Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (7) Issue (5) (2022), (173-177) **online: ISSN 2356–976x**
http://bjas.journals.ekb.eg

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

Basant M. Shafik[1*], Sherif I. Ramadan[1], Mohamed El-Raey[2], Eman M. Abd El Fattah[3], Tahani S. Behour[3], Ayman F. Ashour[4], Amin M. S. Amin[4], and Samah F. Darwish[3]

[1]Animal Wealth Development Department, Faculty of Veterinary Medicine, Benha University, Toukh, Egypt
[2]Department of Theriogenology, Faculty of Veterinary Medicine, Benha University, Toukh, Egypt.
[3]Biotechnology Research Unit, Animal Reproduction Research Institute, Agriculture Research Center, El-Haram, Egypt
[4]Animal 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 anestrum 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-s*) 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 El-sheikh 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 with a little amount of clover hay 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, Ependorf, 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'-TTGGAAGGCAGGGC-ATCTCTGC-3' and reverse 5'-CAGCGTACTTGCGGGTGTTCTC-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 1× Tris-borate EDTA (TBE; 0.002 M EDTA and 0.09 M Tris-boric acid) buffer, alongside a Gene-RulerTM 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 final concentration of 0.5 µg/ml for 30 minutes, then photographed with an FX Molecular Imager apparatus (BIO-RAD, Hercules, CA, USA) [20].

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).

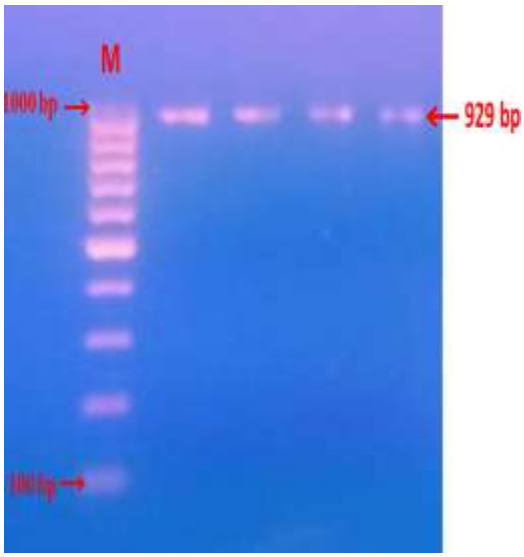

Fig (1) Ethidium bromide-stained agarose gel showing the PCR product of STAT5A gene in Egyptian water buffalo. M: 100-bp ladder. Lanes 1-4: 929-bp PCR product of *STAT5A*

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).

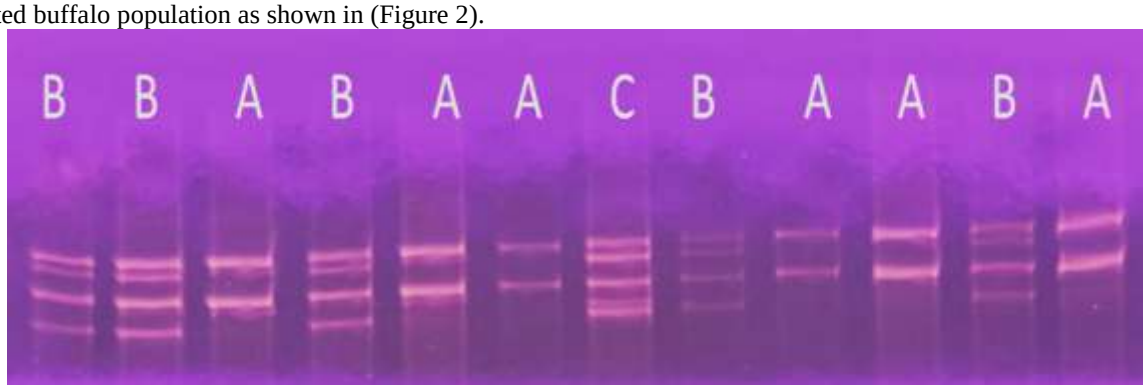

Fig (2) PCR-SSCP patterns showing three conformation patterns of Egyptian buffalo *STAT5A* gene (Lane 1, 2, 8, and 11 = conformation pattern B & Lane 3, 5, 6, 9, 10, and 12 = conformation pattern A & Lan 7 = conformation pattern C)

Pattern B showed the highest observed frequency of 51.7%, while patterns A and C occurred at frequencies of 45% 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.

Breeds	No.	Observed genotype frequency			Expected genotype frequency			Hardy-Weinberg equilibrium	
		A	B	C	A	B	C	X^2-test	*P* value
Egyptian buffalo	60	0.450	0.517	0.033	0.502	0.413	0.085	3.763	0.052^{ns}

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

Similar result was reported by Guo et al. [22], who identified two S*NPs* 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. Ibiş 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.

Acknowledgments

The authors would like to thank the director of the buffalo's farm at Mahallet Mousa for the allowance of blood samples collection. This study was supported by the research project entitled "Tracking the Relationship between Gene polymorphism and Egyptian Buffalo Fertility to Improve Its Profitability Globally" from the Scientific Research Fund (SRF), Benha University, Egypt.

Conflict of Interest Statement

The authors declared that they have no conflict of interest.

References

[1] FAOSTAT. FAO Statistics Division, (2018), Rome, Italy. FAO.

[2] M. El-Salam, H and S. El-Shibiny. A comprehensive review on the composition and properties of buffalo milk, Dairy Sci Technol., Vol. 91, pp. 663-699, 2011

[3] D. Berry and A. Cromie. Associations between age at first calving and subsequent performance in Irish spring calving Holstein–Friesian dairy cows, Livest. Sci., Vol. 123, pp. 44-54, 2009

[4] M. S. El-Tarabany and M. A. Nasr. Reproductive performance of Brown Swiss, Holstein and their crosses under subtropical environmental conditions, Theriogenology. Vol. 84, pp. 559-565, 2015

[5] A. Sosa, K. G. M. Mahmoud, H. Eldebaky, M. Kandiel, A. Abou El-Roos and F. Nawito. Genotyping of follicle stimulating hormone receptor gene in fertile and infertile buffalo, Glob Vet. Vol. 15, pp. 163-168, 2015

[6] M. Ansari-Lari, M. Mohebbi-Fani and A. Rowshan-Ghasrodashti. Causes of culling in dairy cows and its relation to age at culling and interval from calving in Shiraz, Southern Iran, (2012). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. pp. 233.

[7] R. Wakchaure, S. Ganguly, P. Praveen, A. Kumar, S. Sharma and T. Mahajan. Marker assisted selection (MAS) in animal breeding: a review, J. Drug. Metab. Toxicol. Vol. 6, pp. e127, 2015

[8] C. D. C. Barros, R. R. Aspilcueta-Borquis, A. B. Fraga and H. Tonhati. Genetic parameter estimates for production and reproduction traits in dairy buffaloes, Rev. Caatinga. Vol. 29, pp. 216-221, 2016

[9] C. Dario and M. Selvaggi. Study on the STAT5A/AvaI polymorphism in Jersey cows and association with milk production traits, Mol. Biol. Rep., Vol. 38, pp. 5387-5392, 2011

[10] G. Oikonomou, G. Michailidis, A. Kougioumtzis, M. Avdi and G. Banos. Effect of polymorphisms at the STAT5A and FGF2 gene loci on reproduction, milk yield and lameness of Holstein cows, Res. Vet. Sci., Vol. 91, pp. 235-239, 2011

[11] H. Khatib, R. Monson, V. Schutzkus, D. Kohl, G. Rosa and J. Rutledge. Mutations in the STAT5A gene are associated with embryonic survival and milk composition in cattle, J. Dairy Sci., Vol. 91, pp. 784-793, 2008

[12] X. He, M. Chu, L. Qiao, J. He, P. Wang, T. Feng, R. Di, G. Cao, L. Fang and Y. An. Polymorphisms of STAT5A gene and their association with milk production traits in Holstein cows, Mol. Biol. Rep., Vol. 39, pp. 2901-2907, 2012

[13] B. Coizet, S. Frattini, L. Nicoloso, L. Iannuzzi, A. Coletta, A. Talenti, G. Minozzi, G. Pagnacco and P. Crepaldi. Polymorphism of the STAT5A, MTNR1A and TNFα genes and their effect on dairy production in Bubalus bubalis, Ital. J. Anim. Sci., Vol. 17, pp. 31-37, 2018

[14] M. Selvaggi, C. Dario, G. Normanno, G. V. Celano and M. Dario. Genetic polymorphism of STAT5A protein: relationships with production traits and milk composition in Italian Brown cattle, J. Dairy Res., Vol. 76, pp. 441-445, 2009

[15] N. El-Halawany, A. S. Abd-El-Monsif, S. M. Sherif, A. E. El-Wardany, M. A. Abdel-Latif, H. Abdel-Shafy, A. F. Al-Tohamy and Y. El-Sayed.

Investigation of STAT5A, OLR1, LF and TLR4 Variants and Its Association With Milk Yield In Egyptian Buffalo, Res. J. Pharm. Biol. Chem. Sci., Vol. 10, pp. 179-187, 2019

[16] A. Caroli, F. Chiatti, S. Chessa, D. Rignanese, E. Ibeagha-Awemu and G. Erhardt. Characterization of the casein gene complex in West African goats and description of a new αs1-casein polymorphism, Journal of Dairy Science. Vol. 90, pp. 2989-2996, 2007

[17] W. M. Strauss. Preparation of genomic DNA from mammalian tissue, Curr. Protoc. Neurosci., Vol. 6, pp. A. 1H. 1-A. 1H. 3, 1999

[18] E. Ibiş and M. Erdoğan. The determination of polymorphisms between 6-9 exons of the water buffalo STAT5A gene, Eurasian J Vet Sci., Vol. 35, pp. 230-235, 2019.

[19] M. Orita, H. Iwahana, H. Kanazawa, K. Hayashi and T. Sekiya. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms, Proc. Natl. Acad. Sci. . Vol. 86, pp. 2766-2770, 1989.

[20] T. Xie, S. Ho and O. Ma. High resolution single strand conformation polymorphism analysis using formamide and ethidium bromide staining, Mol. Pathol. Vol. 50, pp. 276, 1997

[21] R. Peakall and P. E. Smouse. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research, Mol. Ecol. Notes. Vol. 6, pp. 288-295, 2006

[22] B. Guo, Y. Jiao, C. He, L. Wei, Z. Chang, X. Yue, X. Lan, H. Chen and C. Lei. A novel polymorphism of the lactoferrin gene and its association with milk composition and body traits in dairy goats, Genetics and Molecular Research. Vol. 9, pp. 2199-2206, 2010

[23] M. Naveed, A. Nadeem, M. Javed, M. F. Bhutta and R. B. Khalid. Evolutionary Divergence of Signal Transducer and Activator of Transcription 5A (STAT5A) Gene in Riverine Buffalo, Pak. J. Zool., Vol. 53, pp., 2021

[24] F. Daldaban, K. Arslan, E. G. Aksel and B. Akyuz. Polymorphism of the STAT5A and MYF-5 genes in Anatolian water buffalo, Turk. J. Vet. Anim. Sci., Vol. 44, pp. 284-289, 2020.