POLYMORPHISMS IN THE 5’ FLANKING REGION AND FIRST EXON OF INSULIN-LIKE GROWTH FACTOR 1 GENE IN SOUTHERN POPULATIONS OF BUFFALO

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ABSTRACT

The Insulin-like Growth Factor 1 (IGF-1) system plays a critical endocrine role controlling nutrient metabolism in animals and it is a group of structurally-related polypeptides that regulate the growth of many types of mammalian cells. In liver, IGF-1 is dynamically regulated by lactation and energy balance. Less is known about the regulation of IGF-1 and IGF-binding protein mRNA in reproductive tissues and plays an important role in bovine follicular growth, acquisition of oocyte competence and embryo viability. The present study describes Polymorphisms in the IGF-1 Gene of Buffalo population in south of Iran. In order to characterization polymorphism, blood sample were collected from 95 buffalos from Ahvaz, Dezful, Shushtar, Shadegan and Susangerd cities. DNA extraction was based on Boom method and exon 1 of the IGF-1 gene was amplified to produce a 250 bp fragment. The amplified fragment were digested with Eco105I (SnaBI) restriction endonuclease. Also, the 265 bp of IGF-1 promoter in the 5´ Flanking Region was amplified and subsequently subjected to Single Strand Conformation Polymorphism (SSCP). The results were revealed one pattern (B) in digestion and all the five populations were monomorph. But, the populations exhibited three different SSCP patterns. It is possible that this gene has not exposed to selection and inbreeding is estimated high. 

Keywords: Buffalo, Polymorphism, IGF-1 Gene, Flanking Region, SSCP

1. INTRODUCTION

Genetic evaluation of animal performance has been accomplished by quantitative approaches which depend on molecular technology for identifying genes of economically interesting traits and analysis of the polymorphism of genes whose products are key enzymes in the metabolic pathways of important physiological processes and are related to phenotypes (Beuzen et al., 2000). Candidate genes have known biological functions related to the development or physiology of an important trait (Rothschild et al., 1997). Such genes can encode structural proteins or a member in a regulatory or biochemical pathway affecting the expression of the trait (Bryne and McMullen, 1996) and can be tested as putative QTLs (Yao et al., 1996). The Growth Hormone (GH) and Insulin-like Growth Factor 1 (IGF-1) genes are candidates for growth in bovine, since they play a key role in growth regulation and development (Breier, 1999). Genetic polymorphism in native breeds is a major concern considering the necessity of preserving genetic resources. It is very important to characterize genetically indigenous breeds (Bastos et al., 2001). Insulin-like growth factors 1 and 2 (somatomedins-IGF-1 and IGF-2) are structurally related proteins, playing a key role in cell differentiation, embryogenesis, growth and...
The insulin-like growth factor superfamily

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Receptors</th>
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<tbody>
<tr>
<td>IGF1</td>
<td>Type 1 IGF receptor</td>
</tr>
<tr>
<td>IGF2</td>
<td>Type 2 IGF or IGF 2/ mannose-6-phosphate</td>
</tr>
<tr>
<td>IGFBP1</td>
<td>IGFBP7/MAC25/TAF/PSF</td>
</tr>
<tr>
<td>IGFBP2</td>
<td>IGFBP-rP2 (CTGF)</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>IGFBP-rP3 (NovH)</td>
</tr>
<tr>
<td>IGFBP4</td>
<td>IGFBP-rP4 (CYRI61)</td>
</tr>
<tr>
<td>IGFBP5</td>
<td>IGFBP-rP5 (L56/HTRA1)</td>
</tr>
<tr>
<td>IGFBP6</td>
<td>IGFBP-rP6 (ESM1)</td>
</tr>
<tr>
<td>Potential receptors</td>
<td></td>
</tr>
<tr>
<td>IGFBP(s)</td>
<td>eCOP-1/WISP2/CTGF-L</td>
</tr>
<tr>
<td>IGFBP-rP(s)</td>
<td>IGFBP-rP8 (ELM1/WISP1)</td>
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<td></td>
<td>IGFBP-rP9 (WISP3)</td>
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</table>

Production of these transcripts is controlled by two different promoters both containing canonical regulatory sequences-TATA-box and CCAAT-box (Jansen et al., 1991). It was shown that transcripts of both classes are differentially expressed in various tissues, being, however, most abundant in liver (Wang et al., 2006). In vertebrates, the Insulin-Like Growth Factor 1 (IGF1) or somatomedin gene plays a key role in various physiological and metabolic processes, where IGF1 and growth hormone or somatotrophin is involved in the somatotropic axis. IGF1 is a mediator of many biological effects; for example, it increases the absorption of glucose, stimulates myogenesis, inhibits apoptosis, participates in the activation of cell cycle genes, increases the synthesis of lipids, stimulates the production of progesterone in granular cells and intervenes in the synthesis of DNA, protein, RNA and in cell proliferation (Etherton, 2004). The bovine IGF1 gene was mapped on chromosome 5, in the centimorgan 73.5 (Grosse et al., 1999). The provisional nucleotide sequence is approximately 72 kb (ID number 281239). In humans, pigs, goats, rats and chickens, the IGF1 nucleotide sequence is about 70-90 kb (Rose, 2002). Exon number is different between species; for example, goats, pigs and sheep have 1-6 exons (Mikawa et al., 1995) and humans and rats 1-5 (Rotwein et al., 1986). The IGF-1 gene is extremely conserved among species and few polymorphisms are described. The presence of a microsatellite at the promoter region of this gene in bovine, human and horse allows to analyze genetic variations related to this locus (Kirkpatrick, 1992). Evidence of selection effects on allele frequencies at these two loci was found in the traditional lineage of Canchim (Regitano et al., 1999). Nucleotide sequence variations related to this locus (Kirkpatrick, 1992). Evidence of selection effects on allele frequencies at these two loci was found in the traditional lineage of Canchim (Regitano et al., 1999).
polymorphisms were identified in the bovine IGF-1 gene and their correlations with animals’ growth rate and meat performance traits were found. The Short Tandem Repeat (STR) polymorphism in the 5'-flanking region and the Single Strand Conformation Polymorphism (SSCP) in intron 3 of the IGF-1 were reported by Kirkpatrick (1992). In Hereford cattle the STR polymorphism was shown to be associated with body weight at birth and at weaning and with the growth rate (Moody et al., 1996). Such associations were not found in other beef breeds (Curi et al., 2005). The SSCP in the 5'-flanking region of IGF-1 was found by Ge et al. (1997) in Angus cattle. This polymorphism was then identified as T/C transition, also recognizable as RFLP-SnaBI (Ge et al., 2001). Two alleles and three genotypes were found. Allele A (with nt T at position-472) appeared significantly more frequent than allele B (with nt C) in a group of animals selected for the high IGF-1 content of blood. However, the BB genotype (with nucleotides CC) was found to be associated with higher body weight at weaning (Li et al., 2004). No association was found between IGF-1 RFLP-SnaBI and dairy production traits in Holstein cattle (Hines et al., 1998). Two polymorphisms in the IGF-1 were reported by Lien et al. (2000) in Norwegian cattle: The TTTG insertion/deletion (InDel) in intron 4 and the RFLP-DpnI in intron 5. However, the effects of these polymorphisms on beef or milk production traits were not investigated. The objective of the present study was to investigate in the 5´ Flanking Region and exon 1 of IGF-1 Gene in southern populations of Iranian Buffalo in Khuzestan province of IRAN using PBR (PCR Based RFLP) and SSCP technique.

2. MATERIALS AND METHODS

2.1. Animals and Sampling

In current study, Random blood samples were collected from 95 Buffalos from five populations involve: Ahvaz, Shadegan, Susangerd, Dezful and Shushtar cities in Khuzestan province of Iran (Fig. 1). Approximately, 3 ml blood sample was gathered from venom in EDTA tube and was transferred to-20°C freezer.

2.2. DNA Extraction and PCR Amplification

Genomic DNA was isolated by using DNA Extraction Kit (Diatom) and was based on Boom et al. (1989) method. Quantity was determined by measuring the absorbance at 260 nm and the concentration, purity and quality were determined by measuring the absorbance at 260/280 nm and 230/260 ratios using a NanoDropTM 1000 spectrophotometer (Thermo Scientific). DNA extractions were appropriately labeled and stored at-20°C for analysis. The study was concentrated on a 250 bp fragment of IGF-1 gene spanning over exon 1. Primer sequences for PCR were established by Ge et al. (1997; 2001) (Table 2). The PCR reaction volume of 25 µL contained approximately 100 ng of genomic DNA, 1 unit Taq DNA Polymerase, 1×PCR Buffer, 1.5 mM MgCl2, 200 µM dNTPs and 0.3 pMol of each primer. Amplification conditions included an initial denaturation at 95°C for 5 min, followed by 35 cycles at 94°C for 30 s, 63°C for 30 s and 72°C for 40 s, followed by a final extension at 72°C for 5 min.

2.3. Enzyme Digestion

The amplified fragment was digested with Eco105I (SnaBI). The digestion reaction contained by 5 µL of PCR product, 2 µL Buffer 10×, 5 U of Eco105I (SnaBI) and H2O up to a total volume of 20 µL then were incubated at 37°C for 12-16 h. The digestion products were electrophoresed on 2% agarose gel in 0.5×TBE and visualized by ethidium bromide staining for 40 min at 100 V. The Statistical analysis was calculated using Popgene32 software (version 1.31).

![Fig. 1. Geographical location of the populations studied](image-url)

Table 2. Primer used in the amplification in the bovine IGF1 gene

<table>
<thead>
<tr>
<th>Primer sequence</th>
<th>PCR product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATTACAAAG</td>
<td>CTGCCGCCCCC</td>
<td>Ge et al. (2001)</td>
</tr>
<tr>
<td>ACCTTACCCTAGTG</td>
<td>250</td>
<td>Ge et al. (2001)</td>
</tr>
<tr>
<td>GGCGACGCTCTC</td>
<td>265</td>
<td>Ge et al. (1997)</td>
</tr>
</tbody>
</table>
2.4. Single-strand Conformational Polymorphism Analysis (SSCP)

For SSCP analysis, 5 ml of PCR product and 10 mL of diluting dye were denatured at 95°C for 10 min and immediately plunged into ice for 5 min. The dilution was loaded onto a 12% polyacrylamide gel with 10% glycerol. The gel was run at a constant voltage of 250 V at 20°C for 24 h and gels were silver stained.

3. RESULTS

The amplified of promoter in the 5´ Flanking Region and exon 1 of IGF-1 Gene resulted in a DNA fragment with 250bp and 265bp with PCR technique (Fig. 2). Analysis of exon 1: The results were revealed one pattern (B) in digestion, resulting in one genotype and all the five populations were monomorph. one allele (B) was observed. The \textit{Eco105I} (\textit{SnaB I}) indicated single restriction pattern in all the populations of buffalo and it didn’t produce digestion fragments pattern (bands of approximately 226 and 23 bp) in southern populations of Iranian buffalo. This study had no impact which agrees with the results obtained in cattle with similar gene locus of IFG-1 (Laureano \textit{et al.}, 2006). The animals with one allele were assigned with BB genotype (Fig. 3). A and B allele frequencies were 0.0000 and 1.0000, respectively. The high frequency of the B allele suggests that this allele Variant might have been favored by selection for production traits. The observed Homozygosity and heterozygosity were 1.000 and 0.000, respectively (Table 3). Analysis of promoter in the 5´ Flanking Region: The PCR-SSCP analysis revealed three distinct patterns (Fig. 4).

![Fig. 2. PCR products of IGF-1 (Size obtained: 250 bp)](image1)

![Fig. 3. IGF-1 genotyping by PBR method (2% agarose gel)](image2)

Table 3. The Homozygosity and heterozygosity in different regions

<table>
<thead>
<tr>
<th>Regions</th>
<th>Homozygosity</th>
<th>Heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahvaz</td>
<td>1.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Dezfol</td>
<td>1.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Shushtar</td>
<td>1.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Shadegan</td>
<td>1.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Susangerd</td>
<td>1.000</td>
<td>0.000</td>
</tr>
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</table>

4. DISCUSSION

Candidate genes have known biological functions related to the development or physiology of an important trait. Such genes can encode structural proteins or a member in a regulatory or biochemical pathway affecting the expression of the trait (Bryne and McMullen, 1996) and can be tested as putative QTLs (Yao \textit{et al.}, 1996). The study by Ge \textit{et al.} (2001) characterizes a G→A transition polymorphism within an\textit{Eco130I} site of intron 3 of the IGF1 gene in swamp buffaloes (\textit{Bubalus b. bubalis kerebau}). Polymorphisms in the bovine IGF-I gene are associated with circulating IGF-I concentrations and growth traits. Growth in animals is controlled by a complex system, in which the somatotropic axis plays a key role. Genes that operate in the somatotropic axis are responsible for the postnatal growth, mainly GH that acts on the growth of bones and muscles mediated by IGF-1 (Sellier, 2000).
5. CONCLUSION

The southern populations of Iranian Buffalo showed a low degree of genetic diversity for the IGF-1 locus. Also, random genetic drift can be one of the causes of the homozygosity. Although we observed the low variability for exon 1 but there was high degree of genetic diversity for the 5’ Flanking Region of IGF-1 gene. In the other hand, this data provide evidence that buffalo populations have a good polymorphism for some gene, which opens interesting prospects for future selection programs, especially marker assistant selection between different genotypes of different locus and milk, gain and meat traits. The genetic variation of 265 bp fragment used in Present Results showed that PCR-RFLP (PBR) is appropriate tools for evaluating genetic variability.

6. ACKNOWLEDGMENT

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7. REFERENCES


