

Polymorphism in FSH β 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-Mianji¹ and Z. Ansari-Pirsarai¹
¹ Department of Animal Science, Faculty of Animal and Aquatic Science, Sari Agricultural Science and Natural Resources University, Sari, Iran

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

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ABSTRACT

The objective of the present study was to detect polymorphism in follicle stimulating hormone receptor (FSHR) and beta subunit of follicle stimulating hormone (FSH β) 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 FSH β marker site but detected no polymorphism pattern in this locus. SSCP analysis used for more survey on detection of polymorphism in FSH β 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 these marker loci was carried out using SAS program. Mean comparison by Duncan test showed that the effect of each FSH β 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 FSH β 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 FSH β , 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 heavier market weights. Litter size is a multipolygenic 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 BMPRII (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 species 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-protein coupled receptor. Intracellular portion of receptor of FSH commence series of actions by linkage to G-protein 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 roles in meat production in Iran which is adopted with wide range of dry environmental of eastern Iran (Tahmoorespur and Sheikhlou, 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, Suffolk, 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 (FSH β) 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 Abbasabad 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 FSH β 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 μ L total volume containing approximately 50-100 ng genomic DNA, 3 μ L of MgCl₂, 2.5 μ L of 10x PCR buffer (500 mM KCl and Tris-HCl (pH 8.4)), 0.6 μ L each of forward and reverse primers (10 Pmol each), 0.5 μ L of 10 mM dNTPs (0.2 mM each) and 1 unit of Smart Taq DNA polymerase (CinnaGen, Iran). In PCR optimal conditions for FSH β 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 min, with a final extension at 72 °C for 10 min. After electrophoresis in 1.5% agarose 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 FSH β gene by *ACCI* and *HinfI* enzyme treatment. The amplicons 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™ 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 FSH β 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 mM 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 \times 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* DH5 α 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 FSH β 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 (χ^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:

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

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 FSH β 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 FSH β gene fragment, a high value of heterozygosity for *MSCI* marker site of FSHR gene observed breeds (Table 1 and 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 (Table 1 and 2).

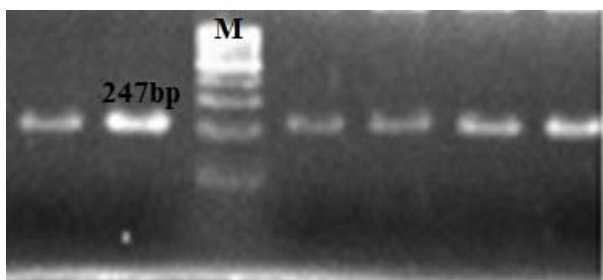


Figure 1 Result of PCR technique for FSH β loci; M: 100bp size marker SM0321



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

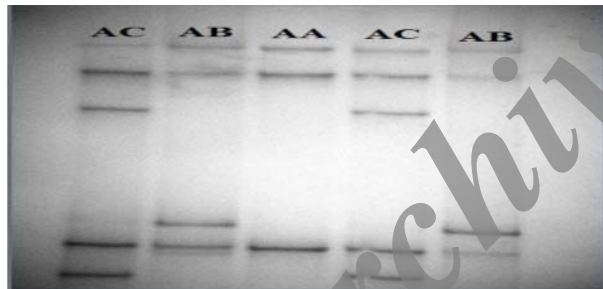


Figure 3 SSCP pattern of analysis of FSH β gene



Figure 4 RFLP pattern of FSH β gene by *ACCI* enzyme

Sequences of different genotype of FSH β gene

Expected sequence length of FSH β 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.

Analysis of nucleotide sequence has been proved three different genotypes.

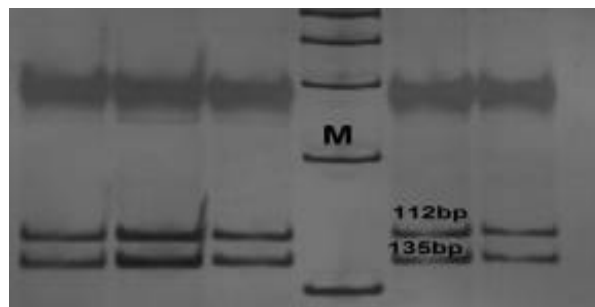


Figure 5 RFLP pattern of FSH β gene by *HinI* enzyme



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

The T nucleotide at position 102 of FSH β 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 FSH β marker site demonstrated that ewes with AC genotype had higher mean of litter size (1.70) than AA genotype (Table 4) and polymorphism 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 FSH β 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 *FSHβ* marker site

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

| Breed | Genotype frequency | | | Allele frequency | | O-Het | χ ² -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 | χ ² -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

| Breed | SE of <i>FSHR</i> loci genotypes±mean | | | | P-value | SE of <i>FSHβ</i> loci genotypes±mean | | | | |
|------------|---------------------------------------|-------------------------|-------------------------|--|---------|---------------------------------------|-----------|-------------------------|--|--------|
| | AA | AB | BB | | | AA | AB | AC | | |
| 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±1.57 | 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 stimulation 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 | 1 st parity | 2 nd 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 FSH β and body weight (Mean \pm SE) traits

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

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

| Breeds | Genotype | BW0 (kg) | BW3 (kg) | BW6 (kg) | BW9(kg) | BW12 (kg) |
|------------|----------|-----------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Baluhci | AA | 4.31 \pm 0.12 | 22.67 \pm 0.94 | 32.14 \pm 1.06 | 33.71 \pm 1.21 | 38.81 \pm 1.26 |
| | AB | 4.07 \pm 0.11 | 21.44 \pm 0.67 | 32.11 \pm 0.71 | 33.63 \pm 0.99 | 37.65 \pm 1.10 |
| | BB | 4.12 \pm 0.33 | 22.89 \pm 0.92 | 33.56 \pm 1.09 | 33.75 \pm 1.56 | 37.63 \pm 2.02 |
| Iran-black | AA | 4.17 \pm 0.26 | 20.11 \pm 1.26 | 31.11 \pm 1.27 | 34.00 \pm 1.05 | 33.89 \pm 1.24 |
| | AB | 3.91 \pm 0.15 | 22.97 \pm 0.66 | 33.03 \pm 0.76 | 34.47 \pm 0.75 | 36.50 \pm 0.77 |
| | BB | 4.14 \pm 0.22 | 21.27 \pm 0.99 | 32.55 \pm 1.05 | 33.82 \pm 0.55 | 35.00 \pm 0.93 |
| Arman | AA | 4.00 \pm 0.14 | 22.12 \pm 0.69 ^a | 32.55 \pm 0.77 ^a | 34.87 \pm 0.84 ^a | 40.45 \pm 1.04 ^a |
| | AB | 4.10 \pm 0.15 | 21.94 \pm 0.62 ^a | 31.33 \pm 0.81 ^{ab} | 34.28 \pm 0.84 ^{ab} | 39.50 \pm 1.79 ^{ab} |
| | BB | 4.75 \pm 0.25 | 19.25 \pm 0.75 ^b | 28.50 \pm 0.87 ^b | 31.00 \pm 1.35 ^b | 33.75 \pm 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 these breeds. In spite of the lack of relationship between FSH β 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 two 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 FSH β 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 FSH β 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 FSH β and ESR loci as major gene affecting litter size in pigs. [Wang et al. \(2006\)](#) investigated polymorphism of FSH β 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 FSH β gene exon 1 and 2 of FSH β 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 FSH β 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 FSH β 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.

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بررسی چند شکلی‌های جایگاه‌های ژنی FSHR و FSH β و ارتباط آنها با عملکرد صفات تولیدی و تولیدمثلی در گوسفندان نژاد ایران بلک، آرمان و بلوچی

ن. نظیفی*، ق. رحیمی میانجی و ز. انصاری پیر سرایی

چکیده

هدف از مطالعه حاضر بررسی چند شکلی در ژن گیرنده هرمون محرک فولیکولی (FSHR) و ژن زیر واحد بتای هرمون محرک فولیکولی (FSH β) و ارتباط آن با صفات نرخ زایش و افزایش وزن بدن در گوسفندان نژاد بلوچی، ایران بلک و آرمان می‌باشد. در ابتدا ردیابی چند شکلی در ژن FSH β به کمک تکنیک PCR-RFLP و آنزیم‌های محدود کننده *HinfI* و *ACCI* انجام شد و هیچ نوع الگوی باندهای چند شکلی در این جایگاه ژنی نشان داده نشد. در مرحله بعد به منظور مطالعات بیشتر در این جایگاه ژنی از تکنیک SSCP استفاده شد که در نتیجه آن ژنوتیپ‌های AA و AC در گوسفندان نژاد بلوچی، ژنوتیپ‌های AA و AB در گوسفندان نژاد ایران بلک مشاهده شد ولی کلیه نمونه‌های مربوط به گوسفندان نژاد آرمان فقط ژنوتیپ AA را نشان دادند. ردیابی چند شکلی در ژن FSHR به کمک تکنیک PCR-RFLP و تیمار آنزیمی *MSCI* انجام شد و سه نوع ژنوتیپ AA، AB و BB در بین نژادهای مورد مطالعه مشاهده شد. آلل B در هر سه نژاد مورد مطالعه بیشترین فراوانی را داشت. آنالیز تجزیه واریانس این جایگاه‌های ژنی به کمک نرم افزار SAS انجام شد. آزمون مقایسه میانگین دانکن نشان داد که چند شکلی‌های هر دو جایگاه ژنی FSHR و FSH β تأثیر معنی داری را روی صفت نرخ زایش در نژاد بلوچی دارند. همچنین نشان داد که آلل جهش یافته می‌تواند متوسط نرخ زایش را به صورت قابل توجهی نسبت به آلل وحشی افزایش دهد. آنالیز داده‌های مربوط به صفت وزن بدن در سنین مختلف و چند شکلی در جایگاه ژنی FSH β ، در هیچ یک از سه نژاد مورد مطالعه اختلاف معنی داری را نشان نداد. مطالعات چند شکلی در جایگاه ژنی FSHR و صفات افزایش وزن بدن نشان دهنده وجود اختلاف معنی داری در نژاد آرمان است، و نشان داد که آلل وحشی عملکرد بهتری دارد. این اطلاعات می‌تواند در برنامه‌های اصلاح نژادی در پرورش گوسفند مورد استفاده قرار بگیرد و سبب بهبود عملکرد تولیدی و تولیدمثلی شود.

کلمات کلیدی FSHR، FSH β ، چند شکلی، نرخ زایش، وزن بدن.