Kafkas Univ Vet Fak Derg
19 (6): 929-933, 2013
DOI: 10.9775/kvfd.2013.8900

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Distribution of Different Alleles of Aromatase Cytochrome P450 *(CYP19)* and Melatonin Receptor 1A *(MTRN1A)* Genes among Native Turkish Sheep Breeds [1]

Cengiz ELMACI [1] Şule ŞAHİN [1] Yasemin ÖNER [1]

[1] *This study was financially supported by the Scientific Research Project Council of Uludag University (Project number: HDP(Z)-2011/27)*

[1] Uludag University, Faculty of Agriculture, Department of Animal Science, TR-16059 Bursa - TÜRKİYE

Makale Kodu (Article Code): KVFD-2013-8900

Summary

In this study, 257 animals from eight native Turkish sheep breeds (Akkaraman, Dağlıç, Gökçeada, İvesi, Karacabey Merino, Karayaka, Kıvırcık, Sakız) were investigated for three SNPs located at *CYP19* and *MTRN1A* genes which are important for reproductive traits. The A→G transition within *CYP19*, the C→T and G→A transitions within *MTRN1A* were investigated by using PCR-RFLP method. To analyze *CYP19* locus *DraI*, for *MTRN1A* locus *MnlI* and *RsaI* restriction enzymes were used. Two alleles were found for all the loci investigated. These alleles named as A and G for *CYP19*, M and m for *MTRN1A-MnlI,* and R and r for *MTRN1A-RsaI*. A and m alleles were predominant at *CYP19* and *MTRN1A-MnlI* loci, respectively. While R allel was prevalent in majority of breeds investigated, r allele was predominant in Sakız, Karacabey Merino, and Karayaka breeds for *MTRN1A-RsaI.* UPGMA test results show that the allele frequencies distributions of these loci can be used for calculating genetic distance between breeds.

Keywords: *Sheep, Reproduction, Polymorphism, CYP19, MTRN1A, Genetic distance*

Aromatase Cytochrome P450 *(CYP19)* ve Melatonin Receptor 1A *(MTRN1A)* Genlerinin Farklı Allelerinin Türkiye Yerli Koyun Irkları Arasındaki Dağılımı

Özet

Bu çalışmada Türkiye yerli koyun ırklarından (Akkaraman, Dağlıç, Gökçeada, İvesi, Karacabey Merinosu, Karayaka, Kıvırcık, Sakız) 257 hayvan üreme özellikleri açısından önem taşıyan *CYP19* ve *MTRN1A* genlerinde bulunan üç adet TNP bakımdan incelenmiştir. *CYP19* lokusunda bulunan A→G transisyonu ile *MTRN1A* lokusunda bulunan C→T ve G→A transisyonları PCR-RFLP metodu kullanılarak incelenmiştir. *CYP19* lokusunu analiz etmek için *DraI, MTRN1A* lokusunu analiz etmek için *MnlI* ve *RsaI* restriksiyon enzimleri kullanılmıştır. İncelenen tüm lokuslar için iki allel bulunmuştur. Bu alleler *CYP19* lokusu için A ve G *MTRN1A-MnlI* lokusu için M ve m, *MTRN1A-RsaI* lokusu için R ve r olarak isimlendirilmiştir. *CYP19* ve *MTRN1A-MnlI* lokusları için sırasıyla A ve m alleleri predominanttır. *MTRN1A-RsaI* lokusu bakımdan incelenen ırkların çoğunda R alleli yaygın iken, Sakız, Karacabey Merinosu ve Karayaka ırklarında r alleli predominanttır. UPGMA test sonuçları bu lokusların allel frekans dağılımlarının ırklar arasındaki genetik mesafenin hesaplanmasında kullanılabileceğini göstermiştir.

Anahtar sözcükler: *Koyun, Üreme, Polimorfizm, CYP19, MTRN1A, Genetik mesafe*

INTRODUCTION

Reproduction is an economically important complex trait influenced by many environmental and genetic components. Classical selection methods for complex composite traits such as reproduction do not result in conclusive genetic improvement. In this sense a gene has major affect can be used for selection criteria for this kind of complex traits to obtain more rapid genetic improvement. Reproductive traits are affected by many genes but a limited

İletişim (Correspondence)
+90 224 2941562
yaseminoner@yahoo.com

number of major genes associated with reproductive traits have been reported in sheep [1-4]. Aromatase cytochrome p450 (*CYP19*) and melatonin receptor 1a *(MTRN1A)* genes are two of the candidates genes for estrogen biosynthesis and seasonality, respectively. While aromatase cytochrome p450 (*CYP19*) is located in chromosome 7, melatonin receptor 1a *(MTRN1A)* is located chromosome 26 [5,6].

Understanding of genetic mechanism of seasonal reproduction in sheep is very important because this seasonal variation in fertility is an important factor limiting efficiency of sheep production. The photoperiod drives their reproductive cycle, which comprises a season of high sexual activity during short days and an anestrous season that occurs during long days. Melatonin is called the "hormone of darkness," because it is released by the pineal gland during the night. The pattern of secretion of melatonin provides information that is apparently 'read' by cells within the brain that have the appropriate receptors and controls reproductive function [7,8]. In mammals, two high-affinity melatonin receptor subtypes have been cloned and characterized, namely the MTNR1A and MTNR1B receptors, also known as the MT1 and MT2 receptors, respectively [9,10]. The MT1 receptor seems to be the only one involved in the regulation of reproductive activity [11,12]. Association studies carried out in the MTNR1A reveal different interesting results for estrus and fertility in the spring [13-15].

During late maturity, the ovaries are in a state of true anestrum. One of the predominant causes of true anestrum is a low level of ovarian estrogens. The aromatase cytochrome P450 enzyme plays an important role in estrogen biosynthesis by conversion of androgens to estrogens, and due to its critical function it is an important hormone both in the controlling of reproduction for male and female [16-18] and of the other traits like fat deposition and growth [19,20]. It maps to bands q24-q31 of the ovine chromosome 7 [5]. In sheep *Cyp19*, there are four different promoter regions (P1.1, P1.4, P1.5 and P2) that show organ-specific activities. As P2 is mainly active in ovarian granulosa cells [21] mutations occurred in these mentioned regions may be significant for obtaining valuable knowledge on reproductive traits. In a previous study some important relationships were determined between the C242T transition and some reproductive traits as litter weight, lambing interval, lambing age, reproductive performance, and maternal ability in Brazilian sheep breeds [22].

There are few studies carried out to light up reproductive characteristic of Turkish native sheep breeds up to present. Due to limited studies the native sheep breeds remain unperceived for reproductive characteristics.

In this study presence and the allelic frequency of A→G transition within P2 at position 113 of *CYP19*, C→T and G→A transitions within exon 2 at position 606 and 612 of *MTRN1A*, respectively were investigated in eight sheep breeds from Turkey. Mutations occurred such loci may be useful tolls as molecular markers in the selection programs. It must be put in mind that to reveal function of these kinds of mutations and to use them in animal production systems it should be understood genetic structure of populations for these loci. Hence understanding of genetic structure of Turkish native sheep breeds for these kinds of loci will contribute to establish organized selection, breeding and conservation programs.

MATERIAL and METHODS

Blood samples used for DNA isolation were collected from 257 animals from both sexes belonging to eight native Turkish sheep breeds and one crossbred population well adapted to local conditions. Total eight sheep breeds; Akkaraman (akk, n=28), Dağlıç (dgl, n=28), Gökçeada (gda, n=28), İvesi (ivs, n= 33), Karacabey Merino (mrns, n=37), Karayaka (kry, n=25), Kıvırcık (kvrck, n=39), Sakız (skz, n=39) were sampled from private farms, conservation farms and research institutes of government at different regions of Turkey.

Total DNA was extracted using a genomic DNA purification kit (K0512, Fermentas, Lithuania) according to the manual instructions. Spectrophotometric methods were used to determine DNA quality and quantity. PCR conditions were carried out according to Zsolnai et al.[23] and Martinez-Royo [4]. Primers and restriction enzymes used for PCR amplifications are given in *Table 1*. The restriction fragments were directly analyzed by electrophoresis in 2% and 2.5% agarose gels in 1× TAE buffer, stained with ethidium bromide, and visualized under UV light.

Direct counting was used to estimate phenotype and allele frequencies of the genetic variants for all loci. The chi-square test ($\chi 2$) was used to check whether the populations were in Hardy-Weinberg equilibrium. All calculations and the $\chi 2$ analyses were carried out using PopGene32 software [24]. Genetic relationships among populations were visualized in a dendrogram constructed by unweighted paired group cluster analysis (UPGMA), from a modified NEIGHBOR procedure implemented in PHYLIP version 3.5 software also using PopGene32. The UPGMA dendrogram of population was constructed based on Nei's genetic distance [25].

Table 1. *Primers sequences and restriction enzymes used in the study*
Tablo 1. *Çalışmada kullanılan primer dizileri ve restriksiyon enzimleri*

Locus	Primers (5′ ⟶ 3′)	R.E	Reference
CYP19-P2	ACAATGGGAGGCTCTGAGAATG GAAAAATTAGAAAATCCCCAAAA	*DraI*	[23]
MNRTA1	TCCCTCTGCTACGTGTTCCT GTTTGTTGTCCGGTTTCACC	*RsaI* *MnlI*	[4]

RESULTS

Two alleles and three genotypes were detected in overall breeds investigated for three loci. In order to genoyping *MTRN1A-Mnl*I and *MTRN1A-Rsa*I the nomenclature of Martinez-Royo was used [4]. To determine SNP 612 in *MTRN1A*, 237 bp PCR products were digested with *Mnl*I restriction endonuclease. M homozygote individuals have 237 and 13 bp fragments, the mm homozygotes have 170 bp, 67 bp, and 13 bp and the heterozygotes have four bands as 237 bp, 170 bp, 67 bp, and 13 bp (the 13 bp could not seen in the gels). The *RsaI* restriction enzyme was used to determinate SNP606 in the same PCR products of *MTRN1A*. RR genotype has 148 bp and 79 bp, and 23 bp (the 23 bp could not seen in the gels), rr genotype has 148 bp and 102 bp, and the heterozygote has all three bands 148 bp, 79 bp, and 102 bp. while the restriction enzyme site absent the allele is named as "r".

To 517 bp PCR fragment was digested by using *Dra*I to analyze CYP 19 P2 region. The G homozygote individuals have only 517 bp uncut fragment. Homozygotes A have 401 bp and 116 bp fragments and the heterozygotes have 517 bp, 401 bp and 116 bp fragments. The fragments obtained from agarose gel electrophoresis are given in the *Fig. 1*.

While in the *CYP19* and *MTRN1A-Mnl*I loci A and m alleles were predominant, respectively, allelic frequencies vary among breeds for *MTRN1A-Rsa*I alleles as given in *Table 2*.

Samples from eight sheep breeds were found to be in Hardy-Weinberg equilibrium at three loci investigated, except Dağlıç and Karayaka sheep breeds. Deviations from the HWE were significant at *CYP19* in Dağlıç breed ($P<0.01$) and *MTRN1A-Mnl*I in Karayaka breed ($P<0.05$).

UPGMA dendrogram obtained revealed two main genetic groups of breeds investigated *(Fig. 2)*.

a)CYP19-*Dra*I b) MNTR1A-*Mn*II c) MNTR1-*Rsa*I

Fig 1. Diagnostic PCR-RFLP fragments of loci investigated (a, b, c)

Şekil 1. İncelenen lokuslara ait PCR-RFLP parçalarının tanımlanması (a, b, c)

Table 2. *Distribution of allelic frequencies among the breeds investigated*
Tablo 2. *Allelik frekansların incelenen ırklar arasındaki dağılımı*

LOCUS	Alleles	BREEDS															
		Fat-tailed						Semi-fat tailed		Thin-tailed							
		ivs	χ^2	dgl	χ^2	akk	χ^2	skz	χ^2	mrns	χ^2	kvrck	χ^2	kry	χ^2	gda	χ^2
Cyp19	A	1	--	0.9286	8.14**	1		0.8462	1.17	0.8378	1.26	0.6795	2.00	0.9600	0.02	0.9286	0.12
	G	-		0.0714		-		0.1538		0.1622		0.3205		0.0400		0.0714	
MT1-*MnlI*	M	0.2576	0.67	0.1071	2.35	0.3214	0.46	0.2308	3.28	0.3108	1.17	0.2564	1.68	0.3200	5.13*	0.4821	0.08
	m	0.7424		0.8929		0.6786		0.7692		0.6892		0.7436		0.6800		0.5179	
MT1-*RsaI*	R	0.7273	2.12	0.6071	0.03	0.6429	1.63	0.4103	0.14	0.4189	0.06	0.5256	0.17	0.2400	3.40	0.7857	1.88
	r	0.2727		0.3929		0.3571		0.5897		0.5811		0.4744		0.7600		0.2143	

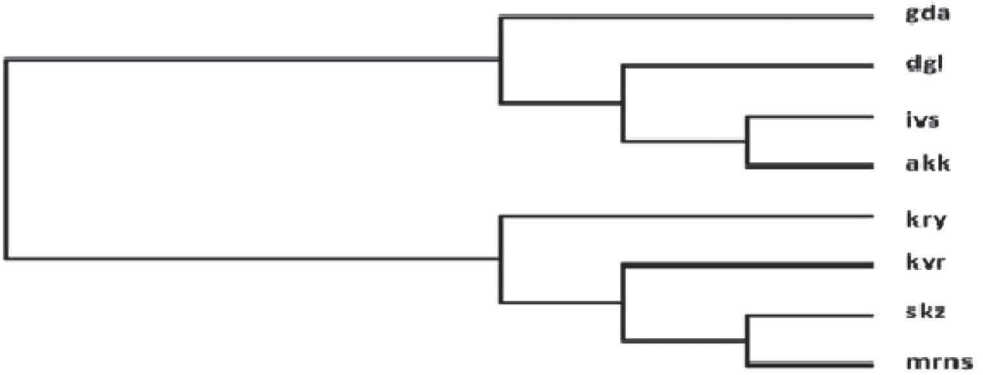

Fig 2. Genetic distance between populations investigated. Dendrogram based on Nei's [25] genetic distance (PopGene program UPGMA PHYLIP Version 3.5, modified NEIGHBOR method)

Şekil 2. İncelenen populasyonlar arasındaki genetik mesafe. Nei'nin [25] genetic mesafesine dayanan UPGMA dendrogramı (PopGene programı UPGMA PHYLIP Version 3.5, adapte edilmiş NEIGHBOR metodu)

Table 3. *Observed and Expected heterozygosity values for all loci in investigated breeds*
Tablo 3. *İncelenen ırklarda tüm lokuslar için gözlenen ve beklenen heterozigotluk değerleri*

LOCUS	BREEDS															
	Fat-tailed						Semi-fat tailed		Thin-tailed							
	ivs		dgl		akk		skz		mrns		kvrck		kry		gda	
	Obs-Het	Exp-Het	Obs-Het	Exp-Het	Obs-Het	Exp-Het	Obs-Het	Exp-Het	Obs-Het	Exp-Het	Obs-Het	Exp-Het	Obs-Het	Exp-Het	Obs-Het	Exp-Het
Cyp19	-	-	0.0714	0.1351	-	-	0.3077	0.2637	0.3243	0.2755	0.5385	0.4412	0.0800	0.0784	0.1429	0.1351
MT1-*MnlI*	0.3333	0.3883	0.1429	0.1948	0.5000	0.4442	0.4615	0.3596	0.4054	0.4343	0.3077	0.3863	0.6400	0.4441	0.5357	0.5084
MT1-*RsaI*	0.3030	0.4028	0.5000	0.4857	0.3571	0.4675	0.4615	0.4902	0.5135	0.4935	0.5385	0.5052	0.2400	0.3722	0.4286	0.3429

Observed heterozygosity for *CYP19* was the highest in Kıvırcık sheep breed and the lowest in Dağlıç sheep breed. Observed heterozygosity for *MTRN1A-Mnl*I was the highest in Karayaka sheep breed and the lowest in Dağlıç sheep breed. For the *MTRN1A-Rsa*I locus the higher value was observed in Kıvırcık breed and the lowest value was observed in Karayaka sheep breed *(Table 3)*.

DISCUSSION

In the present study while Native Turkish sheep breeds were investigated for *CYP19* P2 polymorphism for the first time, Sakız, Akkaraman and İvesi sheep breeds investigated for *MTRN1A Mnl*I and *Rsa*I polymorphisms by Seker et al.[26]. The rest of the breeds were analyzed for the first time for *MTRN1A Mnl*I and *Rsa*I polymorphisms, as well.

The A→G transition within P2 of *CYP19* has been reported in for the first time and the authors analyzed total seven breeds from Spain and Hungaria [23]. For all the breeds investigated, the A allele was found as predominant in the present study. While in the present study the frequencies of A allele were found between 1 and 0.3205, Zsolnai et al.[23] found the frequencies of A allele between 1 and 0.750. In the other study for determinate *CYP19* P2 polymorphism in Tiberian sheep population the *CYP19* P2 locus was found to be monomorphic [27]. The frequencies found for *CYP19* P2 locus were concordance with these previous studies [23,27].

Both *MTRN1A Mnl*I and *Rsa*I loci were found as polymorphic and frequencies for the alleles found were given in *Table 2*. While in the *MTRN1A-Mnl*I locus m alleles were predominat, allelic frequencies vary among breeds for *MTRN1A-Rsa*I alleles. The frequencies of *MTRN1A Mnl*I alleles obtained in the present study were similar to the frequencies reported by Notter et al.[13] and Kaczor et al.[28], but were disagreement with many reports for *MTRN1A Mnl*I polymorphism [4,14,26,29]. In the study carried out on Native Turkish sheep breed the M allele was also found predominant in the Sakız, Akkaraman and İvesi sheep breeds, inconsistency with the present study. On the other hand there is also some discordance between the allele's frequencies for *MTRN1A Rsa*I locus. In the present study frequency of r allele vary from 0.2143 to 0.7600. The results obtained by the other authors also vary breed to breed. For example while in the Columbia and Dorset sheep breeds the r allele frequencies were found 0.97 and 0.65, respectively [13,30] in the Sarda and Tisdale sheep breeds it found as 0.34 and 0.24, respectively [15,29]. These discordances can be explained in the differences in sampling strategies or nomenclature.

Interestingly the dendrogram obtained from analysis of the three loci is quite logical. While all of the majority of thin tail breeds are grouped together except Gökçeada sheep breed, the fat tail breeds are grouped together *(Fig. 2)*. This finding can be accepted as an evidence for utilities of these loci to breed separation.

Majority of breeds investigated in this study have been examined for *MTRN1A- MnlI* and *Rsa*I loci for the first time and for the *CYP19* P2 all of the breeds have been examined for the first time. Results of this study demonstrate that native Turkish sheep breeds have a high level of genetic variation in these three loci investigated. To provide better understanding of function of these mutations it should be needed further analysis to determinate their affects on productive traits by using phenotypic data. Further analysis at expression level it should be also carried out. Number of studies should be increased by using more animals from each breed to be clear the discordance between the allele distributions in *MTRN1A Rsa*I locus to reveal genetic structure of Turkish native sheep breeds for this locus.

REFERENCES

1. Piper LR, Bindon BM: Genetic segregation for fecundity in Booroola Merino sheep. **In,** Barton RA, Robinson DW (Eds): Genetics of Reproduction in Sheep. 159-168. Butterworths, London, 1982.

2. Bradford GE, Quirke JF, Sitorus P, Inounu I, Tiesnamurti B, Bell FL, Fletcher IC, Torell DT: Reproduction in Javanese sheep: Evidence for a gene with large effect on ovulation rate and litter size. *J Anim Sci*, 63, 418-443, 1986.

3. Davis GH, McEwan JC, Fennessy PF, Dodds KG, Farquar PA: Evidence for the presence of a major gene influencing ovulation rate on the X chromosome of sheep. *Biol Reprod*, 44, 620-624, 1991.

4. Martinez-Royo A: Estudio de genes con influencia en prolificidad y estacionalidad reproductiva en la Raza Aragonesa. *PhD Thesis,* Lleida Univ, Spain, 2011.

5. Goldammer T, Brunner RM, Vanselow J, Zsolnai A, Fürbass R, Schwerin M: Assignment of the bovine aromatase encoding gene CYP19 to 10q26 in goat and 7q24-q31 in sheep. *Cytogenet Cell Genet*, 85, 258-259, 1999.

6. Messer LA, Wang L, Tuggle CK, Yerle M, Chardon P, Pomp D, Womack JE, Barendse W, Crawford AM, Notter DR, Rothschild MF: Mapping of the melatonin receptor 1a (MTNR1A) gene in pigs, sheep and cattle. *Mamm Genome*, 8, 368-370, 1997.

7. Barrett P, Conway S, Jockers R, Strosberg AD, Guardiola-Lemaitre B, Delagrange P, Morgan PJ: Cloning and functional analysis of a polymorphic variant of the ovine Mel 1a melatonin receptor. *BBA*, 1356, 299-307, 1997.

8. Arendt J: Melatonin and the pineal gland: Influence on mammalian seasonal and circadian physiology. *Rev Reprod*, 3, 13-22, 1998.

9. Reppert SM, Weaver DR, Ebisawa T: Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. *Neuron*, 13, 1177-1185, 1994.

10. Reppert SM, Godson C, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF: Molecular characterization of a second melatonin receptor expressed in human retina and brain: The Mel1bmelatonin receptor. *P Natl Acad Sci USA*, 92, 8734-8738, 1995.

11. Dubocovich ML: Pharmacology and function of melatonin receptors. *The FASEB J*, 2, 2765-2773, 1988.

12. Weaver DR, Liu C, Reppert SM: Nature's knock-out: The Mel1b receptor is not necessary for reproductive and circadian responses to melatonin in Siberian hamsters. *Mol Endocrinol*, 10, 1478-1487, 1996.

13. Notter DR, Cockett NE, Hadfied TS: Evaluation of melatonin receptor 1a as candidate gene influencing reproduction in an autumn-lambing sheep flock. *J Anim Sci*, 81, 912-917, 2003.

14. Chu MX Ji CL, Chen GH: Association between PCR-RFLP of melatonin receptor 1a gene and high prolificacy in Small Tail Han sheep. *Asian Austral J Anim*, 16 (12): 1701-1704, 2003.

15. Carcangiu V, Mura MC, Vacca GM, Pazzola M, Dettori ML, Luridiana S, Bini PP: Polymorphism of the melatonin receptor MT1 gene and its relationship with seasonal reproductive activity in the Sarda sheep breed. *Anim Reprod Sci*, 116 (1-2): 65-72, 2009.

16. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito YJ, Fisher CR, Michael MD, Mendelson CR, Bulun SE: Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev*, 15, 342-355, 1994.

17. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS: Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *P Natl Acad Sci USA*, 97, 12729-12734, 2000.

18. Jones ME, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao S, Simpson ER: Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *P Natl Acad Sci USA*, 97, 12735-12740, 2000.

19. Simpson E, Rubin G, Clyne C, Robertson K, O'Donnell L, Jones M, Davis S: The role of local estrogen biosynthesis in males and females. *Trends Endocrin Met*, 11, 184-188, 2000.

20. Misztal T, Romanowicz K, Barcikowski B: Melatonin - A modulator of the GnRH/LH axis in sheep. *Reprod Biol*, 2 (3): 267-275, 2002.

21. Vanselow J, Fürbass R, Zsolnai A, Kalbe C, Said HM, Schwerin M: Expression of the aromatase cytochrome P450 encoding gene in cattle and sheep. *J Steroid Biochem Mol Biol*, 79, 279-288, 2001.

22. Lôbo AMBO, Lôbo RNB, Paiva SR: Aromatase gene and its effects on growth, reproductive and maternal ability traits in a multibreed sheep population from Brazil. *Genet Mol Biol*, 32 (3): 484-490, 2009.

23. Zsolnai A, Anton I, Fesus L, Estonba A, Schwerin M, Vanselow J: Allele distributions of two novel SNPs within the sheep *Cyp19* gene. *J Anim Breed Genet*, 119, 402-405, 2002.

24. Yeh F, Yang RC, Boyle T: Popgene (v.1.32), Microsoft Windows-based freeware for Population Genetic analysis. 2000. http://www.ualberta.ca/*fyeh/Pop32.exe, *Accessed:* 03.01.2012.

25. Nei M: Genetic distance between populations. *Am Nat*, 106, 283-293, 1972.

26. Seker I, Ozmen O, Cinar Kul B, Ertugrul O: Polymorphism in melatonin receptor 1A (MTRN1A) gene in Chios, White Karaman and Awassi sheep breeds. *Kafkas Univ Vet Fak Derg*, 17 (5): 865-868, 2011.

27. Wang J, Wang Y, Zheng Y, Xu Y, Hu Y, Liu L, Tao Y: Polymorphism of the intron 9 and promoter 2 of Cyp19 gene in Tibetan Sheep. *Journal of Mianyang Normal University*, 2009. http://en.cnki.com.cn/Article_en/CJFDTOTAL-MYSF200905018.htm, *Accessed:* 10.05.2011.

28. Kaczor U, Kmiecik M, Molik E, Rychlik T: Polymorphism in the melatonin receptor gene MT1 (locus MTNR1A) in sheep. *Arch Tierzucht*, 49, 257-262, 2006.

29. Mura MC, Luridiana S, Daga C, Bini PP, Carcangiu V: Genotype at the MTNR1A locus andresponse to melatonin treatment in Sarda lambs. *Ital J Anim Sci*, 8, 114-116, 2009.

30. Mateescu RG, Lunsford AK, Thonney ML: Association between melatonin receptor 1A gene polymorphism and reproductive performance in Dorset ewes. *J Anim Sci*, 87, 2485-2488, 2009.