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Analysis of Polymorphism of Melatonin Receptor Type 1A (MTNR1A) Gene, in Iraqi Local Sheep Using PCR-RFLP Technique

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Abstract: The gene encoding melatonin receptor type 1A (*MTNR1A*) gene by PCR-RFLP, the aim of the present study of polymorphism *MTNR1A* gene associated with breeding out of season and correlated with prolactin hormone in Iraqi sheep breed,. Blood samples were randomly collected from 60 ewes from *Agriculture Research-Ruminant Researches station* breeding station, Baghdad, and genomic DNA was isolated using a Geneius TM Micro gDNA Extraction Kit. A large fragment of exon 2 of *MTNR1A* gene was amplified by PCR using specific primer pairs and the PCR product was digested with RsaI enzyme which cuts the amplicon to several fragments. There are four cleavage sites (53 bp, 267 bp, 23 bp, 411 bp, 70 bp) for *Rsa* I within the amplification fragment, Digestion with *Rsa*1yielded polymorphic fragments of 267 bp when the cleavage site was present (allele *R*) and a single 290 bp fragment when the cleavage site was (allele *r*). Three genotypes *RR* (411 bp/267 bp), *Rr* (411/290 bp/267 bp) and *rr* (411 bp/290 bp). Three fragments of 23bp, 53 bp and 70bp were been not related allelic polymorphism .Restricted digestion allowed the determination of two alleles (R, r), Result gene frequencies were 0.40 and 0.60 for the alleles *R* and *r* respectively, in local ewes and genotype frequencies of RR (20%), Rr (40%) and rr (40%) in ewes with a significant at ($P<0.01$), the level of prolactin relation to the genotype differences that the highest hormonal level linked to the RR genotype (234.65±17,48ng/ml), and high significant ($P<0.01$) with lower values for prolactin hormone that linked to Rr and rr genotype (217. 02 ± 28.12; 182.91 ± 21.35 ng/ml) in local Iraqi ewes and no-significant values between the out and the in season lambing months, however, higher significant variation ($P<0.01$) of frequencies of R genotype (62.50%) in out-seasonal lambing months than in-seasonal lambing months (37.50%). It was concluded that the alleles frequencies of *MTNR1A* gene 'r' higher than 'R' alleles, Analysis of the *MTNR1A* polymorphism its relationship with prolactin concentration of Moreover show the indirect effect of higher prolactin concentration with genotype RR of the *MTNR1A* gene polymorphism and can be used a genetic selection marker for spring (out-of-season) breeding in Iraqi local sheep.

Key words: *MTNR1A*, PCR-RFLP, Polymorphism, Iraqi sheep.

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Introduction

Sheep live under the influence of seasonal variations in climatic, variables often linked to changes in latitude and altitude of a place with admiration to the total insolation received. The daily photoperiod and the annual cycles in environmental temperatures are the outstanding variables in temperate regions while the annual cycles in rainfall, with the consequential cycles of food availability are found in the tropical region. Sheep do regulate their reproductive activity by feature of differential melatonin secretion in response to photoperiodic (1). Melatonin regulates the circadian rhythmic cycles and reproductive variations in seasonal reproductive ewes (2, 3). Melatonin applies its reproductive and circadian effects by ability of its binding to high affinity melatonin receptors. The pars tuberalis region, where the maximum density of melatonin receptor (MTNR1A) has been documented, was created to be associated with prolactin secretion (4, 5). Melatonin receptor 1A gene (*MTNR1A*), consists of two exons divided by an approximately 8 000 base pair (bp) long intron (6). The first exon (exon I) codes for the first intracellular loop while the second exon (exon II) codes for the rest of the receptor. Amplifications of most of the exon II have been carried out in PCR reactions with primers that start at position 285-304 and ends at position 1108-1089 of the ovine *MTNR1A* gene which has the GenBank number: U14109 (6). The objectives of the present study of polymorphism *MTNR1A* gene associated with breeding out of season and correlated with prolactin hormone in Iraqi sheep breed.

Materials and Methods

Sixty local Iraqi ewes from the state board for *Agriculture Research- Ruminant Researches station- Ministry of Agriculture* (Agurgof) location in 25 Km northwest of Baghdad in latitude 33.325 longitude 44.422 from March 2015 to March 2016 were included. Records and the fertility data collected were; the ewe's date of birth, number of lambing, number of lambs/lambing. Also the sex, weight and number of parity of the ewes were registered. First lambing records were available for all ewes, only 10 ewes had two lambing records while other ewes had three to six lambing. Ewes were divided into 2 main groups depended on the season of lambing as follows: The 1st group include ewes which lambed from February to last April. The 2nd group include ewes which lambed from October to last December. The flock has been managed under same health program and nutritional condition, all open ewes are placed with breeding rams for all time. Lambs were weaned before day 73 of age, then ewes again are placed with breeding rams to begin the next season. Ten ml of blood sample without EDTA were collected from jugular to obtain serum for assay of prolactin concentration using Prolactin kit, single assay from each ewe 60-73 days after lambing is applied. Ten milliliters of blood were collected from the jugular vein in EDTA coated tubes, blood samples transported to laboratory (Molecular department of Biotechnology Research Center-Al-Nahrian University/Baghdad) for DNA extraction using a commercial (Geneaid Biotech Ltd GMB 100) Geneius TM Micro gDNA Extraction Kit/ Korea, and stored at -20°C. The *MTNR1A*

polymorphism was identified using the PCR-RFLP method as described by Messer. A fragment with the size of 824 bp from exon II of *MTNR1A* gene were amplified with a specific primer: forward: 5'-TGT GTT TGT GGT GAG CCT GG-3', and reverse: 5'-ATG GAG AGG GTT TGC GTT TA-3') pairs. The total volume of PCR reaction was 25 µl by PCR Pre Mix Kit. The amplification reactions was started according to Chu *et al.* (8). The amplification was conducted in ABI 2720 thermal cycler (Applied Bio system, USA) with following temperatures profile consisting of an initial denaturation at 94°C for 5 min, followed by at 40 cycle program with denaturation at 94 °C for 1 min, annealing at 54.3 °C for 1 min, elongation at 72 °C for 1 min and final elongation at 72 °C for 12 min. Agarose gel were stained with ethidium bromide 0.5 mg/ml for 20-30 minutes for 3:38h. DNA band was visualized by electrophoresis and captured by gel documentation system to the observed band. PCR products were separated by electrophoresis on 2% agarose gel in 1X TBE buffer alongside with a 50 bp DNA size marker (iNtRON Biotechology, Inc). Agarose gel were stained with ethidium bromide 0.5 mg/ml for 20-30 minutes. Variation in melatonin receptor 1A gene was examined after enzymatic treatment of resulting amplification with *RsaI (Rhodopseudomonase Sphaeroides 1 serotype) restriction* enzymes (7). The digestion reaction was conducted in 10 µL final volume; at 37°C for 30 min by RasI (NEB R0167S) that position of cleavage site at 5'GTAC3' type blunt cut at for genotyping of studied samples, There are four cleavage sites (53 bp, 267 bp, 23 bp, 411 bp, 70 bp) for *Rsa* I within the amplification fragment the digested fragments were electrophoresed on 2.5% agarose gel and stained with ethidium bromide. There are found three genotypes RR (267 bp/267 bp), Rr (267 bp/290 bp) and rr (290 bp/290 bp) (8). The allelic and genotype frequencies and test of Hardy-Weinberg (HW) equilibrium were done sing POPGENE software, version 1.32.

Results and Discussion

Information of these MTNR1A gene or linked genetic indicators would allow more effective and more intensive selection programs for non-seasonal ewe's reproduction (6). The ability to breeding and lambing out-of-season is a feature controlled by gene and is key for improving profits in the ewes industry, by either accelerated lambing or by increasing the quantity of the flock that lambs out-of-season. While accelerated lambing can improve flock efficiency, both accelerated lambing and breeding a proportion of the flock to lamb out-of-season allows a more continuous supply of new ewes, young and market lambs, this should allow for improved market development and improved prices (9). The result showed that in the local Iraqi ewes (n=60), lambing months were October, November, December, February, March and April, and the number of lambing ewes were 9, 21, 6, 9, 9 and 6 respectively. First three months (October, November and December) suspected ewes lambing out of season while in the other three months (February, March and April) suspected ewes lambing within season, the number of lambing in ewes was ranged between 2.00-5.28. The type of lambing in present study were high a significant ($P<0.01$) in single lamb than twins lamb which were 100 %, 80% and 71.43% in December, October and

November respectively, while 33.34% in all month lambing within season. The sex of lamb were high a significant at P<0.01 in male lamb than female in the different lambing months100 % in the October and December, 87.5% in April, 75% in February and March and 55.56% in November. The weight of lamb in the present study were non-significant in the different lambing months, and the weight of lamb ranged between 3.25-4.00kg (Table 1).

Table (1): Data collected showed lambing month with number of lambing and single or twins, weigh of lamb in Iraqi local ewes

Parameter	Lambing month						Chi-square (χ^2)
	October	November	December	February	March	April	
Number of Ewes	9	21	6	9	9	6	--
Number of lambing/ewe (mean)	3	5.28	2	2	3	2	--
No and Type of lambing (%)	(8) 88.88% single (1) 11.12% twins	(15) 71.43% single (6) 28.57% twins	(6) 100% single -	(7) 77.78% single (2) 22.22% twins	(6) 66.67% single (3) 33.33% twins	(4) 66.67% single (2) 33.33% twins	9.331 **
Weigh of lamb (Kg) (mean)	4	3.785	3.25	3.333	3.166	3.25	--
Esters (Month)	May	Jun	July	September	October	November	--
Light (hrs.)	9.7	10.9	11.9	10.7	7.7	7.2	--
Temp °C Max/Min	37.9/22.3	41.7/25.2	44.0/27.3	32.9/23.2	31.6/17.9	22.8/9.9	--

**** (P<0.01)**

No adjustments to individual lamb weights were made for sex of the lamb, and the data consisted of only single and twin-born lambs. The genetic analysis model included fixed effects of ewe age, lambing year, and litter code, effects of lamb sex and type of rearing, and random direct additive, permanent environment effects (10). To summarize, there is potential to genetically improve total weight of lamb weaned by a ewe without major penalties in other production traits. Fertility in out-of-season mating can also be improved, which could lead to more successful accelerated lambing programs, increased numbers of lambs produced by a ewe over her life time, and improved ewe productivity (10). In the present study, the primers for the exon 2 of ovine *MTNR1A* gene were used for amplification genomic DNA of local Iraqi ewes, the PCR products were separated on 2% agarose gels. The result showed that amplification fragment size 824 bp of all samples was amplified successfully a single band by Ethidium Bromide stained bands showed the Figure (1).

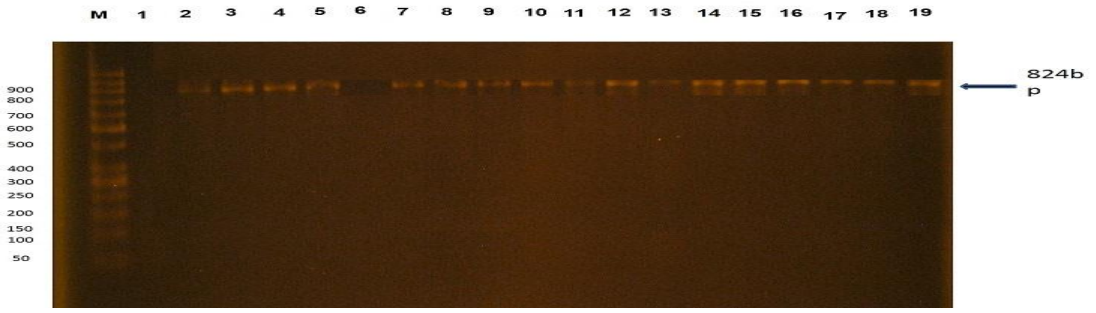

Figure (1): Electrophoresis pattern of PCR product of exon II of *MTNR1A* gene with 824bp size in local Iraqi ewe's. Lane's 1–19 PCR amplification product. 1-6 lanes negative product, M: DNA molecular marker 50 bp size.by Ethidium Bromide stained bands in the 2% agarose gel.

The PCR product was digested with restriction endonucleases Rsa1 and the genetic polymorphisms were investigated by PCR-RFLP. According to the sequence of ovine *MTNR1A* gene in Gen Bank, allelic polymorphism was found with restriction endonuclease Rsa1, which cuts the amplicon to several fragments. There are four cleavage sites (53 bp, 267 bp, 23 bp, 411 bp and 70 bp) for *Rsa* I within the amplification fragment. Digestion with *Rsa*1 yielded polymorphic fragments of 267 bp when the cleavage site was present (allele *R*) and a single 290 bp fragment when the cleavage site was (allele *r*). Three genotypes *RR* (411 bp/267 bp), *Rr* (411/290 bp/267 bp) and *rr* (411 bp/290 bp) according to Chu, et, al (8) were detected in local Iraqi ewes. Three fragments of 23bp, 53 bp and 70bp were been not related allelic polymorphism showed in Figure (2).

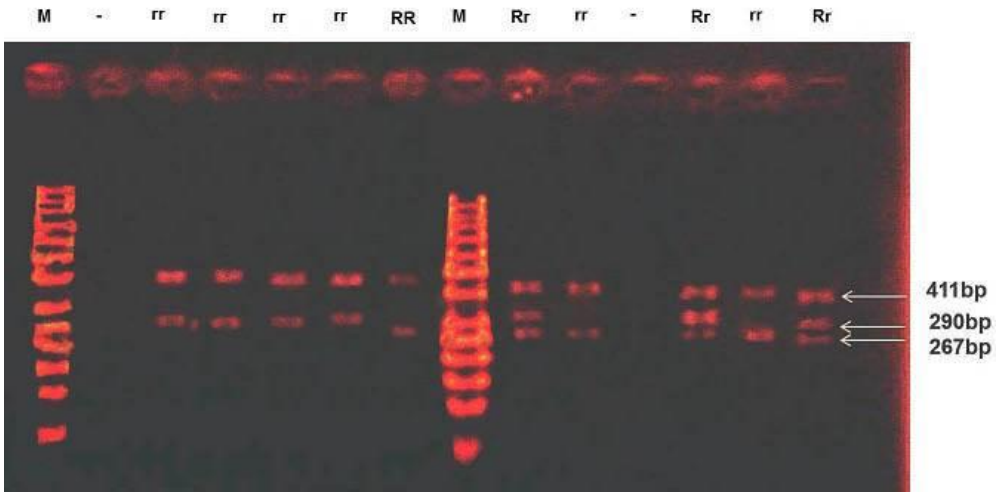

Figure (2): Electrophoresis pattern of PCR product digested with Ras1 restriction enzyme (2% agarose gel) in local Iraqi ewe's s. Lane's 2,3,4,5,8,11 homozygous: rr genotype; Lane's 7,10,12 heterozygous: Rr genotype . Lane's 6 homozygous: RR genotype with. M: DNA molecular marker 50 bp size.by Ethidium Bromide stained bands in the gel.

The recent study showed (Table 2) that the genotype frequency of RR homozygote was 12 (20%), Rr heterozygote in 24 (40%) and of rr homozygote 24 (40%) in Iraqi ewes by digested with restriction endonucleases Rsa1 with a significant at (P<0.01) between the difference frequencies of allele R and r in local Iraqi ewes. The result in Table (3) showed that the allele frequencies were 0.40 and 0.60 for polymorphism of alleles *R* and *r* respectively, in local Iraqi ewes accordance with the Hardy-Weinberg (HW) equilibrium were done sing POPGENE software, version 1.32 . The result was in accord with the findings of (11) in semi-fat talied Sakiz and Thin-tailed Karacabey Merino Turkish sheep Breeds gene frequencies were 0.41 and 0.59 for the *RsaI* polymorphism (alleles *R* and *r*, respectively).also similar to the reported values by (9) in Dorset ewes gene frequencies were 0.35 and 0.65 for the *RsaI* polymorphism genotypic frequencies were 0.44, 0.43, and 0.13 for *rr*, *Rr*, and *RR* genotypes at the *RsaI* polymorphism, also agree with (8) who founded alleles frequencies equal to 0.42 and 0.58 for the *RsaI* polymorphism of the Suffolk sheep in China. Also (12), in Virginia Tech OOS sheep breed in USA obtained data similar to this investigation alleles frequencies (0.34 and 0.66 for the allele polymorphism). (9) showed correlation with RR genotypic to greater spring fertility and an allele R have been hypothesized to be associated with ability of ewes to breeding out of season, some nutritional factors may play a role in improving ovulation and fertility during this period, although ewes are breeding throughout the year in natural photoperiodic conditions of the region. Thus, it has been founded 'R' alleles frequency associated with non-seasonal ewes, needing further investigation, considering the significantly higher frequencies for 'R' alleles (13). Frequencies 'R' alleles were higher significantly than those of the French sheep breeds (14) and Cross-bred Dorset (9).

The sex of lamb were a significantly higher at (P<0.01) in male lamb than female, in the genotype frequency of RR homozygote was 12 male from 12 lambs (100%), Rr heterozygote 15 male from 24 lambs (65%) and of rr homozygote 12 male from 24 lambs (50%), in different lambing month.

Table 2: Distribution of gene polymorphism in male lamb of local Iraqi ewes

Polymorphism	No.	Percentage (%)	Male lamb
RR	12	20.00	12 (100%) **a**
Rr	24	40.00	15 (65%) **b**
rr	24	40.00	12 (50%) **c**
Total	60	100%	39 (65%)
Chi-square value (χ^2)	---	8.250 **	**10.075****
P-value	---	0.00894	0.00765

**** (P<0.01). a significant**

Table 3 Allele frequency of *MTNR1A* gene in local Iraqi ewes

Allele	Frequency
R	0.40
r	0.60
Total	1 (100%)

The present study showed in table (4) the distribution of allele frequency of rr and RR genotype in local Iraqi ewes with no-significant values between the out and the in season lambing months While higher significant variation (P<0.01) of frequencies of R genotype (62.50%) in out-seasonal lambing months than in-seasonal lambing months (37.50%).

Table (4) Distribution of Allele frequency of *MTNR1A* gene in local Iraqi ewes according to season (out or in season)

Season	Total	Genotype distribution				
		RR	Rr	rr	R	r
Out of season	36 (60.00%)	6 (50.00%)	18 (75.00%)	12 (50.00%)	15 (62.50%)	21 (58.33%)
In season	24 (40.00%)	6 (50.00%)	6 (25.00%)	12 (50.00%)	9 (37.50%)	15 (41.67%)
Total	60	12	24	24	24	36
Chi-square value (χ^2)	8.250 **	0.00 NS	12.09 **	0.00 NS	10.43 **	6.82 **
P-value	0.0139	1.000	0.0001	1.000	0.00218	0.0146

**** (P<0.01), NS: Non-significant**

The profiles of prolactin hormone concentration at 60-73 days after local Iraqi ewes lambing were dramatic changes, highest level observed in April while lowest level was in October with high a significant (P<0.01) in prolactin level in difference lambing months, with non-significant differences between October and November, December and February, March and April (Table 5).

Table (5) Relationship between month of lambing and prolactin concentration in local Iraqi ewes

Month of lambing	No. of ewes	Mean ± SE of prolactin conc. (ng/ml)
October	9	169.27 ± 7.48 c
November	21	186.01 ± 28.01 c
December	6	230.98 ± 3.11 b
February	9	231.92 ± 35.17 b
March	9	266.26 ± 15.87 a
April	6	287.33 ± 10.97 a
LSD value	---	32.175 **
P-value	---	0.0149

**** (P<0.01). a significant**

The results obtained for prolactin concentration in the blood of local Iraqi ewes showed in Table (6), the level of prolactin in relation to the genotype differences at the *MTNR1A* locus revealed that the highest hormonal level linked to the RR genotype (234.65±17,48ng/ml), with high significant (P<0.01) heterozygous Rr (217. 02 ± 28.12) and homozygous rr ewes had a little lower values for prolactin hormone linked (182.91 ± 21.35 ng/ml), however, no significant differences founded between lamb birth weights with different genotypes.

Table (6) Relationship between Polymorphism of *MTNR1A* gene with prolactin concentration in local Iraqi ewes and birth weight lambs

Polymorphism	No.	Mean ± SE	
		Prolactin	Birth weight
RR	12	234.65 ± 17.48 **a**	3.38 ± 0.24 **a**
Rr	24	217.02 ± 28.12 **b**	3.50 ± 0.21 **a**
rr	24	182.91 ± 21.35 **c**	3.56 ± 0.14 **a**
LSD value	---	14.677 **	0.601 NS
P-value	---	0.0001	0.762

*** (P<0.01), NS: Non-significant**

The result study agree with (15) in seasonal concentrations of prolactin are paralleled to day length in ewes. The mean concentrations were higher in Mouflon than in Manchega ewes for every month studied, except for the October and November, there were differences between Mouflons and Manchega ewes in the timing of the increase (January vs. March) and attainment of basal levels (October vs. September). Mean levels were significantly(P<0.01) affected by season. Seasonal changes in the prolactin concentrations occur in ewes, with the highest concentrations in the summer and lowest in the winter season, prolactin hormone acts on many target tissues in addition to the mammary gland, and there is evidence indicating a functional role for the seasonal changes in prolactin hormone in the control of gonadotrophin secretion, gonadal activity and sexual behavior (15**).** (16) It has been showed that heat stress may increase prolactin secretion, therefore breeds may differ in the response of prolactin to high ambient temperature. This observation supports previous studies demonstrating seasonal changes in prolactin secretion in lactating and non-lactating ewes, in all these differences in prolactin secretion post lambing could be accounted for by an effect of day length (16). The seasonal changes in prolactin concentration were showed dramatic seasonal changes with the highest levels observed around the summer solstice and the lowest ones around the winter solstice in the Île-de-France sheep breed, the two allelic forms of the *MT1* receptor gene have no direct effect on the seasonal design of several seasonal functions: ovulatory activity, prolactin secretion, follicle activity and melatonin secretion. The influence of this polymorphism in the regulation of seasonal function but may be dependent upon the breed and/or environmental conditions (17). Johnston (18) has been reported that the pituitary melatonin receptor expression exposed a lack of melatonin receptors on lactotroph cells, suggesting an indirect mechanism of melatonin on prolactin

secretion. The importance of the *Pars Tuberalis* PT in the seasonal regulation of prolactin secretion was first demonstrated by the detail that *Pars Tuberalis* PT-conditioned medium stimulates prolactin secretion from pituitary pars distalis cell (19, 20). It was therefore hypothesized that the photoperiodic melatonin signal regulates the release of a prolactin secretagogue, termed tuberalin, from the PT. Later sign indicated that tuberalin secretion is dependent upon photoperiod (21) and regulate seasonal timing mechanisms (22, 23). In spite of evidence for the being of tuberalin, attempts to identify it have not been successful (24, 25).

Generally the non-lactating ewes with increasing photoperiod, day length the level of prolactin concentration increases, while the melatonin concentration declines (26, 27). To the different, during the autumn-winter period, the rise in melatonin concentration contributes to the suppression of prolactin secretion (28; 29). This finding is in agreement with (30) on early postpartum, lactating ewes showed that during increasing photoperiod (day length), the basal concentration of prolactin secretion increased as the stage of lactation progressed. We showed that prolactin secretion increased in ewes rearing lambs from March (spring). However, the treatment of ewes with exogenous melatonin caused the plasma prolactin concentration to decrease. Thus, although the observations during early lactation, plasma prolactin secretion in melatonin-implanted sheep was strongly dependent on the melatonin signal at short photoperiod. In sheep, lambing during the period of shortening daylight length, by usage melatonin implants was less effective in the case of the prolactin secretion. In sheep plasma prolactin concentration reduced gradually. In prolactin secretion concentration increasing in ewes rearing their lambs during periods and decreasing photoperiod, it became apparent that in November-lambing (autumn) ewes, the plasma prolactin concentration during the autumn lambing was over 50% lower compared with lambing in March (spring) (30). This shows that a lengthening melatonin signal contributes to a decline in prolactin secretion, in lactating ewes like to that founded in non-lactating animals. A previous study showed that in lactating ewes under decreasing photoperiod, it is extremely difficult to maintain the plasma prolactin at an adequately high enough level to guarantee milk production, similar to that in winter lambing sheep (31). It has been shown that exposure of ewes to increased artificially photoperiod (16L: 8D), failed to maintain high prolactin concentrations and lactation during decreasing photoperiod. The decline in prolactin secretion was probably dictated by a development of the refractoriness to a continuous, little melatonin signal (32). This suggests that milk production in sheep is highly related to the interaction between melatonin and prolactin.

References

1- Vivien-Roels, B., Pévet, P. (1983). The pineal gland and the synchronization of reproductive cycles with variations of the environmental climatic conditions, with special reference to temperature. Pineal Res. Rev., 1: 91–143.

2- Barrett, P., Conway, S., Jockers, R., Strosberg, A.D., Guardiola-Lemaitre, B., Delagrange, P. and Morgan, P.J. (1997). Cloning and functional analysis of a polymorphic variant of the ovine Mel 1a melatonin receptor. Biochim. Biophys. Acta, 1356 (3): 299–307.

3- Reppert, S.M., Weaver, D.R. and Ebisawa, T. (1994). Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron 13 (5):1177–1185.

4- Dardente, H. (2007). Does a melatonin-dependent circadian oscillator in the Pars Tuberalis drive Prolactin seasonal rhythmicity? J. Neuroendocrinol, 19: 657–666.

5- Dupré, S.M., Burt, D.W., Talbot, R., Downing, A., Mouzaki, D., Waddington, D., Malpaux, B., Davis, J.R., Lincoln, G.A. and Loudon, A.S. (2008). Identification of melatonin-regulated genes in the ovine pituitary pars tuberalis, a target site for

seasonal hormone control. Endocrinology, 149: 527–539.

6- Carcangiu, V., Mura, M.C., Vacca, G.M., Pazzola, M., Dettori, M.L., Luridiana, S. and Bini, P.P. (2009). Polymorphism of the melatonin receptor *MT1* gene and its relationship with sesonal reproductive activity in the Sarda sheep breed. Animal Reproduction Science, 116: 65-72.

7- Messer, L.A., Wang, L., Tuggle, C.K., Yerle, M., Chardon, P., Pomp, D., Womack, J.E., Barendse, W., Crawford, A.M., Notter, D.R. and Rothschild, M.F. (1997). Mapping of the melatonin receptor la (*MTNR1A*) gene in pigs, sheep, and cattle. Mammalian Genome, 8: 368-370.

8- Chu, M.X., Cheng, D.X., Liu, W.Z., Fang, L. and Ye, S.C. (2006). Association between melatonin receptor 1A gene and expression of reproductive seasonality in sheep. Asian-Australasian Journal of Animal Science, 19: 1079–1084.

9- Mateescu, R.G., Lunsford, A.K. and Thonney, M.L. (2009). Association between melatonin receptor 1A gene polymorphism and reproductive performance in Dorset ewes. Journal of Animal Science, 87: 2485-2488.

10- Vanimisetti, H.B. (2006). Genetic evaluation of ewe productivity and its component traits in Katahdin and Polypay sheep. Doctor of Philosophy in Animal and Poultry Sciences, Virginia Polytechnic Institute and State University.

11- Elmaci, C., Sahin, S. and Oner, Y. (2013). Distribution of Different Alleles of Aromatase Cytochrome P450 *(CYP19)* and Melatonin Receptor 1A *(MTRN1A)* Genes among Native Turkish Sheep Breeds. *Kafkas Univ Vet Fak Derg*, 19 (6): 929-933.

12- Notter, D.R. and Cockett, N.E. (2005). Opportunities for detection and use of QTL influencing seasonal reproduction in sheep: a review. Genetics Selection Evolution, 37: S39-S53.

13- Saxena,V. K. , Jha, B. K. , Meena, A. S. and Naqvi S.M.K. (2014) Sequence analysis and identification of new variations in the coding sequence of melatonin receptor gene (MTNR1A) of Indian Chokla sheep breed.. Meta Gene, 2: 450–458.

14- Pelletier, J., Bodin, L., Hanocq, E., Malpaux, B., Teyssier, J., Thimonier, J. and Chemineau, P. (2000). Association between expression of reproductive seasonality and alleles of the gene Mel1a receptor in the ewe. Biology of Reproduction, 62: 1096–1101.

15- Moreno, J.S. Sebastian, A. L.,Bulnes, A.G. ,Brunet, A.G. and Chemineau ,P. (2000). Seasonal changes in ovulatory activity, plasma prolactin, and melatonin concentrations, in Mouon (Ovis gmelini musimon) and Manchega (Ovis aries) ewes. Reprod. Nutr. Dev., 40: 421–430.

16- Rhind, S. M Robinson, J. J. Chesworth J. M. and Crofts R. M. J. (1980) Effects of season, lactation and plane of nutrition on prolactin concentrations in ovine plasma and the role of prolactin in the control of ewe fertility.J.Reprod. Fert. , 58: 145-152.

17- Hernandez, X., Bodin, L., Chesneau, D., Guillaume, D., Allain, D., Chemineau, P., Malpaux, B. and Migaud, M. (2005). Relationship between MT1 melatonin receptor gene polymorphism and seasonal physiological responses in Ile-de-France ewes. Reproduction Nutrition Development, 45: 151–162.

18- Johnston, J.D. and Debra J SkeneD.J. (2015). Regulation of mammalian neuroendocrine physiology and rhythms by melatonin. Melatonin and neuroendocrine physiology Society for Endocrinology Printed in Great Britain Published by Bioscientifica Ltd.

19- Hazlerigg DG, Hastings MH & Morgan PJ. (1996). Production of a prolactin releasing factor by the ovine pars tuberalis. Journal of Neuroendocrinology, 8: 489–492.

20- Morgan PJ, Webster CA, Mercer JG, Ross AW, Hazlerigg DG, MacLean A and Barrett P. (1996). The ovine pars tuberalis secretes a factor(s) that regulates gene expression in both lactotropic and nonlactotropic pituitary cells. Endocrinology, 137: 4018–4026.

21- Stirland JA, Johnston JD, Cagampang FR, Morgan PJ, Castro MG, White MR Davis JR and Loudon AS. (2001). Photoperiodic regulation of prolactin gene expression in the Syrian hamster by a pars tuberalis-derived factor. Journal of Neuroendocrinology, 13: 147–157.

22- Johnston JD, Cagampang FR, Stirland JA, Carr AJ, White MR, Davis JR and Loudon AS. (2003). Evidence for an endogenous per1- and ICER independent seasonal timer in the hamster pituitary gland. FASEB Journal, 17: 810–815.

23- Lincoln GA, Johnston JD, Andersson H, Wagner G and Hazlerigg DG. (2005) Photorefractoriness in mammals: dissociating a seasonal timer from the

circadian-based photoperiod response. Endocrinology, 146: 3782–3790.
24- Johnston JD. (2004). Photoperiodic regulation of prolactin secretion: changes in intra-pituitary signalling and lactotroph heterogeneity. Journal of Endocrinology, 180: 351–356.
25- Dupre SM. (2011). Encoding and decoding photoperiod in the mammalian pars tuberalis. Neuroendocrinology, 94: 101–112.
26- Misztal, T., Romanowicz, K. and Barcikowski, B., (1999). The role of melatonin in prolactin secretion in sheep. Post. Biol. Kom., 26: 117–123.
27- Zieba, D., Szcz¸esna, M., Klocek-Gorka, B., Molik, E., Misztal, T., Williams, G.L., Romanowicz, K., Stepien, E., Keisler, D.H. and Murawski, M. (2008). Seasonal effects of central leptin infusion on melatonin and prolactin secretion and on SOCS-3 gene expression in ewes. J. Endocrinol., 198: 147–155.
28- Morgan, P.J. (2000). The pars tuberalis: the missing link in the photoperiodic regulation of prolactin secretion. J. Neuroendocrinol, 12: 287–295.
29- Morgan, P.J. and Hazlerigg, D.G. (2008). Photoperiodic signaling trough the melatonin receptor turns full circle. J. Neuroendocrinol, 20: 820–826.
30- Molik, E., Misztal, T., Romanowicz, K. and Zieba, D. (2010). The effects of melatonin on prolactin and growth hormone secretion in ewes under different photoperiods, during the early postpartum Period. Small Ruminant Research, 94: 137–141.
31- Molik, E., Misztal, T., Romanowicz, K. and Wierzchos, E. (2007). Dependence of the lactation duration and efficiency on the season of lambing in relation to the prolactin and melatonin secretion in ewes. Livest. Sci., 107: 220–226.
32- Lincoln, G.A., Andersson, H. and Clarke, I.J. (2003). Prolactin cycles in sheep under constant photoperiod: evidence that photo refractoriness develops within the pituitary gland independently of the prolactin output signal. Biol. Reprod., 69: 1416–1423.