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EVALUATION OF POLYMORPHISM IN *IGF-I* AND *IGFBP-3* GENES AND THEIR RELATIONSHIP WITH TWINNING RATE AND GROWTH TRAITS IN MARKHOZ GOATS*

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Abstract

Growth rate and twinning rate are economic traits that can be used in goat breeding objectives. The aim of this study was to investigate polymorphisms in the insulin-like growth factor 1 (*IGF-I*) and insulin-like growth factor binding protein (*IGFBP-3*) genes and their relationship with growth traits and twinning in Markhoz goats. Two sets of specific primers were used to amplify a 249bp fragment of *IGF-I* gene and a 316bp fragment of *IGFBP-3* gene. PCR-SSCP analysis revealed three banding patterns for each gene that confirmed presence of a mutation in position 1617 of the *IGF-I* gene and a mutation in position 58 of *IGFBP-3* gene. The genotype frequencies of *IGF-I* gene were 0.81 (GG), 0.16 (GA) and 0.03 (AA). Also, the genotype frequencies of *IGFBP-3* gene were 0.79 (TT), 0.17 (TC) and 0.04 (CC). The Odds Ratio estimated for twinning rate was 1.11 for second on first parity, 0.19 for third on first parity and 5.71 for second on third parity. The chi-square statistics were 6.46 for *IGF-I* gene and 3.32 for *IGFBP-3* gene. The results also indicated that different genotypes of these genes had no significant effect on birth weight, weight at 6 months, at 9 months and at 12 months but the interactions between different genotypes of *IGF-I* and *IGFBP-3* genes were significant for weaning weight and average daily gain from birth to weaning. These results suggest that twinning rate in Markhoz breed is statistically affected by these genes and can be considered in breeding programs.

Key words: *IGF-I* gene, *IGFBP-3* gene, twinning, growth traits, PCR-SSCP

Growth rate, reproductive performance and milk yield are traits that can be used in goat breeding objectives. Due to the importance of reproductive traits, especially twinning rate, as well as growth traits in breeding industry, it is essential to take these

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traits into consideration as parts of the purpose (Kosgey et al., 2004), but because of the low heritability of reproductive traits and also slow genetic improvement via the traditional selection of livestock, remarkable improvements in the molecular genetics have been achieved to discover candidate genes with significant effects on the traits. These methods include investigation of genetic variation in a set of specific loci and analysis of relationship between genetic variation in region of quantitative trait loci (QTL) and reproductive traits (Van Arendonk et al., 1994).

Insulin-like growth factor-I (*IGF-I*) plays an important role in a variety of physiological processes such as reproduction, embryogenesis, growth and lactation (Adam et al., 2000). *IGF-I* participates in the growth and function of almost every organ in the body (Daughaday and Rotwein, 1989). Studies show that at puberty long-term deficit in the circulating levels of *IGF-I* available to the hypothalamus can lead to a delay in puberty (Estany et al., 2007). In fact, *IGF-I* has an important role in regulating many essential hormones for reproductive system. It stimulates ovarian function by acting with gonadotropins to promote growth and steroidogenesis of ovarian cells (Lucy, 2000) and also increases the estrogens and androgens activity in ovaries and causes positive regulated expression of receptors for LH hormone (Jones and Clemmons, 1995). *IGF-I* gene in goat is located on chromosome 5 and includes 6 exons and 5 introns (Mikawa et al., 1995).

Association of polymorphism in the 5' flanking region of *IGF-I* gene of goat has been studied and the results have shown that this polymorphism is significantly associated with twinning rate (Wang et al., 2011), but the polymorphism of *IGF-I* gene in sheep did not have significant association with twinning rate (He et al., 2012). Also polymorphism in 5' flanking region had a significant effect on growth traits, live weight and carcass weight in Zel sheep (Kazemi et al., 2011). No association was found between the polymorphism in the 5' flanking region and body size, milk yield and birth weight in Chinese dairy goats (Deng et al., 2010; Wang et al., 2011). In Kurdish goat, the polymorphism of *IGF-I* gene was associated with growth traits and yearling fleece weight (Kurdistani et al., 2013).

Insulin-like growth factor binding protein-3 (*IGFBP-3*) gene is a structural gene for growth and reproductive development in mammals and also is responsible for multiple effects of *IGF-I* gene (Thue and Buchanan, 2002; Othman et al., 2014). *IGFBP-3* gene in goat and cattle is located on chromosome 4 (Kappes et al., 1997). The full length of the *IGFBP-3* gene is 8.9 kb and contains 5 exons (Kim et al., 2005). *IGFBP-3* is attached to the *IGF-I* gene and modifies the *IGF-I* effects by regulating *IGF-I* function (Kostecka and Blahovec, 2002; Othman et al., 2014).

Association of *IGFBP-3* gene polymorphisms with reproductive traits in cattle has been studied (Haegeman et al., 1999; Kumar et al., 2004; Maciulla et al., 1997; Sun et al., 2002). The results show that polymorphism of exon 2 in *IGFBP-3* gene is associated with reproductive traits in goat (Lan et al., 2007 b). Also association of the *IGFBP-3* genotypes with production traits has been reported in sheep (Kumar et al., 2006), cattle (Choudhary et al., 2007; Sun et al., 2002) and goat (Lan et al., 2007 a; Lan et al., 2007 b; Li et al., 2008; Sharma et al., 2014).

Up to now, there is no published research relating to investigating association of polymorphisms in the 5' flanking region of *IGF-I* gene and exon 2 of *IGFBP-3* gene

with twinning rate and growth traits in Iranian native goats. Therefore, the aim of this study was to find polymorphisms in these two regions using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) method in Iranian Markhoz goat and also to investigate possible association of these polymorphisms with twinning rate and growth traits in this native breed.

Material and methods

Blood sampling and DNA extraction

The survey was conducted on 152 female goats with recorded pedigree in Kurdistan province, west of Iran. Twinning rate is known as an economically important trait and one of selection goals in breeding programs in this herd. Further, litter size (LS) in kidding was a criterion to select samples for this research. We selected 152 samples as they showed different litter size in kidding, LS=1 or LS>1 in all kidding.

Blood samples for DNA genotyping were collected from the jugular vein using vacuum tubes containing EDTA. The isolation of DNA from whole blood was performed according to the method described by Boom et al. (1990) using DNA extraction kit (Diatom DNA Prep 100, Gen Fanavaran). Quality of DNAs was determined by electrophoresis on 1% agarose gel.

PCR and PCR-SSCP

The primers for amplifying a 249 bp fragment of the 5' flanking region of the *IGF-I* gene were based on Ge et al. (2001) and for amplifying a 316 bp fragment of the exon 2 of the *IGFBP-3* gene were based on Lan et al. (2007 b) (Table 1).

Table 1. The sequence, amplified region, annealing temperature and product size of primers

Gene	Primer sequence	Amplified region	Annealing temperature	Product size
<i>IGF-I</i>	F:5'-ATTACAAAGCTGCCTGCCCC-3' R:5'-ACCTTACCCGTATGAAAGGAATATACGT-3'	5' Flanking region	64°C	249bp
<i>IGFBP-3</i>	F:5'-GAAATGGCAGTGAGTCGG-3' R:5'-TGGGCTCTTGAGTAATGGTG-3'	Exon 2	61°C	316bp

The final volume of 25 µl of each reaction contained 50 ng genomic DNA, 0.25 µM of each primer, 2.5 mM MgCl₂, 0.2 µM dNTPs, 1X PCR buffer and 1 unit Taq DNA polymerase (Sinagene, Iran). Amplification was carried out using thermocycler device (Corbett Life Science, Australia) under the following conditions: for *IGF-I* gene, 5 min at 94°C for initial DNA denaturation, 40 cycles of denaturation at 94°C for 45s, annealing at 64°C for 45s, extension at 72°C for 45s. The final cycle was followed by a 7 min extension at 72°C. The following condition was used for *IGFBP-3* gene: 5 min at 94°C for initial denaturation followed by 35 cycles at 94°C

for 35s, 61°C for 35s and 72°C for 35s. The final extension was followed by a 7 min at 72°C. The PCR products were electrophoresed on 1.5% agarose gel in 1X TAE and visualized by ethidium bromide staining for 1h and 20 min at 80V (Figure 1).

Figure 1. Samples of PCR products on 1.5% agarose gel electrophoresis. A 249bp amplified fragment of the 5' flanking region of *IGF-I* gene (left) and A 316bp amplified fragment of the exon 2 of *IGFBP-3* gene (right); M: 50bp DNA ladder marker

For SSCP analysis, 8 μ l of the PCR products were mixed with 8 μ l denaturing solution (95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol, and 20 mM EDTA) and heated for 10 min at 95°C and chilled on ice for 5 min. The dilution was loaded on a 12.5% polyacrylamide gel with 10% glycerol. The gel was run at a constant voltage of 200V at 4°C for 5 h and 30 min and then stained with 0.1% silver nitrate. The PCR products of different electrophoresis patterns were chosen and sequenced by Gen Fanavaran Co.

Statistical analysis

Allele and genotype frequencies and Hardy-Weinberg equilibrium were estimated by using Popgene (32) software. The linear mixed and multivariable logistical regression models were conducted in SAS (9.1) software to investigate the effects of polymorphisms in the *IGF-I* and *IGFBP-3* genes on growth traits and twinning rate, respectively.

The statistical model for growth traits was as follows:

$$Y_{ijklmo} = \mu + ys_i + bt_j + IGF-I_k + IGFBP-3_l + sire_m + e_{ijklmo}$$

where:

Y_{ijklmo} is the growth trait;

μ is the overall mean;

ys_i is the fixed effect of i^{th} combined effect of year-season of kidding;

bt_j is the fixed effect of j^{th} birth type;

$IGF-I_k$ is the fixed effect of k^{th} *IGF-I* genotype;

$IGFBP-3_l$ is the fixed effect of l^{th} *IGFBP-3* genotype;

$sire_m$ is the random effect of m^{th} sire;

e_{ijklmo} is the random error.

In statistical models for average daily gain from birth to weaning and weaning weight traits, combined effects of *IGF-I* and *IGFBP-3* genes were considered due to a significant interaction.

The generalized statistical model used to test genotype effects on twinning rate was as follows:

$$Y_{ijkl} = \mu + \text{Parity}_i + \text{IGF-I}_j + \text{IGFBP-3}_k + e_{ijkl}$$

where:

Y_{ijkl} is the twinning trait;

μ is the overall mean;

Parity_i is the effect of i^{th} number of parity;

IGF-I_j is the effect of j^{th} *IGF-I* genotype;

IGFBP-3_k is the effect of k^{th} *IGFBP-3* genotype;

e_{ijkl} is the random residual effect of each observation.

Odds Ratio and chi-square parameters were used to show association between effects of the model on twinning rate ($P < 0.05$ or $P < 0.1$). Also, the interaction effects between factors were not significant and were excluded from the statistical model.

Results

PCR-SSCP patterns

Three SSCP polymorphic patterns were observed for the amplified fragments of the 5' flanking region of the *IGF-I* gene (Figure 2). Also, the SSCP method showed three polymorphic patterns for the amplified fragments of the exon 2 of the *IGFBP-3* gene (Figure 2).

Figure 2. Three different SSCP patterns for the 5' flanking region of the *IGF-I* gene (left) and the exon 2 of the *IGFBP-3* gene (right)

Figure 5. Genotype and allele frequencies of *IGF-I* and *IGFBP-3* genes in Markhoz population

The distributions of genotypes and alleles for these genes were not in Hardy-Weinberg equilibrium ($P < 0.05$). The reasons for this disequilibrium can be the sample size in our study and the effect of animal selection for sampling. In the current study, sampling was performed just from some singleton and twin-bearing female goats. Finite number of this Markhoz goat population and random drift probability can be another reason for the disequilibrium.

For the *IGF-I* gene, according to Deng et al. (2010) study, genotype frequencies for GG and GA were 0.85 and 0.15, respectively and allele frequencies were G (0.92) and A (0.08) for Guanzhong goats and the frequencies were GG (0.79), GA (0.21), G (0.89) and A (0.11) for Xinong Saanen goats. In these two breeds, no AA homozygotes were observed which might be due to sampling where they just used one-year-old goats. In another study on *IGF-I* gene in Nanjiang Huang goats, genotype frequencies were GG (0.59), GA (0.372) and AA (0.038) and allele frequencies were G (0.78) and A (0.22) (Wang et al., 2011). This polymorphism had been studied in Kurdish goats of Iran and there were no AA genotypes and genotype frequencies of GG and GA were 0.96 and 0.04, respectively (Kurdistani et al., 2013).

For the *IGFBP-3* gene, Lan et al. (2007) showed that allele frequencies were T (0.82) and C (0.18). Genotype and allele frequencies for exon 2 in the *IGFBP-3* gene were TT (0.58), TC (0.33), CC (0.09), T (0.75) and C (0.25) in Cashmere goats (Liu et al., 2012). Based on the current study and other results published, the reasons for the low number of mutant alleles can include the lower probability of crosses of heterozygous and homozygous recessive individuals in large main populations, the effect of natural selection and removal of these animals from population.

Association analysis for twinning rate

The association results of parity and studied polymorphisms with twinning rate in Markhoz goat were shown in Table 2. In this study, parity had significant effect on twinning rate ($P < 0.05$). This result was consistent with research conducted on Chinese goat breeds (An et al., 2013; Wang et al., 2011). Also, it has been known that parity has significant effect on twinning rate in small tail Han sheep and dairy cattle

(Chu et al., 2004; Nielen et al., 1989). In this study, obtained Odds Ratios were 1.11 for second on the first parity ($P>0.05$), 0.19 for third on the first parity ($P<0.1$) and 5.71 for second on the third parity ($P<0.1$). Based on these results, it is concluded that twinning rate can be increased with increasing parity from the first to second. The reasons for this result can be multiple ovulation in higher parity (Ghavi Hossein-Zadeh et al., 2008), maternal effect and aging (Ligda et al., 2000; Maxa et al., 2007). Although twinning in the third parity is lower than that in the second parity, it can be due to stresses during pregnancy and lactation in the previous large litter size (Rao and Notter, 2000).

Table 2. Estimation of Odds Ratio and chi-square statistics related to parity and genotypes for twinning rate

Variables	Odds Ratio	Chi-Square	Pr > ChiSq
Parity		7.62	0.02**
<u>Parity 2</u>	1.11	0.2	0.65
Parity 1			
<u>Parity 3</u>	0.19	2.93	0.08*
Parity 1			
<u>Parity 2</u>	5.71	3.15	0.07*
Parity 3			
IGF-I		6.46	0.03**
<u>GA</u>	1.35	4.02	0.04**
GG			
<u>AA</u>	0.32	1.44	0.22
GG			
<u>GA</u>	4.2	2.35	0.12
AA			
IGFBP-3		3.32	0.1*
<u>TC</u>	1.41	3.58	0.05**
TT			
<u>CC</u>	1.16	0.72	0.4
TT			
<u>TC</u>	1.21	0.02	0.88
CC			

Superscript **= $P<0.05$ and * = $P<0.1$.

Chi-square statistics equal to 6.46 for *IGF-I* gene ($P<0.05$) and 3.32 for *IGFBP-3* gene ($P<0.1$) showed that twinning rate in Markhoz goats was significantly influenced by *IGF-I* and *IGFBP-3* genes genotypes (Table 2).

The obtained Odds Ratio indicated that twinning rate of heterozygous genotypes in both *IGF-I* and *IGFBP-3* genes was higher than that in two homozygous genotypes. Superiority of these genotypes is shown below: GA>GG>AA for the *IGF-I* gene and TC>CC>TT for the *IGFBP-3* gene (Table 2). It was observed that GA

genotypes had 1.35 twinning rate higher than GG genotypes for *IGF-I* gene with chi-square 4.02 which suggested that there was significant difference among the two genotypes ($P < 0.05$). Also, results indicated that TC genotypes had 1.41 twinning rate higher than TT genotypes for *IGFBP-3* as well as chi-square statistic was estimated 3.58 ($P < 0.05$) that confirmed a significant difference among these two genotypes (Table 2).

Association analysis for growth traits

Association analysis results of the *IGF-I* and *IGFBP-3* polymorphisms with growth traits in Markhoz goats are shown in Figures 6 and 7. The genotypes of *IGF-I* and *IGFBP-3* genes did not show significant effects ($P > 0.05$) on birth weight (BW), weight at 6, 9 and 12 months (W6, W9 and W12) and values were close to each other in each trait.

Figure 6. Least square means ($\pm$ SE) of different genotypes for some growth traits in Markhoz goat (BW: birth weight; W6: weight at 6 months; W9: weight at 9 months; W12: weight at 12 months)

Figure 7. Least square means ($\pm$ SE) of different genotype combinations (*IGF-I* and *IGFBP-3*) for weaning weight (WW) and average daily gain (ADG) in Markhoz goat. Dissimilar letters indicate significant differences at $P < 0.05$

According to statistical analysis, interactions between *IGF-I* and *IGFBP-3* genotypes for two traits of average daily gain (ADG) from birth to weaning and weaning weight (WW) were significant ($P < 0.05$). Therefore, combined effects of genotypes were used to compare the effects of genotypes on these traits (Figure 7). Some combinations of genotypes were not found in samples.

According to the results, GGCC genotypes had lower average daily gain and weaning weight compared to those for other genotypes. However, GACC and GGTC genotypes had the highest average daily gain and weaning weight among other genotypes. The lowest average daily gain and weaning weight were observed when a locus was recessive homozygous and the other one was dominant homozygous. These results represent the interaction (epistasis) between *IGF-I* and *IGFBP-3* genes in these traits. No significant differences were observed among other combinations.

Discussion

The goat *IGF-I* gene has three leader exons (1W, 1A, and 2), which result in four mature mRNAs (class 1W, 1W-1del, 1, and 2) in liver. Class 1 mRNA is expressed in various tissues. Class 1W, 1W-1del, and 2 mRNAs can be detected only in the liver, uterus, and ovary (Mikawa et al., 1995). It is well known that *IGF-I* plays an important role in both embryonic and postnatal growth. It has been shown that there is a correlation between *IGF-I* blood concentration and growth rate in cattle (Schlee et al., 1994; Sirotkin et al., 2000). The actions of *IGF-I* are regulated by *IGF-I* receptor and *IGFBP-3*. *IGF-I* binds to receptor located in the cell membrane and sends a signal to various cytoplasmic substrates. *IGF-I* also binds to *IGFBP-3* to extend its half-life and modulate its binding affinity and transport across vascular barriers.

Twinning rate

So far, no study has been carried out on Markhoz goats in order to examine the relationship between polymorphisms of *IGF-I* and *IGFBP-3* genes with reproduction traits, but the results of other studies have indicated that the *IGFBP-3* gene has a positive effect on reproduction in mammals and can increase the ovulation rate (Lan et al., 2007b). Also, the *IGF-I* gene was effective on reproductive process, and stimulated ovarian activity (Lucy, 2000) and showed to regulate hormones necessary for reproduction (Jones and Clemmons, 1995). This means that the *IGF-I* stimulates the anterior pituitary for secretion of LH which regulates reproductive activity in mammals (Denley et al., 2005).

The mutation in the promoter region of the genes related to litter size may affect ovulation rate in goats, resulting in a corresponding change in prolificacy in small ruminants (He et al., 2012; An et al., 2015). Also, some researchers have observed similar significant association between microsatellite genotypes in 5' flanking region of the *IGF-I* gene and prolificacy in goat (Wang et al., 2011) and sheep breeds (He et al., 2012). However, Zi et al. (2013) reported no significant variation in the relative abundance of IGF1 mRNA between prolific Lezhi black goat and non-prolific Tibetan goat. A similar observation was recorded by Thomas et al. (2016) in the two indigenous goat breeds of South India, low prolific Attappady Black and high prolific Malabari.

It can be hypothesized that the genetic variants in the 5' flanking region of IGF1 gene may affect ovarian folliculogenesis and ovulation in goats (Thomas et al., 2016).

The 5' flanking region polymorphism of the *IGF-I* gene was positively related to twinning rate in cattle and goat (Deng et al., 2010; Kim et al., 2009; Thomas et al., 2016). Likewise, QTL position of twinning rate has been identified between 55 and 65Mb on BTA5 in the Norwegian dairy cattle (Lien et al., 2000) and the North American Holstein populations (Cruikshank et al., 2004). All these reported QTLs had positive effects on reproductive traits of cattle. However, no study on QTL associated with twinning rate in goat and sheep has been previously published.

Our results showed that the *IGF-I* and *IGFBP-3* genes can be considered in marker-assisted selection (MAS) programs and may be useful to improve this trait in Markhoz goat breed of Iran. Review of available literature sources revealed that a few studies have been performed regarding the effects of the *IGF-I* and *IGFBP-3* genes on twinning trait. Concerning the effects of these genes on the stimulation of the reproductive system, further studies are needed in order to reveal the secrets of these genes effects on livestock twinning and also to help improve the performance and efficiency of animal production.

Twinning rate is one of the reproductive traits with low heritability ($h^2 < 0.1$) (Johanson et al., 2001) and is significantly influenced by non-additive genetic factors and proper nutrition before mating (Davis, 2004). Due to this low heritability of twinning trait, not only QTL mapping and effective genes finding methods can be very effective in breeding programs of Markhoz goat breed, but also improving environmental and non-genetic factors such as flushing practices on feeding in the mating season can cause improvement of twinning rate of this breed as well as higher profitability for breeders.

Growth traits

The *IGF-I* gene has been considered to be a candidate marker associated with growth traits in various domestic livestock (Thue and Buchanan, 2002; Zhang et al., 2008; Wu-Jun et al., 2010). For example, the G to C transversion located in intron 4 of the *IGF-I* gene described by Zhang et al. (2008) was shown to have significant association with birth weight, body weight at 6 months and 12 months in Nanjiang Huang goats.

In current study, we found a G to A mutation (g. 1617 G>A, accession D26119.2), in the 5' flanking region of the *IGF-I* gene in Markhoz goats of Iran, and estimated frequencies of the A (mutant) allele and AA genotype were 0.11 and 0.03, respectively. Kurdistan et al. (2013) found this mutation in Kurdish goats with the frequency of 0.04 for mutant allele. The results reported by Deng et al. (2010) showed this mutation in two breeds of dairy goats. Also, the frequencies of the mutant allele were 0.08 and 0.11 for Guanzhong and Xinong-Saanen breeds, respectively. In these two breeds, this polymorphism was not associated with body size and milk yield. The PCR-SSCP analysis that was done by Zhang et al. (2008) revealed no single nucleotide polymorphism (SNP) in the 5' flanking region of the *IGF-I* gene. In the current study, frequency of the AA homozygous genotype was low in the *IGF-I* gene of Markhoz goat. These AA homozygotes were not observed by Deng et al. (2010) and Kurdistan et al. (2013). They reported the lack of AA homozygotes indicating

that the G to A mutation in the goat *IGF-I* gene might have given rise to reduced production in the populations analyzed.

We found a T to C mutation in exon 2 of the *IGFBP-3* gene in Markhoz goats, and calculated frequencies of the C (mutant) allele and CC genotype were 0.125 and 0.04, respectively. Different frequencies of mutant allele C among breeds (0.181 in Cashmere, 0.099–0.015 in dairy and 0 in meat breeds) have been observed by Lan et al. (2007 b). Frequencies of the C allele and CC genotype in Cashmere goats were C (0.25) and CC (0.09) (Liu et al., 2012). In this case, the effect of this mutation in the transcription process in various tissues and its effect on the economic traits must be studied. Regarding all the results reported by researchers, frequencies of the mutant alleles were too low or this allele was not observed in their samples. Reasons for the low numbers of mutant alleles in our samples were emergence of this mutation and effect of natural selection and removal of the animals with the AA and CC genotypes.

Literature reviews showed that results vary in association between *IGF-I* gene polymorphism and growth traits among different breeds. Significant association of the polymorphism in 5' flanking region with average daily gain in Baluchi sheep was reported (Tahmoorespur et al., 2009). On the other hand, polymorphism in the 5' flanking region was associated with growth traits in sheep (Kazemi et al., 2011) and in Kurdish goat (Kurdistani et al., 2013). Wang et al. (2011) observed no significant association between this polymorphism and birth weight in three breeds of goats. Linkage disequilibrium of the *IGF-I* gene with QTLs could be the main reason of the inconsistent results. Therefore, quantitative traits are regulated by the large number of genes and also are affected by the interaction of these genes, so it is usual to observe the different effect of a candidate gene associated with a particular trait in a population. More studies are required in larger populations of Markhoz goats and in other native goat breeds in order to verify the relevant effects of this SNP marker.

Some researchers investigated the association between polymorphism of the *IGFBP-3* gene and growth traits. Kumar et al. (2006) studied polymorphism of the *IGFBP-3* gene by nucleotide sequencing and PCR-restriction fragment length polymorphism (PCR-RFLP) methods in sheep and compared it with that of cattle and buffalo. In another research, the association of *IGFBP-3* polymorphism with growth traits was also reported in cattle (Sun et al., 2002) and goat (Sharma et al., 2014). Lan et al. (2007b) detected polymorphisms in goat *IGFBP-3* gene using PCR-SSCP and DNA sequencing methods. However, one single nucleotide polymorphism in exon 2 and intron 2 of *IGFBP-3* gene has been reported in goat (Lan et al., 2007 b) and a nucleotide transition T>C (nt58 of exon 2) was observed in *IGFBP-3* gene. This transition resulted in the amino acid proline to serine.

In our study, the frequency of C mutant allele was near the frequency reported by Lan et al. (2007b) in Cashmere goats. Our results showed no significant effect of different genotypes of *IGFBP-3* gene with birth weight, weight at 6, 9, and 12 months. However, significant association of the *IGFBP-3* gene with body weight traits has been reported in Indian goat breeds (Sharma et al., 2014)

Literature review reveals that the results of several studies are different to show association of *IGFBP-3* gene polymorphism with growth traits among livestock. So,

complementary researches including QTL studies are needed to better understand the linkage phase and relationship between this locus of *IGFBP-3* gene and growth traits in goat.

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