 (47.5%)	60 (100%)	30 (50.00%)	30 (50.00%)
Chi-Square (χ^2)	--	9.415**	10.487**	9.260**	----	4.381*	5.127*

* (P<0.05), ** (P<0.01)

Table 3. Allele frequency of *MTNR1A* gene in local goats

Allele	Frequency (%)
C	0.6125(61.25 %)
A	0.3875(38.75 %)
Total	1 (100%)
Chi-Square (χ^2)	9.362**

** (P<0.01)

Table 4. Distribution of allele frequency of *MTNR1A* gene in local goats according to birth season (out or in season)

Birth month	Season	Total	CC	CA	AA	C	
January	In	20	7	10	3	12	8
February	season	(50%)	(35%)	(50%)	(15%)	(60%)	(40%)
March							
October	Out of	20	7	11	2	12.5	7.5
November	season	(50%)	(35%)	(55%)	(10%)	(62.5%)	(37.5%)
December							
Total	---	40	14(35%)	21(52.5%)	5(12.5%)	24.5(61.25%)	15.5(38.75%)
Chi-Square (χ^2)	---	0.00 NS	0.00 NS	1.962 NS	1.962 NS	0.672 NS	1.725 NS

NS: Non-Significant

Conflict of Interest

The authors declare that there is no conflict of interest.

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دراسة العلاقة بين التغايرات الوراثية لجين مستقبلات هورمون الميلاتونين 1 مع بعض معاييرالتكاثرفي الماعزالمحلي العراقي

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الخلاصة

الميلاتونين هوالمركب الرئيسي الذي ينقل إشارة التغيرالموسمي الى الجهازالعصبي الغدي في الكائنات الموسمية من خلال مستقبلات هورمون الميلاتونين نوع 1. شملت الدراسة الحالية 40 معزة غيرحامل, تم تربيتها في حقل في اليوسفية / بغداد، إذ أنَّ 20 معزة منها ولدت في شهركانون الثاني, شباط وايار وكانت ولادتها ضمن موسم الولادات المتوقع، و 20 معزة أخرى ولدت في تشرين الاول, تشرين الثاني وكانون الأول حيث كانت ولادتها خارج موسم الولادة المتوقع, المجموعتين المستخدمة في الدراسة تم تسجيل بعض المعايير التكاثر لها بعد الولادة, ثم تم سحب 5 مل من الدم من الوريد الوداجي بواسطة انابيب تحوي مادة مانعة للتخثر, وتم ارسالها الى مختبر التقدم العلمي ACSO في بغداد، تم استخلاص الحامض النووي الرايبوزي منقوص الاوكسجين من الدم اعتماداعلى بروتوكول استخلاص الحامض النووي الرايبوزي منفوص الاوكسجين المعتمد في شركة بروميكا الامريكية لعينات الدم، ومن ثم تم مضاعفة الاكسون الثاني لجين مستقبل هورمون الميلاتونين بواسطة تفاعل سلسه انزيم البلمرة بعد استخدام البادي المناسب للتفاعل, تم بعد ذالك تقطيع القطعة المضخمة (856 قاعدة نايتروجينية) بواسطة انزيم *BsaI* لتحديد مواقع التقيد بالانزيم، لوحظ ان الانزيم *BsaI* يقطع القطعة المضخمة (856 قاعدة نايتروجينية) الى قطعتين (279،577) عندما يكون الاليلC , لكن لايقطع القطعة المضعفة (856) وتبقى قطعة واحدة عندما يكون الاليل A. ولوحظ وجود ثلاث أنماط جينيه النمط الجيني احادي الزايكوتCC عندما توجد قطعتين (279،577) والنمط الجيني احادي الزايكوتAA عند وجود قطعة واحدة (856) والنمط الجيني ثنائي الزايكوتCA عند وجود ثلاث قطع (279 , 577, 856), وكانت نسبة التحليل الجيني لاربعين معزة محلية 35% للنمط الجين CC، 52.5% للنمط الجيني CA و 5.12% للنمط الجيني AA وكانت نسبة الاليلC (61.25%) ونسبةالاليلA (38.75 %) اعتماداعلى قانون هاردي وينبرك . الاليلC مرتبط مع التكاثر خارج الموسم التناسلي والاليل A مرتبط مع التكاثر في الموسم التناسلي. نستنتج من الدراسة الحالية لا توجدعلاقة بين وزن الجنين وجنسه (ذكر،انثى) ونوع الولادة (مفرد، توائم) مع نمط التحليل الجيني لمستقبلات هرمون الميلاتونين 1 في الماعزالمحلي العراقي.

الكلمات المفتاحية: الوراثية، جين، ميلاتونين، الماعز