IJAS Iranian Journal of Applied Animal Science

Polymorphism in FSHβ and FSHR Genes and Their Relationship with Productive and Reproductive Performance in Iran Black, Arman and Baluchi Sheep Breeds

Research Article

N. Nazifi[1*], G. Rahimi-Mianji[1] and Z. Ansari-Pirsaraii[1]

[1] Department of Animal Science, Faculty of Animal and Aquatic Science, Sari Agricultural Science and Natural Resources University, Sari, Iran

Received on: 11 Feb 2014
Revised on: 18 May 2014
Accepted on: 31 May 2014
Online Published on: Jun 2015

*Correspondence E-mail: narges.nazifi@gmail.com

Online version is available on: www.ijas.ir

ABSTRACT

The objective of the present study was to detect polymorphism in follicle stimulating hormone receptor (FSHR) and beta subunit of follicle stimulating hormone (FSHβ) genes and their relation to litter size and body weight trait in Baluchi, Iran black and Arman sheep breeds. PCR-RFLP technique using *ACCI* and *HinfI* enzyme treatments employed to detection of polymorphism in FSHβ marker site but detected no polymorphism pattern in this locus. SSCP analysis used for more survey on detection of polymorphism in FSHβ marker site which resulted in AA and AC genotypes in Baluchi sheep, AA and AB genotypes in Iran black population and no polymorphism pattern detected in Arman breed and all samples showed AA genotype. Polymorphism in FSHR gene has been detected by treatment with *MSCI* restriction enzyme. Three genotypes of AA, AB and BB were observed among studied breeds. The B allele was found with the highest frequency in all tree breeds. Analysis of variance for the effects of theses marker loci was carried out using SAS program. Mean comparison by Duncan test showed that the effect of each FSHβ and FSHR marker sites on litter size has significant effect only in Baluchi sheep population. It seems mutant alleles can improve considerably mean of litter size than the wild type ones. Analysis of data for body weight and polymorphisms in different ages showed that there are no significant differences between genotypes of FSHβ locus and body weight trait in various ages in none of Baluchi, Iran-black and Arman breeds. Survey on polymorphisms of FSHR locus and body weight trait revealed significant difference in some ages only in Arman breed and wild type allele caused better performance. This information can be used to plan breeding programs for sheep breeds aimed at improving production and reproduction performance.

KEY WORDS FSHβ, FFSHR, Polymorphism, litter size, body weight.

INTRODUCTION

The most important aims of sheep breeding are reproduction, growth and fleece traits. Production rate is related to reproduction rate. Out of reproduction traits, litter size (number of lamb born per ewe lambing), is one of the most favored selective trait that is also easy and inexpensive to measure. In general, heritability estimated for litter size is higher than the other reproductive traits such as fertility and lamb survived (weighted mean heritability: 0.10 and mean coefficient of variation: 36%) (Dickerson, 1970). Meat production in sheep can be increased by enhancing rate of reproduction, more favorite meat qualities and more growth to heaver market weights. Litter size is a multipolygenice trait with low heritability, so it is difficult to improve (An *et al.* 2010). Marker assisted selection (MAS) method integrates molecular genetics and artificial selection effects on low heritability traits such as litter size by changing selec-

tion time, selection intensity and accuracy (Simoni *et al.* 1997; An *et al.* 2010). There are some candidate genes which accelerate breeding programs in reproduction. Litter size is affected by some major gene such as BMPRIB (Fogarty *et al.* 2009), BMP-15 (Chu *et al.* 2007), GDF9 (Hanrahan *et al.* 2004), FSH (Faure *et al.* 2005). For example in previous researches on Iranian Breeds, investigation of exon 2 of GDF9 gene in Tali and Beetal goats resulted no nucleotide change in Tali goats, but individuals with high rate of kidding records in Beetal goats were heterozygote by a mutation (C to T) and amino acid variation (alanine to valine) in studied fragment, but the others were wild type (Hadizadeh *et al.* 2013). Another survey beyond to study on BMP15 gene, which is one of candidate gene on increasing ovulation rate and infertility in sheep, on Red Jabalbarez goat which demonstrate no mutation side (Alinaghizadeh *et al.* 2010).

Follicle stimulating hormone (FSH) is one of the most important hormones in mammalian reproduction, development of gonads and their maturation at puberty (Simoni and Nieschlag, 1995; Chappel and Howles, 1991). FSH is composed of two subunits, alpha subunit which determines same in all spacious and hormone-specific beta subunit by specific biological effect which consists of 3 exons and 2 introns (Yaofeng *et al.* 1998). FSH acts by binding to specific receptor on gonads which belonged to G-portion coupled receptor. Intracellular portion of receptor of FSH commence series of actions by linkage to G-portion causes biological effects on gonadotropin (Simoni and Nieschlag, 1995). FSH receptor (FSHR) by its N-terminal domain has high affinity binding of hormone. FSHR provided 10 exons and 9 introns, the extracellular domain encoded by the first 9 exons and the last one encode transmembrane and intracellular domain (Segaloff and Ascoli, 1993). Insufficient FSH causes follicle fail to develop in early stage (Gemzell, 1975).

There is less information about these genes in sheep than the other animals. Baluchi sheep is one of the most numerous Iranian sheep with major rules in meat production in Iran which is adopted with wide range of dry environmental of eastern Iran (Tahmoorespur and Sheikhloo, 2011). Iran-black is synthetic breed resulted from crossing between Baluchi and Chios breed (Rashidi, 2014) and Arman sheep was obtained by crossbreeding of four breeds of Chios, Saffolk, Ghezel and Baluchi sheep (Lotfi *et al.* 2011). Determination of the genetic variability of indigenous Iranian sheep in respect to these important genes has not been sufficiently studied.

The objective of the present study was to detect polymorphism in follicle stimulating hormone receptor (FSHR) and beta subunit of follicle stimulating hormone (FSHβ) genes and their relation with litter size and body weight traits at different ages in Iran black, Arman as synthesized and Baluchi as indigenous sheep breeds.

MATERIALS AND METHODS

Experimental ewes and sampling

Blood samples for the extraction of DNA were randomly obtained from 161 individuals of Iran-black (n=53), Arman (n=50) and Baluchi (n=58) sheep from Abbasabaad sheep breeding station in Mashhad, Khorasan-e-Razavi. Approximately 5 mL blood per sheep was collected aseptically by jugular vein puncture, kept in a tube containing EDTA and transported to the laboratory in cold conditions. Genomic DNA was extracted using salting–out method with a minor modification (Miller *et al.* 1998) and stored in -20 ˚C until used for assay

PCR conditions

To amplify a segment with 304bp length of FSHR gene a pair of PCR primers, forward: 5-CCCATCTTTGGCATCAGC-3 and reverse: 5-ACACAGTGATGAGGGGCAC-3 and a segment with 247 bp length of exon 2 of FSHβ gene a pair of PCR primers, forward: 5-ACTCAGGACTTGGTGTAC-3 and reverse: 5-CTGCTGCTCTTTATTCTC-3 was used as described by An *et al.* (2010), respectively. Polymerase chain reaction (PCR) was conducted in 25 µL total volume containing approximately 50-100 ng genomic DNA, 3 µL of $MgCl_2$, 2.5 µL of 10x PCR buffer (500 m*M* KCl and Tris-HCl (pH 8.4)), 0.6 µL each of forward and reverse primers (10 Pmol each), 0.5 µL of 10 m*M*dNTPs (0.2 m*M* each) and 1 unit of Smart Taq DNA polymerase (CinnaGen, Iran). In PCR optimal conditions for FSHβ gene after denaturation at 94 ˚C for 5 min. 35 amplification cycles were performed as denaturation at 94 ˚C for 30 s, annealing at 47 ˚C for 30 s, extension at 72 ˚C for 45 s, followed by final extension at 72 ˚C for 10 min.

The thermal cycling profile for FSHR gene consisted of 5 min at 95 ˚C followed by 35 amplification cycles of denaturing at 94 ˚C for 45 s, annealing at 56 ˚C for 30 s, extending at 72 ˚C for 1 mine, with a final extension at 72 ˚C for 10 min. After electrophoresis in 1.5% agaros gel the DNA fragments were stained with ethidium bromide and documented using BioRad gel documentation system (BioRad, USA).

Restriction fragment length polymorphism

Restriction fragment length polymorphism method was performed at 37 ˚C for 12 to 14 h for PCR products of FSHβ gene by *ACCI* and *HinfI* enzyme treatment. The ampliers of FSHR genes were digested by *MSCI* restriction enzyme. Digestion reactions were carried out by fol-

lowing profiles: a final volume of 15 µL containing 7 µL of each PCR products were treated with 0.2 µL of each enzyme, 1 µL Buffer and 6.8 µL distilled deionized water. Detection of the products has been done on 2% Agaros gel in the presence of DNA size marker (Gene Ruller^{TM} 100 bp). Then the gel was stained with ethidium bromide and subsequently visualized by gel documentation system (BioRad, USA).

Single-strand conformation polymorphism and cloning

Single strand conformation polymorphism (SSCP) analysis has been used to attempt to determine mutation in FSHβ loci. This analysis was performed as described by some studies, with some modifications. A 2 µL aliquot of each PCR product was mixed with 8 µL of denaturing solution (98% Formamide, 10 m*M* EDTA, 0.025% Bromophenol blue, 0.025% Xylene-cyalon). The mixtures were heated for 10 min. at 98 ˚C and were chilled on ice, immediately. Denatured DNA samples were subjected to PAGE (12%) in 1 × TBE buffer and constant voltage (400V) for 16 h at 4 ˚C. The gel (Acryl amide: bis=29:1) was stained with 0.1% silver nitrate (Bassam *et al.* 1991) and visualized by gel documentation system (BioRad, USA). The PCR product which represent unique PCR-SSCP genotypes were cut from agaros gel and purified using the QIA quick PCR purification Kit (QIA Gene, Germany). Each purified PCR product was ligated into P-GEM-T vector, according to the manufacturer's instructions (Qiagen, Germany). The ligation mixture was transformed into *Escherichia coli* DH5α competent cells. Positive clones were identified by standard techniques (Blue White screening). Insert positive clones were identified by PCR and restriction enzyme digestion. Plasmids from the selected clones were extracted using QIA prep Spin Mini prep Kit (QIA Gene, Germany). Selected samples were sequenced in both directions using M13 forward and reverse primers (Bio Neer, Korea).

Sequence analysis

To survey whether the sequences are FSHβ or not, deduced sequences was subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov/). Alignment of sequences were carried out in multiple sequence alignment (http://multalin.toulouse.inra.fr/multalin/).

Statistical analysis

Allele and genotype frequencies of each candidate genes were calculated by direct counting. Calculation of Chi-square test ($\chi 2$) for deviation from Hardy-Weinberg equilibrium (HWE) has been assessed by Pop-gen (Ver. 1.31) software. The relationship between genotypes and average body weight and litter size has been analyzed with the PROC GLM procedures in Statistical Analysis System (SAS) v. 9.1 version (SAS, 2006). The following fixed effect model was employed for analysis of litter size:

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

Where:
Y_{ijk}: phenotypic value of litter size.
μ: average of litter size in studied population.
P_i: fixed effect of the i_{th} parity (i=1, 2).
G_j: fixed effect of the j_{th} genotype.
e_{ijk}: effect of the random error of each observation.

The following general linear model was used for analysis of body weight gain:

$$Y_{ijklm} = \mu + G_i + S_j + D_k + P_m + b(X_{ijkm} - \overline{X}) + e_{ijkm}$$

Where:
Y_{ijkl}: phenotypic value of body weight traits.
μ: average of favorable trait.
G_i: fixed effect of i_{th} genotype.
S_j: random effect j_{th} sire.
D_k: random effect of k_{th} dam.
P_m: fixed effect of m_{th} sex.
b: linear regression coefficient for the weight of the lamb at birth.
$X_{ijklmny}$: lamb's weight at birth corresponding to Y_{ijkl}.
$\overline{X}$: arithmetic mean of lamb weight at birth.
e_{ijk}: random residual effect.

RESULTS AND DISCUSSION

Allele frequency and genotype

Banding visualization using size marker confirmed correct specific amplification of two studied loci (Figure 1 and 2), but RFLP-PCR analysis of FSHβ marker site showed no polymorphism (Figure 4 and 5) and different banding patterns were identified using SSCP-PCR method (Figure 3). Allele and genotype frequencies, the observed heterozygosity and test for HWE of this marker site presented in Table 1. Three different banding patterns of FSHR gene been observed by 304 bp length when *MSCI* endonuclease was used for digestion. In some samples cleavage site was omitted which resulted to BB genotype, for AA genotype cleavage site was exist and digestion reaction resulted to tow fragments of 90 and 214 bp, whereas in AB genotype three fragments of 90, 214, 304 bp was observed (Figure 6). Allele and genotype frequencies, the observed heterozygosity and test for HWE of FSHR locus shown in Table 2.

Despite of low heterozygosity in FSHβ gene fragment, a high value of heterozygosity for *MSCI* marker site of FSHR gene observed breeds (Table1and 2).

Calculated chi-square test showed significant deviation from Hardy-Weinberg equilibrium for *MSCI* marker site in FSHR locus in Iran-black, Arman and Baluchi population (Table1 and 2).

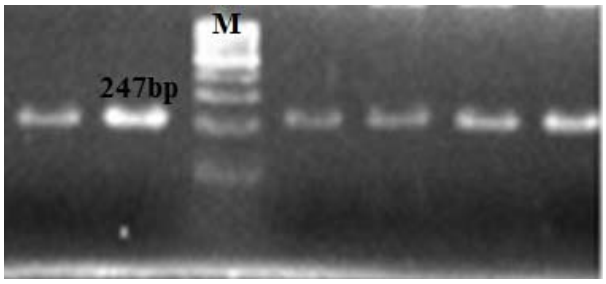

Figure 1 Result of PCR technique for FSHβ loci; M: 100bp size marker SM0321

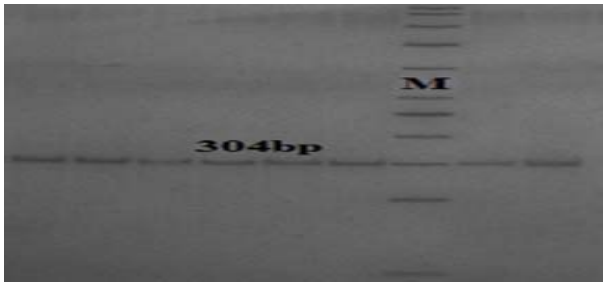

Figure 2 Result of PCR technique for FSHR loci; M: 100bp size marker SM0321

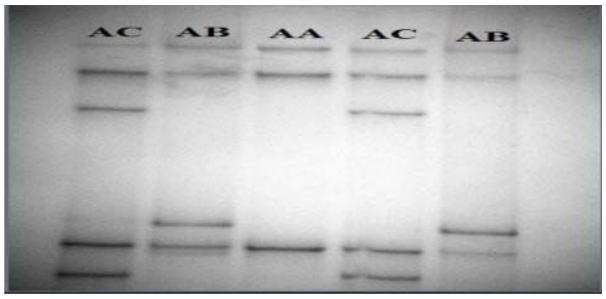

Figure 3 SSCP pattern of analysis of FSHβ gene

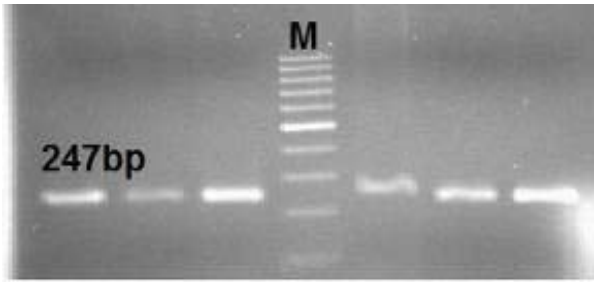

Figure 4 RFLP pattern of FSHβ gene by *ACCI* enzyme

Sequences of different genotype of FSHβ gene

Expected sequence length of FSHβ marker site (247 bp) was confirmed and deposited in GeneBank database (accession number, KF158984-6). Analysis of nucleotide sequence has been proved three different genotypes.

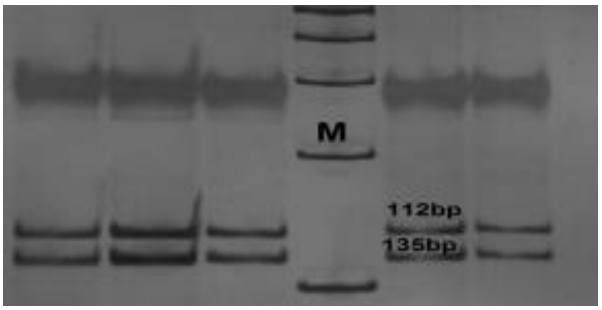

Figure 5 RFLP pattern of FSHβ gene by *HinfI* enzyme

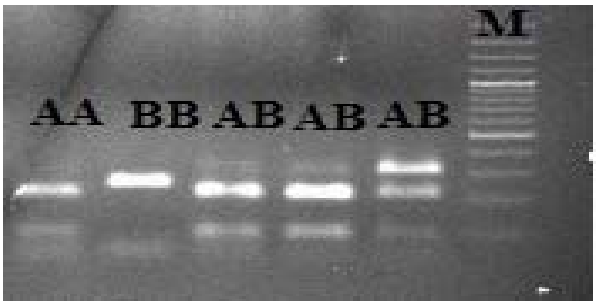

Figure 6 RFLP pattern of FSHR gene by *MSCI* enzyme

The T nucleotide at position 102 of FSHβ marker site changed into A or C in genotypes AA, AB and AC, respectively (Figure 7). These conversions do not result in amino acid changes because of synonymous substitution.

Association analysis between variants and litter size

Association analysis of genotypes and parities on litter size has been shown a significant effect on litter size only in Baluchi sheep breed in both studied genes ($P<0.05$). Polymorphisms of FSHβ marker site demonstrated that ewes with AC genotype had higher mean of litter size (1.70) than AA genotype (Table 4) andpolymorphism patterns of FSHR marker site has shown that ewes with BB genotype (mutant genotype) with higher mean of litter size (1.56) than AA genotype (wild genotype) ($P<0.05$; Table 3).

The highest mean values for the effect of parity on litter size were observed at 2nd parity in Baluchi sheep (1.61) mean of litter size and 1st parity in Iran-Black breed ($P<0.05$). The difference in mean litter size between parities in Arman sheep breed was not significant (Table 4).

Association analysis between variants and body weight traits

Polymorphisms of FSHβ marker site have not significant effect on body weight in none of studied breeds (Table 5).

Figure 7 Sequence comparison of A, *B* and *C* alleles of *FSH*β marker site

Table 1 Gene and genotype frequency of FSHβ gene detected by SSCP-PCR

Breed	Genotype frequency			Allele frequency		O-Het	χ2-value
	AA	AB	AC	A	B or C		
Baluchi	91.38	0	8.62	95	5	0.08	0.09*
Iran-black	94.34	5.66	0	92.46	7.54	0.05	0.03*
Arman	100	0	0	100	0	0	-

* (P<0.05).

Table 2 Gene and genotype frequency of FSHR gene detected by *MSCI* endonuclease

Breed	Genotype frequency (%)			Allele frequency (%)		O-Het	χ2-value
	AA	AB	BB	A	B		
Baluchi	25.86	43.10	31.03	47.42	52.58	0.43	0.97*
Iran-black	10	60	30	40	60	0.61	2.88*
Arman	20.75	50.94	28.30	47	53	0.51	0.07*

* (P<0.05).

Table 3 Association analysis of FSHR and FSHβ genotypes on litter size

	SE of FSHR loci genotypes±mean				SE of FSHβ loci genotypes±mean			
Breed	AA	AB	BB	P-value	AA	AB	AC	P-value
Baluchi	0.8^{b}±1.21	0.05^{b}±1.26	0.07^{a}±1.56	0.003*	0.14^{b}±1.30	-	0.04^{a}±1.70	0.008*
Iran-black	0.10±1.18	0.08±1.08	0.10±1.17	0.7893	0.21±1.66	0.05±1.46	-	0.34
Arman	0.13±1.25	0.8±1.37	0.10±1.51	0.3485	-	-	-	-

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The FSHR marker site showed the same trends as FSHβ on body weight traits in Baluchi and Iran-black population but, a significant effect on body weight observed in Arman breed. The BB genotype showed lower body weight than AA genotype at all 3, 6, 9 and 12 month ages (Table 6).

Reproduction is known as a quantitative trait with polygenic heredity which is affected by different genes. Ovulation rate in mammals determined by complicated exchange hormonal signal between pituitary gland and ovary and also exchange hormonal signal inside the follicles between oocytes and somatic cells (McNatty *et al.* 2005). FSH hormone as a pituitary glycoprotein with alpha and beta subunits has especial role in simulation of regulation follicles in ovary.

Pathway of transmission of FSH signals is through its receptor that counteract with beta subunit, exclusively (Zhao *et al.* 2010).

Infertility in animals is concluded by FSHR insufficient (De Castro *et al.* 2003). Because of more variability in ovulation rate in different strains of sheep, this animal has been considerate as a valuable model in control genetically of ovulation rate (Montgomery *et al.* 1995).

Table 4 Association analysis of parity (Mean ± SE of parity) on litter size

Breeds	1st parity	2nd parity	P-value
Baluchi	0.08b±1.38	0.08a±1.61	0.008*
Iran-black	0.12a±1.58	0.12b±1.36	0.04*
Arman	0.08±1.45	0.08±1.33	0.034

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Detection of polymorphism in Exon 2 of FSHβ marker site using SSCP-PCR resulted AA and AC genotypes in Baluchi breed, AA and AB genotypes in Iran black breed, and AA homozygote genotype in Arman breed.

Table 5 Association of polymorphism loci of FSHβ and body weight (Mean±SE) traits

Breeds	Genotype	BW0 (kg)	BW3 (kg)	BW6 (kg)	BW9 (kg)	BW12 (kg)
Baluhci	AA	4.17±0.9	22.04±0.51	32.11±0.56	33.55±0.70	38.45±0.77
	AC	4.14±0.25	22.80±1.59	34.40±1.69	35.80±2.20	38.2±3.18
Iran-black	AA	4.06±0.11	22.04±0.55	32.64±0.58	34.30±0.52	35.70±0.60
	AB	3.17±0.38	32.67±1.33	31.67±2.73	33.33±2.3	35.67±0.67

Table 6 Association of polymorphism loci of FSHR and body weight (Mean±SE) trait

Breeds	Genotype	BW0 (kg)	BW3 (kg)	BW6 (kg)	BW9(kg)	BW12 (kg)
Baluhci	AA	4.31±0.12	22.67±0.94	32.14±1.06	33.71±1.21	38.81±1.26
	AB	4.07±0.11	21.44±0.67	32.11±0.71	33.63±0.99	37.65±1.10
	BB	4.12±0.33	22.89±0.92	33.56±1.09	33.75±1.56	37.63±2.02
Iran-black	AA	4.17±0.26	20.11±1.26	31.11±1.27	34.00±1.05	33.89±1.24
	AB	3.91±0.15	22.97±0.66	33.03±0.76	34.47±0.75	36.50±0.77
	BB	4.14±0.22	21.27±0.99	32.55±1.05	33.82±0.55	35.00±0.93
Arman	AA	4.00±0.14	22.12±0.69^{a}	32.55±0.77^{a}	34.87±0.84^{a}	40.45±1.04^{a}
	AB	4.10±0.15	21.94±0.62^{a}	31.33±0.81^{ab}	34.28±0.84^{ab}	39.50±1.79^{ab}
	BB	4.75±0±0.25	19.25±0.75^{b}	28.50±0.87^{b}	31.00±1.35^{b}	33.75±1.25^{b}

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Sequence analyzes by Vector NTI software (version. 9) showed that the restriction endonuclease *BsiHKAI* enzyme could be informative marker for the amplified fragment. However, further investigation is needed.

However, the A allele was the most frequent allele in thesebreeds. In spite of the lack of relationship between FSHβ genotypes and body weight traits in these breeds, a significant association was observed between AC genotype and litter size in Baluchi sheep. Similar study carried out by An *et al.* (2010) on this marker site and has shown tow mutations (G40A and T148C) which were associated with litter size in Xinong and Boer goat breeds. Liang *et al.* (2006) found this marker site in Jining Grey goat as a high prolific breed and Cashmere, Boer and Angora goat as low prolific breed.

They have reported that substitution of nucleotide G to A at position 94 in exon 2 of FSHβ gene changed Alanin amino acid into Threonine which creates BB genotype while conversion of C to T at nucleotide 174 didn't have any amino acid variation and results CC genotype. They detected AA, AB and AC genotypes in Jining Grey and Cashmere goats and AA, AC and CC genotypes in Boer goat and AA, BB, CC, AB, AC and BC genotypes in Angora goat. They reported that AA genotype was the best one in prolificacy than AB and AC genotypes in Jining Grey goat, whereas in our study none of the observed mutation caused amino acid variation.

Zhang *et al.* (2011) identified a new mutation (A2645G) in exon3 of FSHβ which changed Glutamine to Arginine and resulted AA genotype in a low prolific Boer and high prolific Matou goat breed. They have reported that the observed genotypes at this marker site had a significant effect on litter size and litter weight at birth in Boer does and better superovulation performances and litter size in Matou breed Since FSH known as essential hormone for growth, propagation and perpetuity follicles, recruitment cycle of development of follicles from early antral stage through maturation up to ovulation (McGee *et al.* 2000) and superovulation, it determined as important factor in higher litter size and heavier litter weight at birth in mammals (Hunter *et al.* 2004). Zhao *et al.* (1998) had a survey in porcine FSH beta subunit and demonstrated that FSH beta locus is closely associated with litter size in commercial pig breeds, such as Yorkshire, Landrace, Durco. They have shown that sows with AA genotype had 1.5 piglets more than sows with BB ones. Also Chen *et al.* (2001) introduced FSHβ and ESR loci as major gene affecting litter size in pigs. Wang *et al.* (2006) investigated polymorphism of FSHβ gene in French Large White and Landrace swine. They have found two AB and BB genotypes but reported there was no significant association between this marker site and litter size trait. Hua *et al.* (2006) reported no polymorphism in FSHβ gene exon 1 and 2 of FSHβ gene in Haimen and Matou goats. Results published from Xu *et al.*

(2003) and Li *et al.* (2004) find no association between various genotypes of FSHβ gene and litter size in Jinhua and Min pigs.

Investigation of possible association between litter size trait and polymorphisms in FSHR gene promised a significant association between litter size and BB genotype in Baluchi breed.

Also, body weight analysis showed that polymorphisms in FSHR gene proved that A allele (the wild type one) in Arman breed causes better performance than the B allele Majority of previous researches showed discordant results such as: Li *et al.* (2010) survey on exon 10 of FSHR gene on Chinese Himan goat using PCR-SSCP technique, Yun *et al.* (2007) on 5' flank region of FSHR gene on Boer goat, Ganjong goat, Xinong Saanen goat, Shannan whait goat using PCR-RFLP technique that showed no polymorphism pattern. Lan *et al.* (2006) which surveyed exon 10 of FSHR gene in Shannan whait, Guanzhong and Boer goat has reported no polymorphism pattern in these goats. Yang *et al.* (2010) detected possible association between polymorphism in FSHR gene and super ovulation Chinese Holstein cows. They have found two different single nucleotide polymorphisms of G-278A and A-320T, where the former mutation resulted CC, CD and DD genotypes and the latter mutation resulted AA, AB and BB genotypes.

The results indicated that the wild type genotype (CC) has significantly increased in the total number of ova ($P<0.01$), and produced more transferable embryos but for second mutation no statistical significant association were found among different genotypes and super ovulation traits.

CONCLUSION

In conclusion, increasing in reproduction activity and lambing rate are very important economic factors in sheep breeding industry. FSHR and FSHβ gens are two major gens in reproduction traits. The possible effect of both studied marker loci based on sheep breeds. The mutant alleles from both loci significantly increased litter size in Baluchi sheep, while the wild type allele showed better performance in body weight traits in Arman sheep breed. It was concluded that selection based on mutant allele of these tow loci in Baluchi sheep as one of the populated indigenous sheep can be exploited in breeding programs for improving reproduction performance in Baluchi sheep. But with the aim of improvement of growth traits selection based on wild type seems be better decision. Then this results can be used in animal breeding programs belonged to Iranian sheep breeds. Further study with higher sample size is needed to clarify the exact role of these marker loci on reproduction and production traits in studied sheep populations.

ACKNOWLEDGEMENT

This research was supported by Laboratory of Molecular genetics and Animal Biotechnology, Department of Animal Science, Sari Agricultural Science and Natural Resources University, Sari, Iran. We thank to researchers in Abbas Abad Sheep Breeding Center for their help in providing samples and data for using in this study.

REFERENCES

Alinaghizadeh H., Mohammadabadi M.R. and Zakizadeh S. (2010). Exon 2 of BMP15 gene polymorphismin Jabal Barez red goat. *J. Agric. Biotechnol*. **2(1),** 69-80.

An X.P., Han D., Hou J.X., Li G., Wang Y.N., Li L., Zhu G.Q., Wang J., Son Y.X. and Cao B.Y. (2010). Polymorphism of exon 2 of FSHβ gene and its relationship with reproduction performance in two goat breeds. *Agricu. Sci. China.* **9(6),** 880-886.

Bassam B.J., Caetano-Anolles G. and Gresshoff P.M. (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem*. **196,** 80-83.

Chappel S.C. and Howles C. (1991). Revaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. *Hum. Reprod*. **6,** 1206-1212.

Chen K.F., Li N., Huang L.S., Zhang Q., Zhang J.S., Sun S.Q., Luo M. and Wu C.X. (2001). The combined genotypes effect of *ESR* and *FSH* beta genes on litter size traits in five different pig breeds. *Chinese Sci. Bull*. **46,** 140-143.

Chu M.X., Jiao C.L., He Y.Q., Wang J.Y., Liu Z.H. and Chen G.H. (2007). Association between PCR-SSCP of bone morphogenetic protein 15 gene and prolificacy in Jining Grey goats. *Anim. Biotechnol*. **18,** 263-274.

De Castro F., Ruiz R., Montoro L., Hernandez D., Padilla E., Real L.M. and Ruiz A. (2003). Role of follicle-stimulating hormone receptor Ser680Asn polymorphism in the efficacy of follicle stimulating hormone. *Fertil. Steril.* **80,** 571-576.

Dickerson G.E. (1970). Efficiency of animal production molding the biological components. *J. Anim. Sci.* **30,** 849-859.

Faure M.O., Nicol L., Fabre S., Fontaine J., Mohoric N., McNeilly A. and Taragnat C. (2005). BMP-4 inhibits follicle stimulating hormone secretion in ewe pituitary. *J. Endocrinol.* **186,** 109-121.

Fogarty N.M. (2009). A review of the effects of the Booroola gene (FecB) on sheep production. *Small Rumin. Res.* **85,** 75-84.

Gemzell C. (1975). Induction of ovulation in infertile women with pituitary tumors. *Am. J. Obstet. Gynecol.* **121,** 311-315.

Hadizadeh M., Mohammadabadi M., Niazi A., Esmailizadeh A., Mehdizadeh Gazooei Y. and Molaei Moghbeli S. (2014). Use of bioinformatics tools to study exon 2 of GDF9 gene in Tali and Beetal goats. *Mod. Genet. J*. **8(3),** 283-288.

Hanrahan J.P., Gergan S.M., Mulsant P., Mullen M., Davis G.H., Powell R. and Galloway S.M. (2004). Mutations in the genes for oocyte derived growth factors GDF 9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biol. Reprod*. **70,** 900-909.

Hua G.H. (2006). Polymorphism analysis of five candidate genes related to reproduction traits and their effects on litter size in four goat breeds. MS Thesis. Huazhong Agric. Univ., Wuhan, Chinese.

Hunter M.G., Robinson R.S., Mann G.E. and Webb R. (2004). Endocrine and paracrine control of follicular development and ovulation rate in farm species. *Anim. Reprod. Sci*. **82,** 461-477.

Lan Y.X., Chen H., Chen C.Y., Pan C.Y., Lie C.Z., Zhang Y.D. and Yu J. (2006). PCR-SSCP detection and DNA sequence analysis of exon 10 goat follicle-stimulating hormone receptor (FSHR) gene. *J. Agric. Biotecnol*. **14,** 484-488.

Li J., Yang R.Q., Meng H. and Pan Y.C. (2004). Study on four candidate genes of litter size in Min pig. *J. Shanghai Jiaotong Univ. Agric. Sci*. **22(1),** 74-77.

Li Y.J., Zhang L., Shang L.Q., Wang H.F., ZOU H., Zhang H. and Ji D.J. (2010). Genetic polymorphisms at three loci of PRLR and FSHR gene correlate with litter size in Chinese Haimen goat. *J. Anim. Vet. Adv*. **9(22),** 2835-2838.

Liang C., Chu M.X., Zhang J.H., Liu W.Z., Fang L. and YE S.C. (2006). PCR-SSCP polymorphism of FSHß gene and its relationship with prolificacy of jining Grey goats. *Hereditas*. **28,** 1071-1077.

Lotfi Farokhad M., Roshanfekr R., Amiri S., Mohamadi K. and Mirzadeh K.H. (2011). Genetic trends estimation for some of the growth traits in Arman sheep. *J. Anim. Vet. Adv*. **10(14),** 1801-1803.

McGee E.A. and Hsueh A.J. (2000). Initial and cyclic recruitment of ovarian follicles. *Endocrinol. Rev*. **21,** 200-214.

McNatty K.P., Smith P., Moore L.G., Reader K., Lun S., Hanrahan J.P., Groome N.P., Laitinen M., Ritvos O. and Juengel J.L. (2005). Oocyte-expressed genes affecting ovulation rate. *Mol. Cell. Endocrinol.* **234(1),** 57-66.

Miller S.A., Dykes D.D. and Polesky H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids. Res.* **16,** 1215-1219.

Montgomery G.W., Tate M.L., Henry H.M., Penty J.M. and Rohan R.M. (1995). The follicle-stimulating hormone receptor and luteinizing hormone receptor genes are closely linked in sheep and deer. *J. Mol. Endocrinol.* **15,** 259-265.

Rashidi A. (2012). Genetic parameter estimates of body weight traits in Iran-Black sheep. *J. Livest. Sci. Technol.* **1(1),** 54-60.

SAS Institute. (2006). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Segaloff D.L. and Ascoli M. (1993). The lutropin / choriogonadotropin (LH.CG) receptor 4 years later. *Endocrinol. Rev.* **14,** 324-347.

Simoni M. and Nieschlag E. (1995). FSH in therapy: physiological basis, new preparations and clinical use. *Reprod. Med. Rev.* **4,** 163-177.

Simoni M., Gromoll J. and Nieschlag E. (1997). The follicle-stimulating hormone receptor biochemistry, molecular biology, physiology and pathophysiology. *Endocrinol. Rev.* **18,** 739-773.

Tahmoorespur M. and Sheikhloo M. (2011). Pedigree analysis of the closed nucleus of Iranian Baluchi sheep. *Small Rumin. Res.* **99(1),** 1-6.

Wang X., Wang A., Fu J. and Lin H. (2006). Effects of ESR1, FSHB and RBP4 genes on litter size in a Large White and a Landrace Herd. *Arch. Tierz. Dummerstorf*. **49(1),** 64-70.

Xu N.Y., Zhang S.Q. and Peng S.H. (2003). Investigation on the distribution and their effects on reproduction traits of three major genes in Jinhua pigs. *Acta Genet. Sin.* **30,** 1090-1096.

Yang W.C., Li S.J., Tang K.Q., Hua G.H., Zhang C.Y., YU J.N., Han L. and Yang L.G. (2010). Polymorphisms in the 5′ upstream region of the FSH receptor gene, and their association with superovulation traits in Chinese Holstein cows. *Anim. Reprod. Sci*. **119,** 172-177.

Yaofeng Z., Ning L.I., Lu X., CaoGengsheng C., Yizhen C., Shun Z., Yongfu C., Changxin W., Jiansheng Z., Shiquan S. and Xueqing X. (1998). FSHB subunit gene is associated with major gene controlling litter size in commercial pig breeds. *Sci. China*. **41,** 665-668.

Yun T., Bin J.I. and Yun C. (2007). Effect of the 5'-flanking region of goat follicole-stimulating hormone receptor gene on yean trail. *J. Northwest A F Univ*. **35,** 9-14.

Zhang C.Y., Wu C.J., Zeng W.B., Huang K.K., Li X., Feng J.H., Wang D., Xu D.Q., Wen Q.Y., Yang L.G. and Hua G.H. (2011). Polymorphism in exon 3 of follicle stimulating hormone beta (FSHB) subunit gene and its association with litter traits and superovulation in the goat. *Small Rum. Res*. **96,** 53-57.

Zhao L.H., Chen J.L., Xu H., Liu J.W. and Xu R.F. (2010). Cloning and expression of FSHb gene and the effect of FSH on the mRNA level of FSHR in the local chicken. *Asian-australas J. Anim. Sci.* **23(3),** 287-416.