doi: 10.17221/58/2016-CJAS

Two Single Nucleotide Polymorphisms in the Caprine *GnIH* Gene Are Associated with Litter Size

*Xiaopeng An[1], Lijuan Bao[1], Jinxing Hou[1], Yueyu Bai[2], Xinyan Zhao[3], Yuxuan Song[1], Binyun Cao[1]**

[1]*College of Animal Science and Technology, Northwest A&F University, Yangling, P.R. China*

[2]*Animal Health Supervision Institute of Henan Province, Zhengzhou, P.R. China*

[3]*Northwest A&F University of Hospital, Northwest A&F University, Yangling, P.R. China*

**Corresponding author: caobinyun@126.com, anxiaopengdky@163.com*

ABSTRACT

An X., Bao L., Hou J., Bai Y., Zhao X., Song Y., Cao B. (2017): **Two single nucleotide polymorphisms in the caprine *GnIH* gene are associated with litter size**. Czech J. Anim. Sci., 62, 269–275.

Gonadotropin-inhibitory hormone (GnIH) can decrease luteinizing hormone and/or follicle-stimulating hormone levels in rat, mouse, sheep, and cattle by the direct suppression of gonadotropin-releasing hormone (GnRH). The present study investigated polymorphisms in the *GnIH* genes of two dairy goat breeds and their association with litter size. Single nucleotide polymorphisms (SNPs) g.1837C>G and g.3195G>A (GenBank Accession Nos. KR778885 and KR819142) were detected in the *GnIH* genes of Xinong Saanen and Guanzhong dairy goat breeds using DNA sequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Furthermore, the g.1837C>G and g.3195G>A loci were closely linked in both breeds ($r^2 > 0.33$). Association analysis showed that these SNPs had significant effects on the litter size of goats ($P < 0.05$). In both breeds, individuals with the *CC/GG* (g.1837C>G/g.3195G>A) genotype showed larger litter sizes in the second and average parities than individuals with the *GG/AA* genotype ($P < 0.05$). Known biochemical and physiological functions, along with our results, indicate that the *CC/GG* genotype may be used in marker-assisted selection to choose individuals with a larger litter size from both breeds.

Keywords: PCR-RFLP; SNPs; combined genotype; candidate gene

Gonadotropin-inhibitory hormone (GnIH) is a hormone that acts in opposition to gonadotropin releasing hormone (GnRH) in the pituitary, inhibiting the synthesis and secretion of luteinizing hormone (LH) and follicle-stimulating hormone(FSH) and suppressing copulation solicitation. It was first discovered in the brain of the Japanese quail in 2000 (Tsutsui et al. 2000; Han et al. 2016). It has been shown that the mammalian counterpart of the avian GnIH, RFamide-related peptide (RFRP), is expressed in hypothalamic neurons that innervate and inhibit GnRH neurons (Ubuka et al. 2012, 2013). *GnIH* gene is located on chromosome 4 in the caprine genome. GnIH precursor polypeptide is the precursor of three mature peptides, named RFamide-related peptide-1, -2, and -3 (RFRP-1, -2, and -3), in birds and two mature peptides (RFRP-1 and -3) in mammals (Bentley et al. 2010; Ubuka et al. 2012). GnIH orthologs have been identified in various vertebrates, including mammals, reptiles, amphibians, and teleosts. Bentley et al. (2008) reported the expression of GnIH and GnIH receptor

X. An and L. Bao contributed equally to this work.

doi: 10.17221/58/2016-CJAS

(GnIH-R) in the avian reproductive system, including gonads and accessory reproductive organs. A mammalian GnIH ortholog (RFRP-3) inhibits LH release in rats (Murakami et al. 2008) and cattle (Kadokawa et al. 2009). Clarke et al. (2008) also found that peripheral administration of the deduced ovine GnIH homolog, RFRP-3, reduced the amplitude of LH pulses in sheep. Likewise, in culture, RFRP-3 inhibited GnRH-stimulated LH and FSH secretion and was associated with a reduction in LHβ- and FSHβ-subunit expression (Clarke et al. 2008). Thus, it is thought that GnIH and its mammalian homolog RFRP-3 act to inhibit gonadotropin release in both birds and mammals. It was confirmed that different doses of GnIH/RFRP-3 inhibit the release and synthesis of testosterone and impact the expression of the genes encoding 3β-hydroxysteroid dehydrogenase (3β-HSD) and cytochrome P450 17A1 (CYP17A1), enzymes that play key roles in the synthesis of testosterone (Zheng et al. 2015). These findings suggest that GnIH may act as a neurohormone that affects reproductive traits.

Based on these findings, we hypothesized that the *GnIH* gene may be a candidate to select reproductive traits in goats. Considering these findings, we detected single nucleotide polymorphisms (SNPs) in the caprine *GnIH* gene using DNA sequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis and investigated the associations between these genetic markers and the litter size of dairy goats.

MATERIAL AND METHODS

Collection of blood samples and isolation of DNA. To detect polymorphisms in the caprine *GnIH* gene, blood samples were obtained from 622 goats belonging to the following two breeds: Guanzhong (GZ, n = 316) and Xinong Saanen (SN, n = 306) that were reared in the Fuping and Qianyang counties of Shaanxi Province, respectively. Their diets were based on alfalfa, corn silage, and a combination of concentrates, including corn, soya meal, and bone meal. Their health, fertility, and production records had been maintained by dairymen and veterinarians. Litter size data from the first to fourth parities were obtained from production records. Blood (5 ml) was aseptically collected from the jugular vein of each goat and stored in tubes containing the anticoagulant ACD (citric acid : sodiumcitrate : dextrose, 10 : 27 : 38). All of the samples were delivered to the laboratory on ice. Genomic DNA was extracted from white blood cells using a standard phenol-chloroform extraction protocol. All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Sequence analysis and SNP investigation. Based on the sequences of the caprine and bovine *GnIH* genes (GenBank Accession Nos. JF327669 and AC_000161), 14 pairs of primers were designed to amplify the caprine *GnIH* gene. Optimal annealing temperatures are listed in Table 1. The samples were then screened using pooled DNA sequencing to identify putative SNPs of this gene (Bansal et al. 2002). Approximately 5 µl of DNA (100 ng/µl) per sample were obtained from each goat to create a DNA pool for each goat breed. PCR products were forwarded to Invitrogen (Shanghai, China) for sequencing in both directions. SNPs were identified using Chromas 2.31 and DNAstar 7.0 software, and then the SNPs of the caprine *GnIH* gene were genotyped using PCR-RFLP. The reaction volume (25 µl) contained 50 ng of genomic DNA, 12.5 µl of 2 × reaction mix (including 500 µM of each dNTP; 20 mM Tris-HCl, pH 9; 100 mM KCl; and 3 mM $MgCl_2$), 0.5 µM of each primer, and 0.5 U of *Taq* DNA polymerase (TIANGEN, China). Cycling was performed as follows: 5 min at 95°C, 35 cycles of denaturation at 94°C for 30 s, annealing at X°C (for the values of X see Table 1) for 30 s, extension at 72°C for 35 s, and a final extension at 72°C for 10 min (Pasandideh et al. 2015). PCR products (5 µl) obtained using different primer pairs were mixed with 0.7 µl of 10 × RE buffer, 2.5 U of restriction enzyme (NEB, UK), and 3.8 µl of sterilized ddH_2O. The reaction mixtures were subsequently incubated for 1.5 h at 37°C. The restriction enzymes used in this study are shown in Table 2. Digestion products were subjected to electrophoresis on a 3.5% horizontal agarose gel. The genotypes were visualized on agarose gels stained with ethidium bromide.

Statistical analysis. SN and GZ breeds were analyzed separately. The allelic frequencies, heterozygosity (H_e), and polymorphism information content (PIC) of both breeds were calculated using the Popgene (Version 1.31) software. Linkage disequilibrium was analyzed using the SHEsis software (Shi and He 2005). An association analysis between

doi: 10.17221/58/2016-CJAS

SNPs and litter size for both breeds was performed using univariate analysis in the General Linear Model procedure performed using SPSS 16 software. Multiple comparisons of means were performed using the least significant difference method. The applied model was expressed as follows:

$$Y_{ikm} = \mu + G_i + S_k + e_{ikm}$$

where:

Y_{ikm} = measured trait from each of the ikm^{th} animal
μ = overall population mean
G_i = fixed effect associated with the i^{th} genotype or combined genotype
S_k = fixed effect associated with the k^{th} sire
e_{ikm} = random error

Effects associated with farm, birth year, and birth season are not matched in the linear model as preliminary statistical analyses indicated that these effects did not have a significant influence on the variability of traits in the analyzed populations.

RESULTS

SNPs identification and genotypes. Two SNPs were identified in *GnIH* after sequencing the amplicons arising from the use of different primer pairs: g.1837C>G (GenBank Accession No. KR778885) in intron 1 and g.3195G>A (GenBank Accession No. KR819142) in exon 2 (Figure 1). Although

Table 1. Primer information of *GnIH* gene for screening polymorphisms

Primer	Sequence (5'→3')	Gene region	Product size (bp)	T_m (°C)
PF1	ATGGTAGGTGAGCGAGAG	33	315	50
PR1	CCTGGTGGCACAATGAAT	347		
PF2	CATTCATTGTGCCACCAG	329	465	50
PR2	ATCCTACCTTCCACCTCAA	793		
PF3	GAGGTGGAAGGTAGGATGT	777	401	52
PR3	GCGTCAATTCTTGCGAGA	1177		
PF4	GACGCAAACACCAAAGAC	1173	421	50
PR4	GCTTGAAGTGGCTAACATC	1593		
PF5	ATGAAATTATTTCATTAAAACGAT	1540	550	51
PR5	GCGAATTTACATGAAGGCATAG	2089		
PF6	TAGATGGTGCAATTCCACTT	2032	486	50
PR6	CTCCCTAACTATCCCTCCTT	2517		
PF7	ACTGAAGGAGGGATAGTTAGG	2494	400	51
PR7	AGCAATCTAGTTTCTCCATCC	2893		
PF8	CGTTCCTCCTCCTTGAATAC	2798	370	51
PR8	TTGTTGACGGCAGGTGTA	3167		
PF9	TAGGCTGGGAGAAAGAAAG	3080	323	52
PR9	ATTGGTAGATGGTGAATGC	3402		
PF10	CCAAGACCCTGAGTAATTTG	3350	366	50
PR10	GCAGGCAGGTTCAGTAAT	3715		
PF11	GCACCGCAATTACTGAAC	3690	311	50
PR11	AGGCAAGAATACTGGAGTG	4000		
PF12	CAGTATTCTTGCCTGGAGA	3987	321	50
PR12	ATCTGAATTTGAAACCTGGG	4307		
PF13	ACCCAGGTTTCAAATTCAGA	4287	477	51
PR13	CTCATCCATCACAACAATAGC	4763		
PF14	CGGTAGAGTATGGCAATGA	4540	319	50
PR14	GACAGGCTCCAGATTTCTT	4858		

PF = forward primer, PR = reverse primer, T_m = annealing temperature

doi: 10.17221/58/2016-CJAS

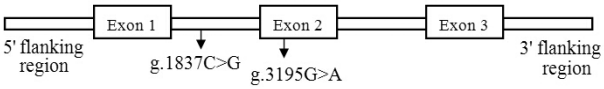

Figure 1. Schematic diagram of mutation sites in the caprine *GnIH* gene

the g.3195G>A SNP is present in an exon, it is a synonymous mutation (Pro89Pro of GnIH). The two SNPs were genotyped in the SN and GZ dairy goat breeds (Figures 2 and 3). The PICs were 0.37 at the g.1837C>G and g.3195G>A loci in both dairy goat breeds (Table 2). The genotypic distribution and allele frequencies of the two SNPs are shown in Table 2. To reveal the linkage relationships between the two SNPs, the degree of linkage disequilibrium was also estimated in both dairy goat breeds (Table 2). If r^2 was > 0.33 (Ardlie et al. 2002), the linkage disequilibrium was considered strong. Our results showed that the g.1837C>G and g.3195G>A loci were closely linked in both breeds (Table 2).

Table 2. Genotypic distribution of two single nucleotide polymorphisms loci in the *GnIH* gene

Locus	Restriction enzyme			Breed	
				SN	GZ
g.1837C>G	*Rsa*I	genotype	*CC*	76	102
			CG	173	137
			GG	57	77
		allele	*C*	0.53	0.54
			G	0.47	0.46
		He		0.57	0.43
		PIC		0.37	0.37
		equilibrium χ^2 test		$P = 0.02$	$P = 0.02$
g.3195G>A	*MspA1*I	genotype	*AA*	42	45
			GA	167	172
			GG	97	99
		allele	*A*	0.41	0.41
			G	0.59	0.59
		He		0.55	0.54
		PIC		0.37	0.37
		equilibrium χ^2 test		$P = 0.03$	$P = 0.03$
		LD		$r^2 = 0.42$	$r^2 = 0.41$
				$P = 0.00$	$P = 0.00$
				$D' = 0.73$	$D' = 0.70$

SN = Xinong Saanen, GZ = Guanzhong, LD = linkage disequilibrium of g.1837C>G and g.3195G>A, *He* = heterozygosity, PIC = polymorphism information content

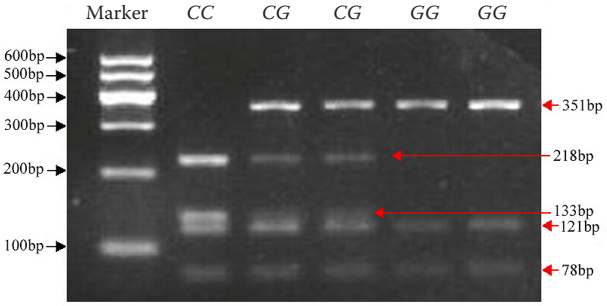

Figure 2. Agarose gel electrophoresis patterns obtained after digestion with *Rsa*I endonuclease at the g.1837C>G locus of *GnIH* gene

Effect of SNPs on litter size. At the g.1837C>G locus, individuals of the SN and GZ breeds with the *CC* genotype exhibited larger litter sizes than those with the *GG* genotype in the second, fourth, and average parities ($P < 0.05$; Table 3). At the same locus, individuals of the SN breed with the *CG* genotype showed larger litter sizes than those with the *GG* genotype in the average parity ($P < 0.05$). At the g.3195G>A locus, individuals of the SN breed with *GG* or *GA* genotypes showed larger litter sizes than those with the *AA* genotype in the second, third, and average parities ($P < 0.05$). At the same locus, individuals of the GZ breed with the *GG* genotype exhibited larger litter sizes than those with the *AA* genotype in the second, fourth, and average parities ($P < 0.05$). The results of the association analysis of the combined genotypes showed that individuals of the SN breed with the *CC*/*GG* (g.1837C>G/g.3195G>A) genotype had larger litter sizes than those with *CG*/*GA*, *CG*/*GG*,

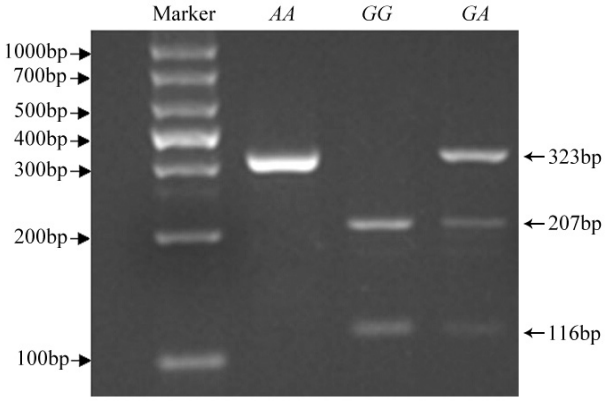

Figure 3. Agarose gel electrophoresis patterns obtained after digestion with *MspA1*I endonuclease at the g.3195G>A locus of *GnIH* gene

doi: 10.17221/58/2016-CJAS

Table 3. Association analysis of two single nucleotide polymorphisms with litter size of dairy goats (means ± standard errors)

Locus	Breed	Genotype	*n*	Litter size				
				1st parity	2nd parity	3rd parity	4th parity	average
g.1837C>G	SN	*CC*	76	1.65 ± 0.06^{b}	2.04 ± 0.05^{b}	2.07 ± 0.05	2.18 ± 0.04^{b}	1.98 ± 0.03^{c}
		CG	173	1.51 ± 0.04^{ab}	1.92 ± 0.03^{b}	2.08 ± 0.04	2.08 ± 0.03^{ab}	1.89 ± 0.02^{b}
		GG	57	1.46 ± 0.07^{a}	1.72 ± 0.06^{a}	1.95 ± 0.06	1.98 ± 0.05^{a}	1.78 ± 0.04^{a}
	GZ	*CC*	102	1.61 ± 0.05	1.93 ± 0.04^{b}	2.05 ± 0.05	2.28 ± 0.05^{b}	1.97 ± 0.03^{b}
		CG	137	1.53 ± 0.04	1.79 ± 0.03^{a}	1.97 ± 0.04	2.07 ± 0.04^{a}	1.84 ± 0.02^{a}
		GG	77	1.56 ± 0.06	1.73 ± 0.05^{a}	1.95 ± 0.06	1.96 ± 0.06^{a}	1.80 ± 0.03^{a}
g.3195G>A	SN	*AA*	42	1.52 ± 0.08	1.71 ± 0.07^{a}	1.86 ± 0.07^{a}	2.05 ± 0.06	1.79 ± 0.04^{a}
		GA	167	1.53 ± 0.04	1.93 ± 0.04^{b}	2.07 ± 0.04^{b}	2.07 ± 0.03	1.90 ± 0.02^{b}
		GG	97	1.55 ± 0.05	1.96 ± 0.05^{b}	2.10 ± 0.05^{b}	2.14 ± 0.04	1.94 ± 0.03^{b}
	GZ	*AA*	45	1.56 ± 0.08	1.71 ± 0.07^{a}	1.91 ± 0.08	1.98 ± 0.08^{a}	1.79 ± 0.04^{a}
		GA	172	1.52 ± 0.04	1.80 ± 0.03^{ab}	1.97 ± 0.04	2.05 ± 0.04^{a}	1.84 ± 0.02^{a}
		GG	99	1.63 ± 0.05	1.90 ± 0.05^{b}	2.06 ± 0.05	2.28 ± 0.05^{b}	1.97 ± 0.03^{b}

SN = Xinong Saanen, GZ = Guanzhong

$^{a–c}$values with different superscripts within the same column and mutation locus in particular breed differ significantly at $P < 0.05$

GG/*AA*, or *GG*/*GA* genotypes in the average parity ($P < 0.05$; Table 4). Likewise, individuals of the GZ breed with the *CC*/*GG* genotype had a larger litter size than those with *GG*/*AA*, *CC*/*GA*, *CG*/*GA*, *GG*/*GA*, or *GG*/*GG* genotypes in the fourth and average parities ($P < 0.05$).

Table 4. Combined effect of two single nucleotide polymorphisms loci on litter size in both dairy goat breeds

Breed	Combined genotype	*n*	Litter size				
			1st parity	2nd parity	3rd parity	4th parity	average
SN	*CC/AA*	3	2.00 ± 0.29	2.00 ± 0.26	1.67 ± 0.27	2.00 ± 0.22	1.92 ± 0.15
	CC/GA	18	1.61 ± 0.12	2.00 ± 0.11^{bc}	1.94 ± 0.11	2.11 ± 0.09	1.92 ± 0.06^{bc}
	CC/GG	55	1.64 ± 0.07	2.06 ± 0.06^{b}	2.13 ± 0.06^{b}	2.22 ± 0.05^{b}	2.01 ± 0.04^{b}
	CG/AA	7	1.57 ± 0.19	1.86 ± 0.17	1.86 ± 0.17	2.29 ± 0.14^{bc}	1.89 ± 0.10
	CG/GA	131	1.53 ± 0.04	1.94 ± 0.04^{bc}	2.10 ± 0.04^{b}	2.08 ± 0.03^{ac}	1.91 ± 0.02^{c}
	CG/GG	35	1.43 ± 0.09	1.86 ± 0.08^{ac}	2.03 ± 0.08	2.06 ± 0.06	1.84 ± 0.04^{ac}
	GG/AA	32	1.47 ± 0.09	1.66 ± 0.08^{a}	1.88 ± 0.08^{a}	2.00 ± 0.07^{ac}	1.75 ± 0.05^{a}
	GG/GA	18	1.44 ± 0.12	1.83 ± 0.11	1.94 ± 0.11	1.94 ± 0.09^{a}	1.79 ± 0.06^{ac}
	GG/GG	7	1.43 ± 0.19	1.71 ± 0.17	2.29 ± 0.17^{b}	2.00 ± 0.14	1.86 ± 0.10
GZ	*CC/AA*	2	1.50 ± 0.36	1.50 ± 0.32	2.00 ± 0.36	1.50 ± 0.35^{a}	1.63 ± 0.20
	CG/AA	7	1.57 ± 0.19	1.86 ± 0.17	2.14 ± 0.19	2.00 ± 0.19	1.89 ± 0.11
	GG/AA	36	1.56 ± 0.09	1.69 ± 0.08^{a}	1.86 ± 0.09^{a}	2.00 ± 0.08^{a}	1.78 ± 0.05^{a}
	CC/GA	26	1.54 ± 0.10	1.89 ± 0.09	1.89 ± 0.10	2.12 ± 0.10^{a}	1.86 ± 0.06^{a}
	CG/GA	115	1.51 ± 0.05	1.78 ± 0.04^{a}	1.97 ± 0.05	2.06 ± 0.05^{a}	1.83 ± 0.03^{a}
	GG/GA	31	1.55 ± 0.09	1.81 ± 0.08	2.07 ± 0.09	1.94 ± 0.09^{a}	1.84 ± 0.05^{a}
	CC/GG	74	1.64 ± 0.06	1.96 ± 0.05^{b}	2.11 ± 0.06^{b}	2.37 ± 0.06^{b}	2.02 ± 0.03^{b}
	CG/GG	15	1.60 ± 0.13	1.80 ± 0.12	1.93 ± 0.13	2.13 ± 0.13	1.87 ± 0.07
	GG/GG	10	1.60 ± 0.16	1.60 ± 0.14^{a}	1.90 ± 0.16	1.90 ± 0.16^{a}	1.75 ± 0.09^{a}

SN = Xinong Saanen, GZ = Guanzhong

$^{a–c}$values with different superscripts within the same column in particular breed differ significantly at $P < 0.05$

doi: 10.17221/58/2016-CJAS

DISCUSSION

In this study, the g.1837C>G and g.3195G>A loci were in Hardy–Weinberg disequilibrium in the SN and GZ goat breeds ($P < 0.05$), which means that the population analyzed may be subject to evolutionary forces such as selection, mutation or migration. PIC is related to the use efficiency and selection potential of genetic markers; the greater the PIC values and heterozygous ratio, the greater the potentials of the genetic markers, and their effects are better for animal genetic breeding (Botstein et al. 1980). PIC values are classified as low polymorphism when PIC is < 0.25, moderate polymorphism when 0.25 < PIC < 0.50, and high polymorphism when PIC is > 0.50 (Botstein et al. 1980). The two goat breeds have moderate genetic diversity at the g.1837C>G and g.3195G>A loci; therefore, both loci have a moderate potential as genetic markers.

The identification of candidate genes responsible for variation in quantitative traits has been a challenge in modern genetics (Fontanesi et al. 2014). Until now, no references have been given on the role of GnIH in the control of litter size in animals. In our study, SNPs g.1837C>G and g.3195G>A located in the *GnIH* gene are interesting markers for litter size in both goat breeds, but nothing indicates that these SNPs are more than simple markers. Reproductive traits are complex quantitative traits involving multiple genes, loci, and their interactions (Dimauro et al. 2015); therefore, the combined effects of multiple genes or loci on reproductive traits should be analyzed. In the present study, associations between both loci and litter size were analyzed from the first to fourth parities. The mean litter size of goats tended to increase in later parities. In both dairy goat breeds, individuals with the *CC*/*GG* genotype showed larger litter sizes than those with the *GG*/*AA* genotype in the second and average parities ($P < 0.05$). The litter size at the second parity is often a valuable index to determine whether a goat is prolific (Yuqin et al. 2011). Indeed, the *CC*/*GG* genotype may be used in marker-assisted selection to choose individuals with larger litter sizes from both dairy goat breeds. These results are consistent with the single SNP-trait association study and confirm that our previous single SNP-trait association was reliable.

CONCLUSION

This study explored the genetic polymorphism of the *GnIH* gene in the Guanzhong and Xinong Saanen breeds of dairy goat and indicated that SNPs g.1837C>G and g.3195G>A had significant effects on the litter size ($P < 0.05$). The *CC*/*GG* genotype may be used in marker-assisted selection to choose individuals with larger litter sizes from both dairy goat breeds.

REFERENCES

Ardlie K.G., Kruglyak L., Seielstad M. (2002): Patterns of linkage disequilibrium in the human genome. Nature Reviews Genetics, 3, 299–309.

Bansal A., van den Boom D., Kammerer S., Honisch C., Adam G., Cantor C.R., Kleyn P., Braun A. (2002): Association testing by DNA pooling: an effective initial screen. Proceedings of the National Academy of Sciences of the United States of America, 99, 16871–16874.

Bentley G.E., Ubuka T., McGuire N.L., Chowdhury V.S., Morita Y., Yano T., Hasunuma I., Binns M., Wingfield J.C., Tsutsui K. (2008): Gonadotropin-inhibitory hormone and its receptor in the avian reproductive system. General and Comparative Endocrinology, 156, 34–43.

Bentley G.E., Tsutsui K., Kriegsfeld L.J. (2010): Recent studies of gonadotropin-inhibitory hormone (GnIH) in the mammalian hypothalamus, pituitary and gonads. Brain Research, 1364, 62–71.

Botstein D., White R.L., Skolnick M., Davis R.W. (1980): Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics, 32, 314–331.

Clarke I.J., Sari I.P., Qi Y., Smith J.T., Parkington H.C., Ubuka T., Iqbal J., Li Q., Tilbrook A., Morgan K., Pawson A.J., Tsutsui K., Millar R.P., Bentley G.E. (2008): Potent action of RFamide-related peptide-3 on pituitary gonadotropes indicative of a hypophysiotropic role in the negative regulation of gonadotropin secretion. Endocrinology, 149, 5811–5821.

Dimauro C., Nicoloso L., Cellesi M., Macciotta N.P.P., Ciani E., Moioli B., Pilla F., Crepaldi P. (2015): Selection of discriminant SNP markers for breed and geographic assignment of Italian sheep. Small Ruminant Research, 128, 27–33.

Fontanesi L., Calo D.G., Galimberti G., Negrini R., Marino R., Nardone A., Ajmone-Marsan P., Russo V. (2014): A candidate gene association study for nine economically important traits in Italian Holstein cattle. Animal Genetics, 45, 576–580.

doi: 10.17221/58/2016-CJAS

Han X., Li J., Cao X., Du X., Meng F., Zeng X. (2016): Surgical castration but not immunocastration is associated with reduced hypothalamic GnIH and GHRH/GH/IGF-I axis function in male rats. Theriogenology, 86, 657–665.

Kadokawa H., Shibata M., Tanaka Y., Kojima T., Matsumoto K., Oshima K., Yamamoto N. (2009): Bovine C-terminal octapeptide of RFamide-related peptide-3 suppresses luteinizing hormone (LH) secretion from the pituitary as well as pulsatile LH secretion in bovines. Domestic Animal Endocrinology, 36, 219–224.

Murakami M., Matsuzaki T., Iwasa T., Yasui T., Irahara M., Osugi T., Tsutsui K. (2008): Hypophysiotropic role of RFamide-related peptide-3 in the inhibition of LH secretion in female rats. Journal of Endocrinology, 199, 105–112.

Pasandideh M., Mohammadabadi M.R., Esmailizadeh A.K., Tarang A. (2015): Association of bovine PPARGC1A and OPN genes with milk production and composition in Holstein cattle. Czech Journal of Animal Science, 60, 97–104.

Shi Y.Y., He L. (2005): SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Research, 15, 97–98.

Tsutsui K., Saigoh E., Ukena K., Teranishi H., Fujisawa Y., Kikuchi M., Ishii S., Sharp J.P. (2000): A novel avian hypothalamic peptide inhibiting gonadotropin release. Biochemical and Biophysical Research Communications, 275, 661–667.

Ubuka T., Son Y.L., Tobari Y., Tsutsui K. (2012): Gonadotropin-inhibitory hormone action in the brain and pituitary. Frontiers in Endocrinology, 3, 148.

Ubuka T., Son Y.L., Bentley G.E., Millar R.P., Tsutsui K. (2013): Gonadotropin-inhibitory hormone (GnIH), GnIH receptor and cell signaling. General and Comparative Endocrinology, 190, 10–17.

Yuqin W., Li Y.X., Zhang N.N., Wang Z.B., Bai J.Y. (2011): Polymorphism of exon 2 of BMP15 gene and its relationship with litter size of two Chinese goats. Asian-Australasian Journal of Animal Sciences, 24, 905–911.

Zheng L.C., Su J., Fang R., Jin M.M., Lei Z.H., Hou Y.L., Ma Z.Y., Guo T.T. (2015): Developmental changes in the role of gonadotropin-inhibitory hormone (GnIH) and its receptors in the reproductive axis of male Xiaomeishan pigs. Animal Reproduction Science, 154, 113–120.

Received: 2016–07–01
Accepted after corrections: 2017–02–13