Polymorphism of the Signal Transducer and Activator of Transcription 5A (STAT5A) gene in Egyptian water buffaloes using the SSCP technique

Basant M. Shafik1*, Sherif I. Ramadan1, Mohamed El-Raey2, Eman M. Abd El Fattah3, Tahani S. Behour4, Ayman F. Ashour5, Amin M. S. Amin6, and Samah F. Darwish3

1Animal Wealth Development Department, Faculty of Veterinary Medicine, Benha University, Toukh, Egypt
2Department of Theriogenology, Faculty of Veterinary Medicine, Benha University, Toukh, Egypt.
3Biotechnology Research Unit, Animal Reproduction Research Institute, Agriculture Research Center, El-Haram, Egypt
4Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Dokki, Giza, Egypt
E-mail: Basant.shafeek@gmail.com

Abstract

Buffalo is the main source of white milk and contributes 45% of the annual milk production in Egypt. The STAT5A protein has an important role in the signal transduction process within uterine epithelial cells and mammary glands affecting milk yield, fertilization, and embryonic survival rates in cattle. The studies conducted for the improvement of the genetic potentiality of productive and reproductive traits of Egyptian buffalo are still scanty. Data on polymorphisms of the buffaloes STAT5A gene is limited. Hence, the present study aimed to identify novel polymorphisms in the STAT5A gene and to validate its genotypic frequencies in Egyptian buffalo. A 929 bp including part of intron 7 to part of intron 9 and the intervening exons of the STAT5A gene was amplified and genotyped by the SSCP method. Three different conformation patterns in the investigated 60 buffaloes were observed. Pattern B showed the highest observed frequency of 51.7%, while patterns A and C occurred at frequencies of 45% and 3.3% respectively. A Chi-square test showed that the Egyptian water buffalo population was in Hardy-Weinberg equilibrium. STAT5A gene might be utilized as a potential molecular marker for effective animal selection and breeding programs.

Key Words: Buffalo, Conformation patterns, Polymorphism, SSCP, STAT5A gene

1. Introduction

Water Buffaloes (Bubalus bubalis) are considered one of the most important pillars of national food security strategies in developing countries including Egypt. Buffalo is considered the main source of white milk and supply about 45% of the annual milk production in Egypt [1]. Buffalo’s milk is more acceptable to the Egyptian citizen due to its acceptable flavor, white color, and high-fat content [2]. Farm profitability is greatly influenced by the reproductive performance of its dairy animals [3]. Reproductive disorders such as anestrus and repeat breeding are the major infertility problem in Egyptian buffalo that greatly decrease farm profitability by increasing the calving interval length [4, 5]. Improvement of reproductive efficiency in dairy farms is a very important issue because poor reproductive performance is the main factor for dairy animals culled after low milk production [6]. It is claimed that the improvement of buffalo’s reproductive efficiency by traditional selection methods, is a very difficult concern, due to low heritability, sex-limited nature of reproductive traits in addition to long generation interval of buffaloes [7, 8]. Advances in molecular genetics have allowed animal breeders to characterize and identify molecular markers associated with dairy reproductive traits.

Signal transducer and activator of transcription (STAT) proteins include seven STAT proteins; STAT1, STAT2, STAT3, STAT4, STAT5 (A & B), and STAT6 acting as transcription factors and signal transducers in different mammals [9]. STAT5A protein is also called mammary gland factor (MGF) and is considered the main mediator of growth hormone action [10]. The STAT5A protein has an effective role in signal transduction within uterine epithelial cells and in the mammary gland. The STAT5A is a member of the placental lactogen (PL) and interferons (IFN-α) signal transduction pathway within uterine epithelial cells and mammary gland, which is very important in milk production, fertilization, and embryonic survival rates [11-13]. In bovine, there was a relationship between STAT5A gene polymorphisms and both of milk yield, fertilization, and embryonic survival rates [11, 14, 13]. The research efforts conducted for the improvement of the genetic potentiality of productive and reproductive traits of Egyptian buffalo are still scanty. Studies on polymorphisms of the Egyptian buffaloes STAT5A gene are scarce [15]. Single strand conformation polymorphism (SSCP) is considered an effective molecular technique for investigating DNA mutations in the amplified fragments and could be used as a powerful tool for selecting the economically important traits [16]. In the current study, polymorphisms of STAT5A gene in Egyptian buffalo were
detected in terms of genotype frequency for potential use as molecular combined marker in successful animal breeding program.

2. Materials and Methods

Animals

The current study was conducted on a total number of 60 Egyptian buffaloes at Mahallet Mousa farm in Kafr Elsheikh province; this station belongs to the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt. Buffaloes were naturally mated for the first time when they are 24 months of age and/or 300 to 350 kg of body weight. Animals were mated naturally. After 60 days from the last mating, pregnant ones were investigated by rectal palpation and sonar. Buffaloes were kept under semi-open sheds. Hand Robotic milking was applied twice per day at 7.00 a.m and 4.00 p.m. during the lactation period then the yielded milk was estimated daily. Buffaloes were fed by the routine feeding program of Mahallet Mousa farms. Animals were supplied with their requirement by Egyptian clover (Trifolium Alexandrinum), concentrate mixture, and rice straw from December to May period according to their requirement on a concentrate mixture and rice straw. The concentrate feed mixture was offered twice per day, while clover hay and rice straw were given twice a day also. Drinking water was offered freely all the time a day.

DNA extraction and polymerase chain reaction (PCR)

Sterilized vacutainer tubes containing EDTA were used for collecting blood samples from the jugular veins of 60 candidate animals. The genomic DNA was extracted from the leucocytes using an organic phenol/chloroform technique [17]. The quantity and quality of DNA were measured using a spectrophotometer (Biophotometer, Eppendorf, Germany) at 260 nm and 280 nm before being kept at -20°C.

DNA fragment of 929 bp of STAT5A gene including part of intron 7 to part of intron 9 was amplified using the following primers; forward 5’-TTGGAAGGCAGGCCATCTCTGC-3’ and reverse 5’-CAGCGTACTTGCAGGGTGTTTCTC-3’ [18]. To each 50 ng DNA template, 20 pmol of each specific primer, (2×) DreamTaq™ Green PCR Master Mix (Thermo Scientific, Germany) were added in the amplification reaction mixture (20 microliters). The conditions of thermal cycling were as the following: 95°C for 5 min for initial denaturation step, then 35 cycles at 94°C for 30 s, 65°C for 30 s, 72°C for 1 min and a final extension step at 72°C for 10 min. Amplified product was verified by electrophoresis on 2% agarose gels in 1x Tris-borate EDTA (TBE; 0.002 M EDTA and 0.09 M Tris-boric acid) buffer, alongside a Gene-Ruler™ 100-bp ladder (Thermo Fisher Scientific, Waltham, MA, USA) as a molecular weight ladder to confirm the length of the PCR products. Gels were stained with ethidium bromide (Gibco-BRL, Waltham, MA, USA) and visualized on a UV trans-illuminator.

Single-stranded conformation polymorphism (SSCP)

For SSCP, ten microliters of each PCR product of STAT5A gene was mixed with 10 µl denaturing loading dye (95% formamide, 20 mM EDTA pH 8.0, 0.05% bromophenol blue, 0.05% xylene cyanol) in a PCR tube. Samples were denatured in a thermal cycler (Nexus Gradient, Eppendorf, Germany) for 10 min at 95°C. Denatured samples were kept at -20°C for 30 minutes in an ice-chilled box before being separated on 8% polyacrylamide gel (29:1) containing 20% formamide. The electrophoresis was carried out at 120 V for 10 h in 1x Tris borate EDTA (TBE) buffer [19]. Then, the gel was stained to be seen and analyzed using ethidium bromide stain with a molecular weight ladder to confirm the length of the PCR products.

Statistical analysis

Estimation of genotypes frequency, observed and expected heterozygosities, in addition to the Hardy-Weinberg equilibrium was carried out using GENALEX software version 6.0 [21].

3. Results and Discussion

We successfully amplified 929-bp including part of intron 7 to part of intron 9 and the intervening exons of the STAT5A gene as shown in (Figure 1).
The SSCP analysis of this study showed the presence of three different conformation patterns (three genotypes) in the investigated buffalo population as shown in (Figure 2).

Pattern B showed the highest observed frequency of 51.7%, while patterns A and C occurred at frequencies of 45.0% and 3.3% respectively. Pattern A showed the highest expected heterozygosity frequency of 50.2%, while patterns B and C occurred at frequency of 41.3% and 8.5% respectively. A Chi square test showed that Egyptian water buffalo population was in Hardy-Weinberg equilibrium as shown in (Table 1).

Table (1) Genetic polymorphism of STAT5A gene in Egyptian buffalo.

<table>
<thead>
<tr>
<th>Breeds</th>
<th>No.</th>
<th>Observed genotype frequency</th>
<th>Expected genotype frequency</th>
<th>Hardy-Weinberg equilibrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Egyptian buffalo</td>
<td>60</td>
<td>0.450</td>
<td>0.517</td>
<td>0.033</td>
</tr>
</tbody>
</table>

A, B, and C = conformation patterns A, B, and C

Similar result was reported by Guo et al. [22], who identified two SNPs in STAT5A exon8 (C924T) and intron8 (C989T) in Italian buffaloes using the direct sequencing technique. The observed and expected
heterozygosities of these two SNPs were 43.9% and 46.0% for (C924T) and 41.0% and 43.6% for (C989T) respectively. İbiş and Erdoğan [18] recorded ten SNPs between the 6th to 9th exons of the STAT5A gene in the Anatolian water buffaloes and reported a range of 4.20% to 76.0% for the observed heterozygosities and a range of 4.10% to 50.0% for the expected heterozygosities. Although, Naveed et al. [23] identified nine polymorphisms of the STAT5A gene in Nili Ravi Buffalo; three in exonic and six in intronic regions, and the G/A SNP in exon 5 that cause a non-synonymous mutation changing Serine to Asparagine, the observed heterozygosities were very low and ranged between 2% and 18%.

By contrast, Daldaban et al. [24] showed no polymorphism in a 215 bp segment of STAT5A gene in Anatolian water buffaloes after digestion with AvaI restriction enzyme, where all of the examined animals recorded monomorphic pattern in terms of CC genotype. Genetic polymorphism or variability of signal transducers and activators of transcription 5A might be used as potential markers for future animal selection and breeding programs. The construction of SNP markers could be aided by identifying the precise SNPS by variant sequencing. Further, genetic association studies for investigating the possible relationship between STAT5A gene polymorphisms with productive and reproductive traits of Egyptian buffalo are eagerly anticipated.

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Conflict of Interest Statement

The authors declared that they have no conflict of interest.

References


