Asian-Aust. J. Anim. Sci.
Vol. 19, No. 8 : 1079 - 1084
August 2006

www.ajas.info

Association between Melatonin Receptor 1A Gene and Expression of Reproductive Seasonality in Sheep

M. X. Chu*, D. X. Cheng[1], W. Z. Liu[1], L. Fang and S. C. Ye

Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100094, P. R. China

ABSTRACT : To determine whether a link exists between reproductive seasonality and the structure of the melatonin receptor 1A (*MTNR1A*) gene, the latter was studied in nonseasonal estrous breeds (Small Tail Han and Hu ewes) and seasonal estrous breeds (Dorset, Suffolk and German Mutton Merino ewes). A large fragment of the exon 2 of the *MTNR1A* gene was amplified and a uniform fragment of 824 bp was obtained in 239 ewes of five breeds. The 824 bp PCR product was digested with restriction endonucleases *Mnl* I and *Rsa* I, and checked for the presence of restriction sites. The presence (allele *M*) or absence (allele *m*) of an *Mnl* I site at base position 605 led to three genotypes *MM* (236 bp/236 bp), *Mm* (236 bp/303 bp) and *mm* (303 bp/303 bp) in five sheep breeds. The presence (allele *R*) or absence (allele *r*) of a *Rsa* I site at base position 604 led to three genotypes *RR* (267 bp/267 bp), *Rr* (267 bp/290 bp) and *rr* (290 bp/290 bp) in five sheep breeds. Frequencies of *MM* and *RR* genotypes were obviously higher, and frequencies of *mm* and *rr* genotypes were obviously lower in nonseasonal estrous sheep breeds than in seasonal estrous sheep breeds. Sequencing revealed four mutations (G453T, G612A, G706A, C891T) in *mm* genotype compared to *MM* genotype and one mutation (C606T) in *rr* genotype compared to *RR* genotype. For polymorphic *Mnl* I and *Rsa* I cleavage sites, the differences of genotype distributions were very highly significant ($p<0.01$) between Small Tail Han ewes and seasonal estrous sheep breeds. In each group, no significant difference ($p>0.05$) was detected. These results preliminarily showed an association between *MM*, *RR* genotypes and nonseasonal estrus in ewes and an association between *mm*, *rr* genotypes and seasonal estrus in ewes. (**Key Words :** Sheep, Reproductive Seasonality, Melatonin Receptor 1A Gene, PCR-RFLP)

INTRODUCTION

Melatonin synthesis by the pineal gland occurs only during the hours of darkness, and gates photoperiodic information in mammals. Melatonin regulates circadian rhythms and reproduction changes in seasonally reproductive mammals (Ortavant et al., 1985; Reppert et al., 1994; Weaver and Reppert, 1996; Barrett et al., 1997). The circadian effects of melatonin appear to be mediated by melatonin receptors in the hypothalamic suprachiasmatic nucleus, the site of a circadian clock (Weaver and Reppert, 1996), and the reproductive effects mediated by melatonin receptors in the hypophyseal pars tuberalis (Reppert et al., 1994). Annual fluctuations in timing and duration of the nocturnal elevation in circulating melatonin is known to be a key factor influencing seasonal reproduction in sheep (Malpaux et al., 1996). Melatonin exerts its reproductive and circadian effects through the binding to pharmacologically specific, high-affinity, G-protein-coupled receptors (Dubocovich and Takahashi, 1987; Reppert et al., 1988; Vanecek, 1988; Roca et al., 1996). Melatonin receptor 1a (MTNR1A), a high-affinity melatonin receptor that mediates these two major biological functions of melatonin in mammals had been cloned by Reppert et al. (1994). By microsatellite markers and two-point linkage analysis, Messer et al. (1997) mapped *MTNR1A* gene to ovine chromosome 26, between microsatellites CSSM43 and BM6526. Discovery by Messer et al. (1997) of two polymorphic RFLP sites within the ovine MTNR1A gene provided opportunity to evaluate the influence of this gene on seasonal reproduction. Pelletier et al. (2000), Ji et al. (2003), Chu et al. (2003) and Notter et al. (2003) reported that the homozygous genotype for the absence of a polymorphic *Mnl* I site at position 605 of exon 2 of *MTNR1A* gene was associated with seasonal anovulatory activity in ewes.

In most sheep breeds, ovulatory activity of ewes is

* Corresponding Author: M. X. Chu. Tel: +86-10-62816001, Fax: +86-10-62895351, E-mail: mxchu@263.net

[1] College of Animal Science and Technology, Shanxi Agricultural University, Taigu 030801, P. R. China.

Received September 26, 2005; Accepted March 15, 2006

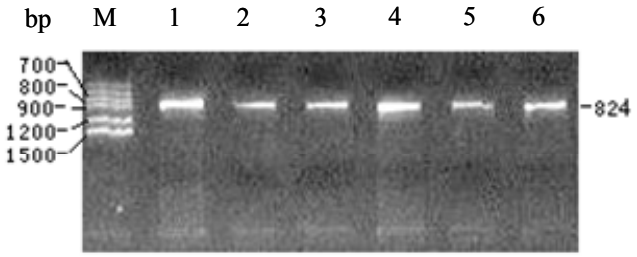

Figure 1. Electrophoresis of PCR amplification of exon 2 of *MTNR1A* gene in sheep (1.5% agarose gel). M: SD013 DNA marker; 1-6: PCR amplification product.

generally inhibited for several consecutive months of the year, referred to as the anestrous season, which occurs in spring. However, both Small Tail Han sheep and Hu sheep, that are excellent local breeds in China, display significant characteristics of non-seasonal estrus (Tu, 1989). Small Tail Han ewes continue to cycle throughout the year (Zhang, 1995; Wang et al., 1997; Jia et al., 2005). The objectives of the present study were firstly to detect the PCR-RFLP polymorphism of *MTNR1A* gene in the non-seasonal estrous breeds (Small Tail Han and Hu ewes) and seasonal estrous breeds (Dorset, Suffolk and German Mutton Merino ewes), and secondly to investigate the associations between *MTNR1A* gene and reproductive seasonality in ewes. This achievement could provide a theoretical basis for genetically controlling the ovine estrus, and lay a foundation for both changing the ovine estrous seasonality and improving the reproductive performance by means of gene knockout.

MATERIALS AND METHODS

Genomic DNA preparation

Jugular blood samples (10 ml per ewe) were collected from 137 Small Tail Han ewes (Jiaxiang Sheep Breeding Farm located in Jiaxiang County, Shandong Province, China), 30 Dorset, 24 Suffolk and 21 German Mutton Merino ewes (HITEK Ranch (Beijing) Ltd. Co. located in Dasungezhuang Town, Shunyi District, Beijing, China), and 27 Hu ewes (Yuhang Hu Sheep Breeding Farm located in Yuhang District, Hangzhou City, Zhejiang Province, China) using acid citrate dextrose as an anticoagulant. These ewes were chosen at random. Genomic sheep DNA was extracted from whole blood by phenol-chloroform method, and then dissolved in TE buffer (10 mmol/L Tris·Cl (pH 8.0), 1 mmol/L EDTA (pH 8.0)) and kept at -20°C.

Primer sequences

Primers for PCR of Messer et al. (1997) were employed corresponding to positions 285-304 (sense primer) and 1108-1089 (antisense primer) of the sequence (GenBank U14109) of exon 2 of ovine *MTNR1A* gene from Reppert et al. (1994), and synthesized by Shanghai BioAsia Biotechnology Ltd. Co. (Shanghai, China). The expected amplification fragment size was 824 bp. The primer sequences were as follows:

Forward: 5'-TGTGTTTGTGGTGAGCCTGG-3';
Reverse: 5'-ATGGAGAGGGTTTGCGTTTA-3'.

Genotyping samples

The polymerase chain reactions were carried out in 25 μl volume containing approximately 10×PCR buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.0), 0.1% Triton X-100) 2.5 μl, 1.2 mmol/L $MgCl_2$, 2 mmol/L each dNTP, 2 μmol/L each primer, 50 ng ovine genomic DNA, and 2 U *Taq* DNA polymerase (Promega, Madison, WI, USA). PCR conditions were as follows: denaturation at 94°C for 4 min, followed by 32 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 50 s, extension at 72°C for 70 s, with a final extension at 72°C for 10 min on Mastercycler® 5333 (Eppendorf AG, Hamburg, Germany). The resultant products were kept at 4°C and detected by electrophoresis on 1.5% agarose gels (Promega, Madison, WI, USA).

PCR products of 7 μl were digested separately with 5 U *Mnl* I (New England Biolabs, Beverly, MA, USA) and 5 U *Rsa* I (Promega, Madison, WI, USA) at 37°C overnight. The resultant fragments were separated by electrophoresis on 8% polyacrylamide gels in parallel with a pBR322/*Msp* I marker. The gels were stained with silver nitrate (silver staining), photographed and analyzed using an AlphaImager™ 2200 and 1220 Documentation and Analysis Systems (Alpha Innotech Corporation, San Leandro, CA, USA).

Genotyping was performed in the 239 DNA samples from 137 Small Tail Han, 30 Dorset, 24 Suffolk, 21 German Mutton Merino and 27 Hu ewes.

Cloning and sequencing

After RFLP analysis, PCR products of different homozygous genotypes were separated on 1.5% agarose gels and recovered using Geneclean II kit (Promega, Madison, WI, USA). Each DNA fragment was ligated into the pGEM-T Easy vector (Promega, Madison, WI, USA) according to the manufacturer's instructions. The ligation reactions were carried out in 10 μl volume containing PCR product 1 μl, pGEM-T Easy vector (50 ng/μl) 1 μl, T_4 ligase (3 U/μl) 1 μl, 2×ligation buffer 5 μl, ddH_2o 2 μl. Each DNA fragment was then transformed into *Escherichia coli* DH5α competence cell. Positive clones of transformed cells were identified by restriction enzyme digestion. Two clones of each homozygous genotype were selected and sequenced. Each clone was sequenced for twice. The target DNA fragments in recombinant plasmids were sequenced from

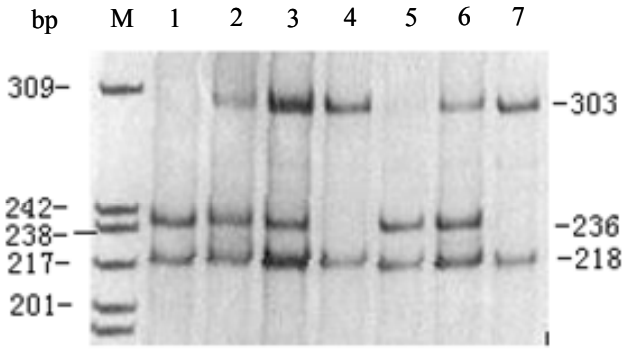

Figure 2. Polymorphism of the *Mnl* I cleavage site at position 605. M: pBR322/*Msp* I marker; Lanes 1, 5: *MM* genotype (Small Tail Han ewes); Lanes 2, 3, 6: *Mm* genotype (Hu ewes); Lanes 4, 7: *mm* genotype (Suffolk ewes).

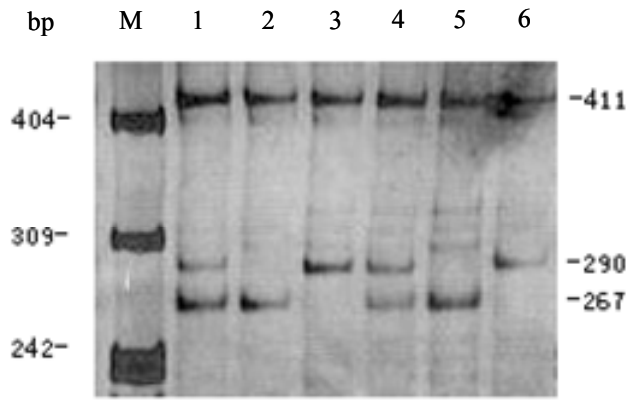

Figure 3. Polymorphism of the *Rsa* I cleavage site at position 604. M: pBR322/*Msp* I marker; Lanes 2, 5: *RR* genotype (Small Tail Han ewes); Lanes 1, 4: *Rr* genotype (Hu ewes); Lanes 3, 6: *rr* genotype (Suffolk ewes).

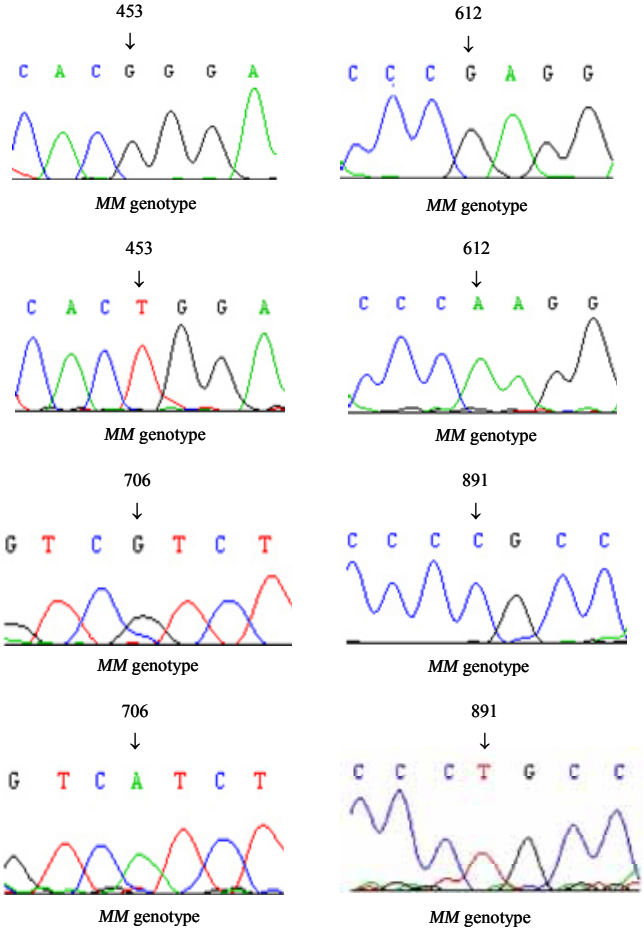

Figure 4. Sequence comparison of *MM* and *mm* genotypes of *MTNR1A* gene in sheep.

both directions using an automatic ABI 377 sequencer (Perkin Elmer Applied Biosystems, Foster City, CA, USA) by Beijing Dingguo Biotechnology Ltd. Co. (Beijing, China).

Statistical analysis

Distribution of genotypes between different sheep breeds was analyzed using the chi-square method.

RESULTS

PCR amplification of exon 2 of ovine *MTNR1A* gene

In the present study, the primers for the exon 2 of ovine *MTNR1A* gene were used for amplification genomic DNA of different sheep breeds and the PCR products were separated on 1.5% agarose gels. The result showed that amplification fragment size 824 bp was consistent with the target one and had good specificity (Figure 1), which could be directly analyzed by RFLP.

RFLP analysis of exon 2 of ovine *MTNR1A* gene

There are seven cleavage sites (218 bp, 36 bp, 67 bp, 236 bp, 22 bp, 28 bp, 82 bp, 135 bp) for *Mnl* I within the amplification fragment, but only one was shown to be polymorphic (Chu et al., 2003). This site was at position 605 in the reference sequence (Reppert et al., 1994). Digestion with *Mnl* I yielded polymorphic fragments of 236 bp and 67 bp when the cleavage site was present (allele *M*) or a single 303 bp fragment if the cleavage site was absent (allele *m*). Three genotypes *MM* (236 bp/236 bp), *Mm* (236 bp/303 bp) and *mm* (303 bp/303 bp) were detected in five sheep breeds (Figure 2).

There are four cleavage sites (53 bp, 267 bp, 23 bp, 411 bp, 70 bp) for *Rsa* I within the amplification fragment, but only one was shown to be polymorphic (Chu et al., 2003). This site was at position 604 in the reference sequence (Reppert et al., 1994). Digestion with *Rsa* I yielded polymorphic fragments of 267 bp and 23 bp when the cleavage site was present (allele *R*) or a single 290 bp fragment when the cleavage site was absent (allele *r*). Three genotypes *RR* (267 bp/267 bp), *Rr* (267 bp/290 bp) and *rr* (290 bp/290 bp) were detected in five sheep breeds (Figure 3).

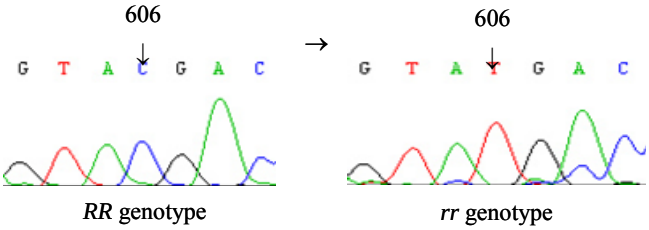

Figure 5. Sequence comparison of *RR* and *rr* genotypes of *MTNR1A* gene in sheep.

Sequencing of different homozygous genotypes

Sequencing verified the presence (or absence) of the polymorphic *Mnl* I and *Rsa* I cleavage sites as assessed by polyacrylamide gel electrophoresis. The sequencing results indicated that genotype *MM* had the same sequence as that of U14109 in GenBank and was referred to as wild type. Genotype *mm* had four mutations（G453T, G612A, G706A, C891T）compared to *MM* genotype and was referred to as mutation genotype. Mutation at position 706 resulted in the substitution of a valine by an isoleucine in the amino acid sequence. The other mutations were silent. Genotype *RR* had the same sequence as that of U14109 in GenBank and was referred to as wild type. Genotype *rr* had one mutation (C606T) compared to *RR* genotype and was referred to as mutation genotype. Mutation at position 606 was silent. In all, a total of 5 different mutations were registered (see Table 1, Figures 4 and 5).

Table 1. Positions of mutations and base and amino acid changes in exon 2 of *MTNR1A* gene in five sheep breeds

Position of mutations[a]	Base change	Amino acid change and position[a]
453	G→T	None
606	C→T	None
612	G→A	None
706	G→A	Val→Ile 220
891	C→T	None

[a] Base or amino acid positions corresponding to U14109 of GenBank.

Allele and genotype frequencies of *MTNR1A* gene in different sheep breeds

Allele and genotype frequencies of *MTNR1A* gene in five sheep breeds were presented in Table 2.

Frequencies of *MM* and *RR* genotypes were obviously higher, and frequencies of *mm* and *rr* genotypes were obviously lower in nonseasonal estrous sheep breeds than in seasonal estrous sheep breeds. These results preliminarily showed an association between *MM*, *RR* genotypes and nonseasonal estrus in ewes, an association between *mm*, *rr* genotypes and seasonal estrus in ewes.

Test of difference for *MTNR1A* genotype distribution in different sheep breeds

The test result of difference for *MTNR1A* genotype distribution in five sheep breeds was summarized in Table 3.

For polymorphic *Mnl* I and *Rsa* I cleavage sites, the differences of genotype distributions were very highly

Table 2. Allele and genotype frequencies of PCR-RFLP of the *MTNR1A* gene in five sheep breeds

Breed	No.	*Rsa* I					*Mnl* I				
		Allele frequency		Genotype frequency			Allele frequency		Genotype frequency		
		R	*r*	*RR*	*Rr*	*rr*	*M*	*m*	*MM*	*Mm*	*mm*
Small Tail Han sheep	137	0.71	0.29	0.46 (63)	0.50 (68)	0.04 (6)	0.75	0.25	0.52 (71)	0.47 (64)	0.01 (2)
Hu sheep	27	0.65	0.35	0.41 (11)	0.48 (13)	0.11 (3)	0.80	0.20	0.63 (17)	0.33 (9)	0.04 (1)
Suffolk sheep	24	0.42	0.58	0.04 (1)	0.75 (18)	0.21 (5)	0.56	0.44	0.33 (8)	0.46 (11)	0.21 (5)
Dorset sheep	30	0.48	0.52	0.13 (4)	0.70 (21)	0.17 (5)	0.57	0.43	0.23 (7)	0.67 (20)	0.10 (3)
German Mutton Merino sheep	21	0.48	0.52	0.24 (5)	0.48 (10)	0.28 (6)	0.55	0.45	0.19 (4)	0.71 (15)	0.10 (2)

The numbers in the brackets are the genotype individuals.

Table 3. Test of difference of *Mnl* I (above diagonal) and *Rsa* I (below diagonal) genotype distributions of *MTNR1A* gene in five sheep breeds

Sheep breed	Small Tail Han sheep	Hu sheep	Suffolk sheep	Dorset sheep	German Mutton Merino sheep
Small Tail Han sheep		2.05	19.06***	12.22**	11.02**
Hu sheep	2.01		5.95	9.20*	9.28**
Suffolk sheep	19.54***	9.50**		2.54	3.05
Dorset sheep	14.10***	5.51	1.38		0.15
German Mutton Merino sheep	16.13***	2.94	4.86	2.60	

* p<0.05, ** p<0.01, *** p<0.001.

significant ($p<0.01$) between Small Tail Han ewes and seasonal estrous sheep breeds. In each group, no significant difference ($p>0.05$) was detected.

DISCUSSION

Polymorphism of ovine *MTNR1A* gene

The 824 bp product of exon 2 of the ovine *MTNR1A* gene was digested with restriction endonucleases *Mnl* I and *Rsa* I in sheep populations. For *Mnl* I site, the 286 bp and 236 bp fragments were polymorphic (Messer et al., 1997; Notter et al., 2003), the 303 bp and 236 bp fragments were polymorphic (Pelletier et al., 2000; Chu et al., 2003; Ji et al., 2003). For *Rsa* I site, the 295 bp and 290 bp fragments were polymorphic (Messer et al., 1997; Notter et al., 2003), the 290 bp and 267 bp fragments were polymorphic (Chu et al., 2003; Ji et al., 2003). The results of this study were consistent with those of Pelletier et al. (2000), Ji et al. (2003) and Chu et al. (2003).

Five point mutations（G453T, C606T, G612A, G706A, C891T）of exon 2 of ovine *MTNR1A* gene were observed within the limits studied here (positions 305-1088 excluding the primers). These five mutations were identical to those reported by Pelletier et al. (2000). Two mutations at positions 606 and 891 had been evidenced by Barrett et al. (1997). Two mutations at positions 606 and 612 had been evidenced by Messer et al. (1997). Eight point mutations (C426T, G555A, G783A, G801A, C893A, C1101T, A1121G, A1129G) reported by Barrett et al. (1997) and Pelletier et al. (2000) were not detected in the present study. No new mutations were detected in the present study.

Functional differences in MTNR1A were not anticipated for different genotypes. This study and Pelletier et al. (2000) demonstrated that mutation at position 612, responsible for the absence of *Mnl* I site, was always found to be associated with the 3 other mutations at positions 453, 706, and 891. This study and Pelletier et al. (2000) revealed that the mutation at position 706 leaded to the substitution of a valine at position 220 by an isoleucine in the fifth transmembrane domain. Moreover, isoleucine 220 is close to histidine 211, whose mutation modifies the K_d value of ^{125}I-melatonin binding to $Mel_{1\alpha\beta}$ receptor (Conway et al., 1997). The importance of this particular amino acid merits further study by mutagenesis.

Reproductive seasonality and ovine *MTNR1A* gene

Spontaneous ovulation in early spring in the Merinos d'Arles ewes had been studied by Hanocq et al. (1999), and this trait was found to have a significant heritability ($h^2 = 0.20$) in a model taking into account several physiological parameters such as weight and age of animals. Montgomery and Kawker (1987) and Hanocq et al. (1999) suggested the existence of a common overall factor involved in the control of reproductive seasonality. One possible candidate could be *MTNR1A* gene.

Pelletier et al. (2000), Ji et al. (2003), Chu et al. (2003) and Notter et al. (2003) reported that the homozygous genotype for the absence of a polymorphic *Mnl* I site at position 605 of exon 2 of *MTNR1A* gene was associated with seasonal anovulatory activity in ewes. This study showed an association between *MM* genotype and nonseasonal estrus in ewes, an association between *mm* genotype and seasonal estrus in ewes. The next step in the present study will be to establish whether the association between genotype and ovarian seasonality is a true genetic linkage. The fact that this association remained within families supported this hypothesis (Pelletier et al., 2000).

This study showed an association between *RR* genotype and nonseasonal estrus in ewes, an association between *rr* genotype and seasonal estrus in ewes. The next step in the present study will be to establish whether the association between genotype and ovarian seasonality is a true genetic linkage.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (No. 30300248) and by Beijing Municipal Science and Technology Program of China (No.Y0705003041131).

REFERENCES

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

Chu, M. X., C. L. Ji and G. H. Chen. 2003. Association between PCR-RFLP of melatonin receptor 1a gene and high prolificacy in Small Tail Han sheep. Asian-Aust. J. Anim. Sci. 16(12):1701-1704.

Conway, S., S. J. Canning, P. Barrett, B. Guardiola-Lemaitre, P. Delagrange and P. J. Morgan. 1997. The roles of valine 208 and histidine 211 in ligand binding and receptor function of the ovine $Mel_{1\alpha\beta}$ melatonin receptor. Biochem. Biophys. Res. Commun. 239(2):418-423.

Dubocovich, M. L. and J. S. Takahashi. 1987. Use of 2-[^{125}I] iodomelatonin to characterize melatonin binding sites in chicken retina. Proc. Natl. Acad. Sci. USA. 84(11):3916-3920.

Hanocq, E., L. Bodin, J. Thimonier, J. Teyssier, B. Malpaux and P. Chemineau. 1999. Genetic parameters of spontaneous spring ovulatory activity in Merinos d'Arles sheep. Genet. Sel. Evol. 31(1):77-90.

Ji, C. L., M. X. Chu, G. H. Chen, G. L. Zhou and Y. Zhu. 2003. Polymorphism analysis for exon 2 of melatonin receptor 1a gene in four sheep breeds by PCR-RFLP. J. Huazhong Agric. Univ. 22(2):105-109.

Jia, C. L., N. Li, X. B. Zhao, X. P. Zhu and Z. H. Jia. 2005.

Association of single nucleotide polymorphisms in exon 6 region of *BMPRIB* gene with litter size traits in sheep. Asian-Aust. J. Anim. Sci. 18(10):1375-1378.

Malpaux, B., C. Viguie, D. C. Skinner, J. C. Thiery, J. Pelletier and P. Chemineau. 1996. Seasonal breeding in sheep: mechanism of action of melatonin. Anim. Reprod. Sci. 42(1):109-117.

Messer, L. A., L. Wang, C. K. Tuggle, M. Yerle, P. Chardon, D. Pomp, J. E. Womack, W. Barendse, A. M. Crawford, D. R. Notter and M. F. Rothschild. 1997. Mapping of the melatonin receptor 1a (*MTNR1A*) gene in pigs, sheep, and cattle. Mammalian Genome. 8(5):368-370.

Montgomery, G. W. and H. Kawker. 1987. Seasonal reproduction in ewes selected on seasonal changes in wool growth. J. Reprod. Fertil. 79(1):207-213.

Notter, D. R., N. E. Cockett and T. S. Hadfield. 2003. Evaluation of melatonin receptor 1a as a candidate gene influencing reproduction in an autumn-lambing sheep flock. J. Anim. Sci. 81(4):912-917.

Ortavant, R., J. Pelletier, J. P. Ravault, J. Thimonier and P. Volland-Nail. 1985. Photoperiod: main proximal and distal factor of the circannual cycle of reproduction in farm mammals. Oxf. Rev. Reprod. Biol. 7:305-345.

Pelletier, J., L. Bodin, E. Hanocq, B. Malpaux, J. Teyssier, J. Thimonier and P. Chemineau. 2000. Association between expression of reproductive seasonality and alleles of the gene for Mel_{1a} receptor in the ewe. Biol. Reprod. 62(4):1096-1101.

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

Reppert, S. M., D. R. Weaver, S. A. Rivkees and E. G. Stopa. 1988. Putative melatonin receptors in a human biological clock. Sci. 242(4875):78-81.

Roca, A. L., C. Godson, D. R. Weaver and S. M. Reppert. 1996. Structure, characterization, and expression of the gene encoding the mouse Mel_{1a} melatonin receptor. Endocrinol. 137(8):3469-3477.

Tu, Y. R. 1989. The Sheep and Goat Breeds in China. Shanghai Science and Technology Press, Shanghai, P. R. China. pp. 50-52, 55-58.

Vanecek, J. 1988. Melatonin binding sites. J. Neurochem. 51(5):1436-1440.

Wang, J. M., Z. J. Qin, X. X. Qu, Z. H. Wang and F. C. Li. 1997. Advance on germplasm characteristics and utilization ways of Shandong Small Tail Han sheep. Grass-feeding Livestock. (3):6-10.

Weaver, D. R. and S. M. Reppert. 1996. The Mel_{1a} melatonin receptor gene is expressed in human suprachiasmatic nuclei. Neuroreport. 8(1):109-112.

Zhang, H. Q. 1995. Development and utilization on reproductive performance of Small Tail Han sheep. China Sheep and Goat Farming. (2):27-29.