



ASSOCIATION BETWEEN GENETIC POLYMORPHISM OF EXON 10 OF PROLACTIN RECEPTOR GENE AND LITTER SIZE OF SHEEP

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ABSTRACT

Improvement of reproductive traits in livestock species has become of increasing interest, especially in Sheep where moderate increases in litter size can gain large profit. Prolactin receptor (PRLR) is an anterior pituitary peptide hormone involved in different physiological activities and is essential for reproductive success. The associations of PRLR gene with litter size in 4 Chinese sheep breeds was studied using PCR-SSCP. Genotypes and alleles frequency of PRLR gene among four sheep breeds at locus P2 were at HWE equilibrium ($P>0.05$) while the genotype at locus P3 were significantly deviated from HWE ($P<0.01$) except for Altry sheep. At P2 locus Sheep with genotype CC had a larger litter size than sheep with BB and BC genotype, while at locus P3 sheep with DD genotype had a larger litter size compared with DE genotype, although the results for P2 and P3 were statistically non significant ($P>0.05$). Hu sheep with DD genotype had a larger litter size than that of DE genotype. However, the association of prolactin receptor gene with litter size was non significant ($P>0.05$). Our results showed that PRLR gene couldn't be selected as a candidate gene for the prolificacy of sheep.

Keywords: Sheep, PRLR gene, Litter size.

INTRODUCTION

Domestic sheep (*Ovisaries*) have played important roles in diverse human societies as a source of food, hide, and wool, and are one of the major components of agro-pastoral societies since the Neolithic [1]. Fertility and fecundity increased progressively as the type of birth of lambs increased from single to triplets [2]. Producing ability on fecundity of ewes, obtained from calculating repeatability of litter size, is a good genetic parameter, which could measure potential inheritability of fecundity. Genetic improvement of reproductive traits has traditionally been restricted to the use of quantitative genetic methods but using these methods led to the limited gain only. The use of molecular genetic markers in Marker Assisted Selection (MAS) could potentially be employed in conjunction with traditional selection

methods to accelerate the rate of change in economically important traits [3].

Prolactin (PRL) is an anterior pituitary peptide hormone involved in many endocrine activities, essential for reproduction and mediated by its receptor (PRLR) detected in various tissues including brain, ovary, placenta and uterus in several mammalian species [4-7]. Prolactin receptor (PRLR) is thought to play a central role in signal transmitting from prolactin to milk protein genes. It binds prolactin and contributes to activation of JAK2 kinases and subsequent phosphorylation of STAT5 transcription factors which bind to recognition sequences located in promoters of milk protein genes [8, 9]. An importance of the gene was confirmed in transgenic mice experiments, mice homozygous for a null mutation in the

PRLR gene were sterile due to a failure of embryonic implantation, demonstrated irregular cycles, reduced fertilization rates and defective embryonic development [10]. These characteristics make PRLR a strong candidate gene for reproductive traits [11-13].

Prolactin receptor gene (PRLR) was investigated as a candidate gene influencing litter size in several studies [14-17]. Recent study has evidenced that the PRLR gene locus influences the prolificacy in sheep [18], preweaning survivability of piglets [19] and affect ovulation and ejaculate in pig, which might lead to litter size differences [20]. Very recently, the PRLR has been identified as candidate gene affecting key quantitative traits, like reproduction and growth in livestock [21]. Our aim was to study single nucleotide polymorphism of the PRLR gene exon 10 and its relation with litter size in sheep breeds.

MATERIALS AND METHODS

Experimental Animals

Ear tissue samples were collected from four sheep breeds, included 208 Hu sheep, 101 Wadi sheep, 115 Small Tail Han sheep and 98 Altay sheep. Samples for 119 individuals of Hu sheep were collected from Xuzhou ShenNing sheep industry Co., LTD, and 89 were collected from Suzhou Sheep Breeding Farm. Samples of Wadi sheep, Small Tail Han sheep and Altay sheep were collected from Binzhou area of Shandong province, Yuncheng region of Shandong province and Altay region of Xinjiang, respectively. Litter size was estimated from breeding records.

DNA Extraction and PCR-SSCP

Genomic DNA was extracted by phenol-chloroform methods, dissolved in TE buffer and stored at -80°C. Four pairs of primers were designed in exon 10 of PRLR gene according to the genomics sequence of sheep (AF041257) [22] and cow (NM_174155) [23] published on Genbank. PCR kits, DNA Ladder Marker etc. were purchased from Shanghai Shenggong Biological Engineering Technology Company. Primers were synthesized by Shanghai Shenggong Biological Engineering Technology Company (Table 1).

The PCR reactions were performed in 20µl contained 10×PCR buffer, 2.5mmol/l dNTP, 10 pmol/µl of each primer, 5 U/µl of Taq DNA polymerase and 1µl genomic DNA, double-distilled water was added to a final volume of 20µl. PCR condition was 5 min at 94°C followed by 32 cycles of 30 s at 94°C, 30s at the respective annealing temperature (54.8°C, 53.8°C, 59.3°C and 65°C) and 30 s at 72°C. The final extension step was 10 min at 72°C, and then PCR products were preserved at 4°C. A mixture of 7 µl PCR product and 3 µl buffers was denatured for 15min at 98°C, plugged into ice, and then analyzed by 12% non-denaturing polyacrylamide gel electrophoresis and silver stained. The results were copied

and saved by Kodak Gel image analysis system. Analysis of the genetic polymorphism was conducted by Kodak Digital Science ID Image Analysis Software based on the standard PBR322 /Msp Marker.

DATA ANALYSIS

Hardy-Weinberg Equilibrium of Different Varieties and Sites

The χ^2 compatibility test was applied using the following the formula:

$$\chi^2 = \sum_{i=1}^n \frac{(O_i - E_i)^2}{E_i}$$

Where:

Ei = the theoretical value

Oi = the actual observed values

n = the number of alleles

df = k-1, k = classification number. If df = 1, we should correct it continuously.

Significance Test of Genotype Frequencies of Different Varieties

The χ^2 independence test was applied using the formula:

$$\chi^2 = \sum_{i=1}^n \frac{\left(\left| O_i - E_i \right| - \frac{1}{2} \right)^2}{E_i} (df = 1)$$

Where:

Ei = the theoretical value

Oi = the actual observed values

n = the number of alleles

df = k-1, k = classification number. If df = 1, we should correct it continuously.

Analysis of Different Genotypes and Litter Size of Sheep

We used least squares means and constructed the general linear model (GLM) as follow:

$$Y_{ijk} = \mu + G_j + I_k + G_j * I_k + e_{ijk}$$

Where:

Yijk = the observed value

μ = the population mean

Gj = the genotype effect

Ik = the varieties effect

eijk = the random error

Analysis of Different Genotypes and Litter Size of HU Sheep

We used least squares means and constructed the general linear model (GLM) as follow:

$$Y_{ijk} = \mu + G_j + I_k + e_{ijk}$$

Where:

Yijk = the observed value

μ = the population mean

Gj = the genotype effect

I_k = the family effect

e_{ijk} = the random error

All data was analyzed using SPSS software.

RESULT

Genetics polymorphisms were detected by PCR-single strand conformation polymorphism (SSCP) in 4 Chinese sheep breeds. The polymorphisms were used to study the associations with litter size. Among four primers studied primer 2 and 3 were shows single strand conformation polymorphism. In primer 2 (P2) three genotypes BB, BC and CC were detected, while in primer 3 (P3) two genotypes DD and DE were detected (Fig 1).

Genotypes and alleles frequency of PRLR gene among four sheep breeds were presented in Table 2. The genotypes of P2 were at HWE equilibrium ($P > 0.05$) while the genotype of P3 were significantly deviated from HWE ($P < 0.01$) except for Altay sheep (Table 3).

The numbers in brackets represent individual number over the total number of individuals of different genotypes.

Df = 1, $\chi^2_{0.05} = 3.84$, $\chi^2_{0.01} = 6.63$. The difference was not significant ($\chi^2_{2i} < \chi^2_{0.05}$, $P > 0.05$); the difference was significant ($\chi^2_{0.05} < \chi^2_{2i} < \chi^2_{20.01}$, $0.01 < P < 0.05$); the difference was extremely significant ($\chi^2_{2i} > \chi^2_{20.01}$, $P < 0.01$).

Df = 2, $\chi^2_{20.05} = 5.99$, $\chi^2_{20.01} = 9.21$. The

difference was not significant ($\chi^2_{2i} < \chi^2_{0.05}$, $P > 0.05$); the difference was significant ($\chi^2_{0.05} < \chi^2_{2i} < \chi^2_{20.01}$, $0.01 < P < 0.05$); the difference was extremely significant ($\chi^2_{2i} > \chi^2_{20.01}$, $P < 0.01$).

The χ^2 independence test of different genotypes in four sheep breeds of the amplified fragments of P2 showed non significant different ($P > 0.05$) (Table 4). Similarly, the genotype frequency of Hu sheep, Small Tail Han sheep and Wadi sheep has no significance for P3 ($P > 0.05$) while these three breeds were significantly different from Altay sheep ($P < 0.01$) (Table 5).

The values in table was chi-square and P was stand for probability caused by sampling error. The difference was not significant ($P > 0.05$); the difference was significant ($0.01 < P < 0.05$); the difference was extremely significant ($P < 0.01$).

The least squares mean of litter size of sheep breeds corresponding different genotypes of the amplified fragments of P2 showed that Sheep with genotype CC had a larger litter size than sheep with genotype BB and BC, while for P3 sheep with genotype DD had a larger litter size compared with DE, although the results for P2 and P3 were statistically non significant ($P > 0.05$) (Table 6).

Hu sheep with DD genotype had a larger litter size than that of DE genotype. However, the association of prolactin receptor gene with litter size was not significant ($P > 0.05$) (Table 7).

Table 1. Primer sequences and allele size of prolactin receptor (PRLR) gene in sheep

Primer	Primer sequence(5'→3')	Allele size (bp)
P1	F 5'- AAGGGCAAGTCCGAA GAACT- 3'	248bp
	R 5'- TGAGGTTCATCACACTTTTC- 3'	
P2	F 5'- TGTCTGAAAA GTGTGATGAA- 3'	233bp
	R 5'- A GCAATGTTGTGTAAGAATA - 3'	
P3	F 5'- CTTACCACAACATTGCTGACG - 3'	231bp
	R 5'- GTTTA GCAGA GAACAA GGGGG - 3'	
P4	F 5'- AAACCCCCTTGTCTCTGCTA- 3'	315bp
	R 5'- CCCAACCCAACCTGGAGTCTGC- 3'	

Table 2. Genotype and allele frequencies of prolactin receptor (PRLR) gene in sheep breeds

Loci Breeds	Primer 2 of PRLR gene				Primer 3 of PRLR gene		
	BB	BC	CC	B(C)	DD	DE	D(E)
Hu	0.7212 (119/161)	0.2596 (39/161)	0.0192 (3/161)	0.8602 (0.1398)	0.0385 (6/161)	0.9615 (155/161)	0.5186 (0.4814)
Small	0.7391 (85/114)	0.2174 (24/114)	0.0435 (5/114)	0.8509 (0.1491)	0.0601 (7/114)	0.9399 (107/114)	0.5304 (0.4696)
Wadi	0.7030 (71/101)	0.2277 (23/101)	0.0693 (7/101)	0.8168 (0.1832)	0.0693 (7/101)	0.9307 (94/101)	0.5347 (0.4653)
Altay	0.7245 (71/98)	0.2350 (23/98)	0.0405 (4/98)	0.8418 (0.1582)	0.9490 (93/98)	0.051 (5/98)	0.9745 (0.0255)

Hu: Hu sheep; Small: Small Tail Han sheep; Wadi: Wadi sheep; Altay: Altay sheep

The numbers in brackets represent individual number over the total number of individuals of different genotypes.

Table 3. The χ^2 compatibility test of different genotypes for primer 2 and 3

Project	Hu	Small	Wadi	Altay
P2 (df=2)	0.0113	2.8524	5.7668	1.3795
Equilibrium or not	Yes	Yes	Yes	Yes
P3 (df=1)	138.6498	90.1104	76.5375	0.0674
Equilibrium or not	Not	Not	Not	Not

Table 4. The χ^2 independence test of different genotypes in four sheep breeds of the amplified fragments of primer 2

	Hu	Small	Wadi
Small	1.607(P=0.448)		
Wadi	4.343(P=0.448)	0.769(P=0.681)	
Altay	1.141(P=0.565)	0.095(P=0.954)	0.773(P=0.679)

The values in table was chi-square and P was stand for probability caused by sampling error.

The difference was not significant ($P>0.05$); the difference was significant ($0.01<P<0.05$); the difference was extremely significant ($P<0.01$).

Table 5. The χ^2 independence test of different genotypes in four sheep breeds of the amplified fragments of primer 3

	Hu	Small	Wadi
Small	0.833(P=0.362)		
Wadi	1.351(P=0.245)	0.063(P=0.802)	
Altay	214.443**(P=0)	167.555**(P=0)	153.960**(P=0)

The values in table was chi-square and P was stand for probability caused by sampling error. The difference was not significant ($P>0.05$); the difference was significant ($0.01<P<0.05$); the difference was extremely significant ($P<0.01$).

Table 6. The least square mean of litter size for four sheep breeds

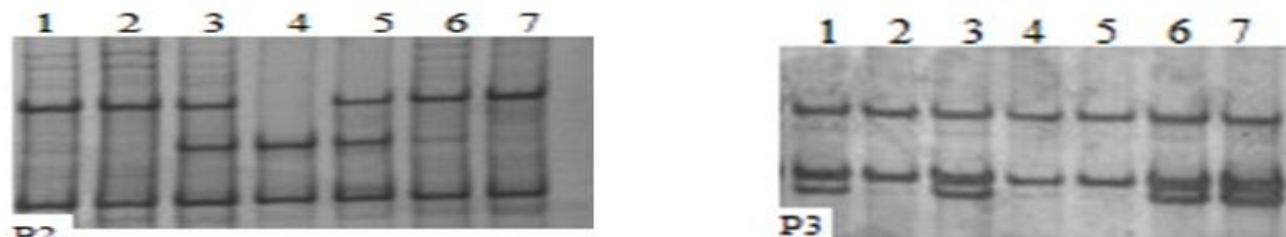
Primer	Genotype	Individual number	$\mu \pm e_{ijk}$
P2	BB	346	2.1796 \pm 1.0179a
	BC	109	2.0636 \pm 0.8933a
	CC	19	2.4562 \pm 0.9572a
P3	DD	113	2.5134 \pm 0.0918a
	DE	361	2.0559 \pm 0.0510a

The same lowercase letter expressed there was no significant difference among genotypes ($P>0.05$).

Table 7. The least squares mean of litter size for Hu sheep

Primer	Genotypes	Individual number	$\mu \pm e_{ijk}$
P2	BB	119	2.0664 \pm 0.5994a
	BC	39	2.1778 \pm 0.5485a
	CC	3	2.1667 \pm 0.5774a
P3	DD	6	2.5001 \pm 0.8165a
	DE	155	2.0796 \pm 0.5727a

The same lowercase letter expressed there was no significant difference among genotypes ($P>0.05$).

Fig 1. PCR-SSCP analysis of prolactin receptor (PRLR) gene in sheep using P2 and P3 primers.

P2 primer 1, 2, 6, 7: BB genotype; 3, 5: BC genotype; 4: CC genotype

P3 primer 1, 3, 6, 7: DE genotype; 2, 4, 5: DD genotype

DISCUSSION

Improvement of reproductive traits in livestock species has become of increasing interest, especially in Sheep where moderate increases in litter size can gains large profit. Prolactin receptor (PRLR) is an anterior pituitary peptide hormone involved in different physiological activities and is essential for reproductive success. PRL is involved in many endocrine activities including key functions related to reproduction and lactation in mammals via JAK/STATs signal transduction pathway [24].

In the present study single strand conformation polymorphism was detected in four Sheep breeds. In locus P2 three genotypes BB, BC and CC were detected, while in locus P3 two genotypes DD and DE were detected. In the previous studies polymorphisms in the amplified region of PRLR gene was found in German pig [25], four sheep populations (Small Tailed Han, Suffolk, Dorset, F1 of Dorset ♂ crossed with Small Tailed Han ♀) [26], 314 Small Tail Han ewes and in exon 10 and part of 3'untranslated region of PRLR gene in goats [12]. PCR-SSCP showed that the frequency of allele A was larger than that of G and the difference of least square method between two genotypes (AA and AB) of Jining goat is not significant difference. Genotypes and alleles frequency of PRLR gene among four sheep breeds at locus P2 were at HWE equilibrium ($P>0.05$) while the genotype at locus P3 were significantly deviated from HWE ($P<0.01$) except for Altry sheep. Hardy-Weinberg deviation of locus P3 was probably caused by the selection on these three breeds. Moreover, herdsman in pastoral area didn't have selection criteria for sheep breeding. Altry sheep always kept the low prolific trait, which makes the amplified products of P3 stay in Hardy Weinberg equilibrium state.

Mice with null mutations in PRLR, eliminating functional receptors, are sterile due to a failure of embryonic implantation and also demonstrate irregular cycles, reduced fertilization rates, and defective embryonic development [10]. DNA polymorphism of the ovine prolactin receptor gene (PRLR) was investigated and used to study its effect on litter size in sheep [14]. Chen *et al.* (2010) indicated that the prolactin receptor locus was either a major gene that influences the prolificacy in Small Tail Han sheep and Chinese Merino (Xinjiang type) prolific strain sheep or was in close linkage with such a gene [27]. In the present study the least squares mean of litter size of the four sheep breeds showed that at locus P2 Sheep with genotype CC had a larger litter size than sheep with genotype BB and BC, while at locus P3 sheep with genotype DD had a larger litter size compared with DE, although the results were statistically non significantly ($P>0.05$). In addition we analyzed Hu sheep and found that there are no significant difference on litter size between genotypes at locus P2

and P3 ($P>0.05$), but sheep with genotype DD had a larger litter size than that of DE genotype. The Small Tail Han ewes with genotype BB or AB had 0.64-0.76 or 0.44-0.54 more lambs than those with genotype AA, respectively [16]. These results preliminarily showed that the prolactin receptor locus is either a major gene that influences the prolificacy in Small Tail Han sheep or is in close linkage with such a gene [14]. The correlation analysis showed that the Chinese Merino sheep with genotype CE and DD had 0.31($P=0.05$) lambs more than those with genotype CD or DE; the average birth weight of genotype AA was 0.66 kg heavier than genotype BB($P=0.05$); the average three-month weight of genotype DE was 2.42 kg heavier than genotype CD($P=0.05$) [17]. Zhang *et al.* (2007) showed that PRLR gene may be an effective gene or a closely related marker controlled the reproduction traits of Jining goat [12]. Mu *et al.* (2011) found that sheep of genotype AD were better than the other three in average little size and had a significant difference compared with the individuals of genotype AB ($P<0.05$) [26]. Several polymorphic sites have been detected within PRLR gene and statistically significant associations between PRLR variants and milk production traits have been described in dairy cattle [9, 28]. In addition, PRLR gene is a promising candidate gene that affects growth traits in cattle. Individuals with genotype BB had greater hucklebone width, body weight and average daily gain than those with genotype AA at 6 months old ($P<0.01$), as well as better body height, body length and heart girth when 6 months ($P<0.05$) [16]. Our results suggested the PRLR gene couldn't be selected as a candidate gene for the prolificacy of sheep.

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CONFLICT OF INTEREST

Authors declared no conflict of interest

REFERENCES

1. Chen SY, Duan ZY, Sha T. Origin, genetic diversity, and population structure of Chinese domestic sheep. *Gene*, 376 , 2006, 216-223.
2. Musa HH, Elamin FM, Suleiman AH. Reproduction and production performance of West African sheep in Sudan. *J Anim Vet Adv*, 4, 2005, 750-754.
3. Di R, Yin J, Chu MX. DNA polymorphism of introns 1 and 2 of prolactin receptor gene and its association with litter size in goats. *Animal Science Papers and Reports*, 29(4), 2011, 343-350.
4. Shiota M, Banville D, Ali S. Expression of two forms of prolactin receptor in rat ovary and liver. *Mol Endocrinol*. 4, 1990, 1136-1143.
5. Cassy S, Charlier M, Belair L. Developmental expression and localization of the prolactin receptor (PRLR) gene in ewe mammary gland during pregnancy and lactation: estimation of the ratio of the two forms of PRLR messenger ribonucleic acid. *Biology of Reproduction* 58(5), 1998, 1290-1296.
6. Kelly PA, Binart N, Freemantle M. Prolactin receptor signal transduction pathways and actions determined in prolactin receptor knockout mice. *Biochem Soc Trans*, 29, 2001, 48-52.
7. Omelka R, Martiniakova M, Peskovicova D. Associations between Alu I polymorphism in the prolactin receptor gene and reproductive traits of slovak large white, white meaty and landrace pigs. *Asian Aust J Anim Sci*, 21, 2008, 484- 488.
8. Bole-Feysot CH, Goffin V, Edery M. Prolactin (PRL) and its receptor: Actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev*, 19, 1998, 225-268.
9. Brym P, Kaminski S, Wojcik E. Polymorphism within the bovine prolactin receptor gene (PRLR). *Anim Sci Pap Rep*, 23, 2005, 61-66.
10. Ormandy CJ, Camus A, Barra J. Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. *Genes Dev*, 11, 1997, 167-178.
11. Van Rens BT, Evans GJ, van der Lende T. Components of litter size in gilts with different prolactin receptor genotypes. *Theriogenology*, 59, 2003, 915-926.
12. Zhang GX, Chu MX, Wang JY. Polymorphism of exon 10 of prolactin receptor gene and its relationship with prolificacy of Jining Grey goats. *Hereditas (Beijing)*, 29, 2007, 29- 336.
13. Barreras Serrano A, Herrera Haro JG, Hori-Oshima S. Prolactin receptor (PRLR) gen polymorphism and associations with reproductive traits in Pigs. *J Anim Vet Adv*, 8, 2009, 469-475.
14. Chu MX, Mu YL, Fang L. Prolactin receptor as a candidate gene for prolificacy of small tail han sheep. *Anim Biotechnol*, 18, 2007, 65-73
15. Li GL, Zhao ZS, Xue AY. Polymorphism Analysis for Exon 10 of Prolactin Receptor Gene in Sheep. *China Anim Husband Vet Med*, 36, 2009, 45-49.
16. Lu A, Hu X, Chen H. Single nucleotide polymorphisms of the prolactin receptor (PRLR) gene and its association with growth traits in Chinese cattle. *Mol Biol Rep*, 38, 2011, 261-6.
17. Wu HB, Wu HQ, Wang ZB. Relationship between polymorphism of exon 10 of prolactin receptor gene and reproduction trait of Chinese Merion Sheep. *J Shihezi Uni (Natural Sci)*, 29(1), 2011, 45-49.
18. Chu MX, Zhang GX, Wang JY. Polymorphism of prolactin receptor gene and its relationship with litter size of some goat breeds. *J Agri Biotech*, 16, 2008, 725-726.
19. Tomas A, Casellas J, Ramirez O. High amino acid variation in the intracellular domain of the pig prolactin receptor (PRLR) and its relation to ovulation rate and piglet survival traits. *J Anim Sci*, 84, 2006, 1991-1998.
20. Kmiec M, Terman A. Associations between the prolactin receptor gene polymorphism and reproductive traits of boars. *J Appl Genet*, 47(2), 2006, 139-141.
21. Iso-Touru T, Kantanen J, Li MH. Divergent evolution in the cytoplasmic domains of PRLR and GHR genes in Artiodactyla. *BMC Evol Biol*, 9, 2009, 172.
22. Bignon C, Binart N, Ormandy C. Long and short forms of the ovine prolactin receptor: cDNA cloning and genomic analysis reveal that the two forms arise by different alternative splicing mechanisms in ruminants and in rodents. *J Mol Endocrinol*, 19, 1997, 109-120.
23. Auchtung TL, Rius AG, Kendall PE. Effects of photoperiod during the dry period on prolactin, prolactin receptor, and milk production of dairy cows. *J Dairy Sci*, 88(1), 2005, 121-127.
24. Clevenger CV, Kline JB. Prolactin receptor signal transduction. *Lupus*, 10, 2001, 706-718.
25. Drogemuller C, Hamann H, Distl O. Candidate gene markers for litter size in different German Pig lines. *J Anim Sci*, 79, 2001, 2565- 2570.
26. Mu YL, Chu MX, Sun SH. PCR-SSCP Analysis on Prolactin Receptor Gene in Sheep. *Acta Veterinaria et Zootechnica Sinica*, 37, 2006, 956-960.
27. Chen Y, Luo QJ, Yang JQ. Relationships between Genetic Polymorphisms of Intron 9 and Exon 10 of Prolactin Receptor Gene and Litter Size of Sheep. *China Anim Husband Vet Med*, 37, 2010, 100-106.

28. Zhang JL, Zan LS, Fang P. Genetic variation of PRLR gene and association with milk performance traits in dairy cattle. *Can J Anim Sci*, 88, 2008, 33–39.