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Detection of FSHR gene variants associated with sperm motility in Egyptian buffalo bulls

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ABSTRACT

This study's goal was to use the DNA sequence techniques to find new mutations in the follicle-stimulating hormone receptor exon 10 (FSHR) in Egyptian buffalo bulls with different sperm motility. A total of 50 Egyptian buffalo bulls aged 3–4 years old, at Kafr El-Sheikh Governorate (breeding station of Mahallet Mousa), Egypt were investigated. The bulls were divided into two groups according to spermatozoa motility: the first group of high sperm motility (> 60%, n = 32) and the second group of low sperm motility (< 60%, n = 18). Blood samples were collected to obtain genomic deoxyribonucleic acid, and to perform polymerase chain reaction with an annealing temperature of 57 °C to amplify 306 bp of the FSHR exon 10 gene. The results showed that all the investigated buffalo bulls were monomorphic and had the same genotype. We may conclude that there no different variants of FSHR gene associated with different sperm motility, therefore we need to study more genes in relation to sperm motility.

1. INTRODUCTION

Buffalo is one of the most essential multipurpose agricultural animals. Infertility issues are widespread among this species, resulting in a significant decrease in their production.Profitable dairy farming begins with successful reproductive performance. Increased calving intervals and lower milk output sequel of reproductive inefficiency, resulting in major economic losses for the dairy sector(Inchaisri et al. 2010).

Reproduction is a difficult process that requires careful coordination of all elements. Although both parents have an impact on reproductive success, much of the study on dairy cattle has focused on cow fertility, with the bull's potential contributions generally neglected. It is become clear that low-fertility bulls' diminished capacity to establish pregnancy is caused by several factors, including ability of sperm for fertilization, embryonic development before implantation,development of the placenta and embryo after conception and pregnancy recognition (Immler 2018, Ortega et al. 2018)

Bull fertility has significant impacts on productivity (Braundmeier and Miller 2001), it is regulated by both genetic and environmental factors(Huang et al. 2011, Corbet et al. 2012).

Because of the poor heritability of qualitytraits of semen, direct selection for these characteristics is not practicable (Mathevon et al. 1998). As a result, MAS (Marker Assisted Selection) might be utilized to find superior sires at a young age.The candidate gene strategy may give unique indicators to predict sperm quality features in bulls as molecular biology techniques advance .

Hormones have a significant impact on how animals' reproductive systems develop and operate, both in females and males (Zhao et al. 2020, Sun et al. 2012),Polymorphisms in their receptor genemight be useful indicators of spermatogenesis. Follicle-Stimulating Hormone is one of the most important hormones insperm formation process in bulls (Sun et al. 2012). Inactivating mutations in the FSH and its receptor (FSHR) genes has been resulted in Azoospermia,low sperm count, and subfertility(Huhtaniemi 2006).However, there are few investigations into the relationship between FSHR genetic variants and Egyptian buffalo fertility(Ramadan, Saker, and Shafik 2020).The goal of this research was to investigate polymorphism in Follicle Stimulating Hormone Receptor (FSHR) gene by a direct

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DNA sequencing method in Egyptian buffalo bullsclassified according to sperm motility..

2. MATERIAL AND METHODS

All the experimental procedures were performed at the laboratory of Animal Wealth Development Department and the Central Laboratory of the Faculty of Veterinary Medicine, Benha University, Egypt, and were approved by the Animal Ethical Committees of Fac. Vet. Med., Benha University with ethical approval number (BUFVTM03-05-22).

2.1. Animals :

This study was conducted on 50 Egyptian buffalo bulls aged 3–4 years old and weighted 450-550 kgbelonged to Kafr El-Sheikh Governorate(breeding station of Mahallet Mousa), Egypt. All buffalo bulls were exposed to the same management and nutrition programs. The bulls were classified according to individual sperm motility into highand low quality semen; the first group had more than 60% sperm motility (high; n = 32) and the second had less than 60% sperm motility (low; n = 18) according to (Mahmoud et al. 2021).

2.2. Blood samples and DNA extraction :

EDTA-containing vacutainer tubes were used to collect blood samples from the jugular veins. All samples were labelled, placed in an ice box, and transferredto the lab for keeping at -20 °Cdegrees Celsius until further processing .

The genomic deoxyribonucleic acid was extracted from the blood using the G-spinTM total DNA extraction Kit (iNtRON Biotechnology, KOREAN) (Lot. No 105250852) which is based on silica membrane technology and following the manufacturer's procedure (www.intronbio.com). After introducing a specific buffer system, centrifugation eliminates impurities. The CE buffer was used to elute purified DNA, which was then ready to be used. The DNA content was measured using a Spectro-Star-Nano absorbance plate-reader (BMG Lab Tec GmbH, Ortenberg, Germany).

2.3. Polymerase chain reaction (PCR):

The Primer set for amplification of 306 bp fragment of FSHR gene was described by (Othman and Abdel-samad 2013) as the following sense primer5'-CTG CCT CCC TCA AGG TGC CCC TC-3' and antisense primer5'-AGT TCT TGG CTA AAT GTC TTA GGG GG-3 .'

The polymerase chain reaction was practiced in a total volume of 25 µl, with 500µg Deoxyribonucleic acid template, 0.8 µl of each sense and antisense primers (10 pmol), 12.5µl Master mix (Dream Taq Green PCR mix 2x, Thermo Scientific, USA) (Lot. No. 00584522) and up to 25 µl nuclease free water. PCR reaction was conducted as following: PCR tubes containing the mixture were subjected to initial denaturation for 5 minutes at 95 °C, followed by 35 cycles of amplification (denaturation at 94 °C for 60 seconds, annealing at 57 °C for 60 seconds and extension at 72 °C for 60 seconds) and final extension at 72 °C for 5 minutes.

The size and quality of polymerase chain reaction amplicons were detected by agarose gels (2 %) stained with ethidium-bromide (Invitrogen™ UltraPure™ ethidium) (Invitrogen™, USA) (Lot. No. 18H135301) alongside a Gene Ruler TM 50 bp Ladder (iNtRON Biotechnology, KOREA) (Lot. No. 0010-100803.51) as a molecular weight marker to confirm the length of the PCR products and checked with UV light of BioRad-Gel Documentation System (Gel DOC XR+ System, Hercules, CA, USA).

2.4 .DNA Sequencing:

After getting the purified PCR amplicons by using polymerase chain reaction purification kit (QIAquick PCR Purification Kit, Qiagen Company, Germany). The sequencing was done by using the same forward primer with Big Dye Terminator (Big Dye X Terminator™, USA) in 3500xlDNA Sequencers (Applied biosystems, Foster City, CA,USA). BLAST software (http://www.ncbi.nlm.nih.gov/) was used for the identification of the obtained sequences. Finch TV 1.4.0 (http://www.geospiza.com/finchtv/) and MEGA 7 software were used for sequence alignment and DNA polymorphism identification(Kumar, Stecher, and Tamura 2016)..

3. RESULTS

In our study, PCR-DNA sequencing was used to demonstrate the polymorphism of FSHR exon 10 gene in Egyptian buffalo bulls and their association with sperm motility.

The length of PCR product was a 306-bp fragment of the FSHR exon 10 gene (Figure 1). The sequence analysis of the PCR samples of all tested groups (high and low-motility spermatozoa) showed one genotype. All the investigated samples were monomorphic, as shown in figure 2

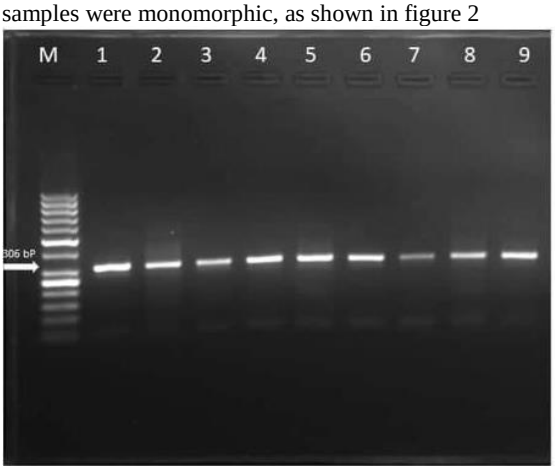

Fig. 1Agarose gel stained with ethidium bromide showed PCR product of FSHR gene in Egyptian buffaloes. M stands for a 50-bp ladder. Lanes 1- 9: 306-bp PCR product of FSHR gene.

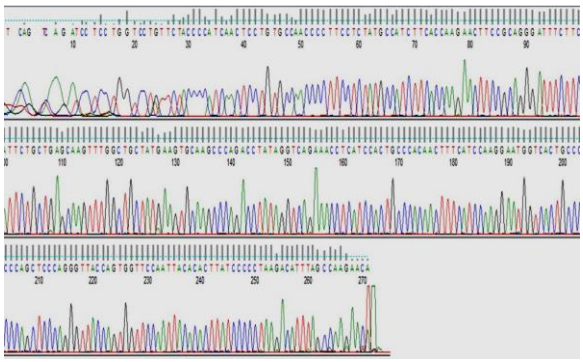

Fig. 2 Sequence chromatogram of 306 bp fragment of FSHR exon 10 of Egyptian buffalo.

The sequence identification and confirmation of 306 bp of the buffalo FSHR gene sequence with reference sequenced retrieved from Gene-Bank (EF650049.1)was performed by

BLAST and showed that the segment possessed identities of 96% (Figure 3).

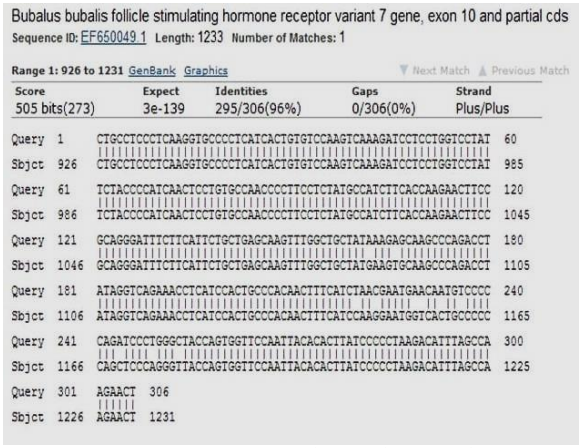

Fig.3 Nucleotide sequence alignment of FSHR fragment of 306 bp with reference sequence of Bubalus bubalis (EF650049.1) showed 96% identify using BLAST.

4. DISCUSSION

The polymorphism of FSHR in exon 10 was examined in this study and their possible association with sperm motility in Egyptian buffalo bulls.

Our findings revealed that the FSHR gene has no polymorphisms, in all bulls. On the contrary, Nikitkina et al. (2021) reported that FSHR gene showed polymorphic inversions by RFLP and sequencing between groups of Russian Holstein bulls which were classified according to semen quality and the polymorphisms in the FSHR gene were also showed to had significant ($p< 0.05$) correlations with double ejaculate volume, concentration, and total number of spermatozoa and non-significant association with sperm motility.

Sang et al. (2011) demonstrated a new single nucleotide polymorphism in Chinese Holstein of FSHR A-234500T (rs43676359) and substantiated thispolymorphism significantly associated to sperm concentration and semen volume per ejaculate, while non-significantly associated with sperm motility.

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