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DNA polymorphism of FSHR gene and its association with infertility traits in Egyptian buffaloes

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ABSTRACT

The objectives of this study were to detect genetic polymorphism in exon 10 of Follicle Stimulating Hormone Receptor gene (FSHR) by using Restriction Fragment Length Polymorphism (RFLP) and direct sequence methods, also to investigate their possible association with infertility traits in Egyptian buffaloes. A total number of 50 female Egyptian buffaloes at Mahallet Mousa experimental farms in Kafr El-sheikh province were investigated. Animals were divided into three groups: normal fertile (20 animals), infertile due to Anestrum (15 animals) and infertile due to repeat breeding (15 animals). Genomic DNA was extracted from blood samples, then PCR was performed with annealing temperatures of 57 ºC to amplify 231 bp of FSHR exon 10 gene. The amplicons were digested with Hin1II (NlaIII) restriction enzyme. The results showed that all the investigated buffaloes were monomorphic and genotyped as A/A genotype. Moreover, the monomorphism of buffaloes FSHR exon 10 gene was confirmed by sequencing three samples from each buffaloes' group. It seems that FSHR gene are highly conserved in the investigated buffalo's population.

1. INTRODUCTION

Water Buffalo (*Bubalus bubalis*) is considered the most important dairy animal in Egypt from the socioeconomic point of view. Buffaloes are considered the main source of white colored milk in Egypt (Abou-Bakr, 2009). Buffalo's milk is greatly preferred by the Egyptian consumer due to its acceptable flavor, white color and high fat percent (El-Salam and El-Shibiny, 2011). Reproductive performance of dairy animals has a great impact on the farm profitability (Berry and Cromie, 2009). Among the reproductive performance, infertility problems such as anestrum and repeat breeding are the major reproductive disorders in Egyptian buffaloes that impact on farms profitability because of their effect on calving interval length (Ahmed et al., 2010; El-Tarabany and Nasr, 2015; Sosa et al., 2015). Improvement of fertility performance is a very important concern in dairy farms, because poor fertility is the main reason for dairy animals culling after low milk production (Ansari-Lari et al., 2012).

It is believed that the improvement of buffalo's reproductive performance by classical selection programs, is a very difficult task, due to long generation interval, sex limitation and low heritability of reproductive traits in buffaloes (Wakchaure et al., 2015; Barros et al., 2016). The advancement of molecular biology enabled the animal breeders for the identification and characterization of molecular markers that were associated with reproductive traits in farm animals. The reproductive efficiency of Egyptian buffaloes is greatly influenced by infertility disorders such as anestrus, inactive ovaries, repeat breeding (Ahmed et al., 2010; Sosa et al., 2015). Genes that encode the endocrine hormones may affect the reproductive efficiency and overall productivity of the dairy animals (Meyer et al., 1990).

Follicle Stimulating Hormone Receptor gene (FSHR) is composed of 10 exons and 9 introns (Simoni et al., 1997). The development, growth, differentiation, maturation and ovulation of ovarian follicles are conducted by Follicle Stimulating Hormone (FSH) through binding to its specific receptor (FSHR) in the surface of the ovary (Chu et al., 2012; Yang et al., 2012). Genetic selection based on molecular markers allows for accurate selection of specific DNA variant that is associated with desirable traits, so that it is very useful method for selection of low heritable traits such as reproductive and fertility traits (Pedersen et al., 2009). However, studies on the association of FSHR genetic variations with reproductive performance of Egyptian buffaloes still few. The aims of this study were to identify genetic polymorphism in Follicle Stimulating Hormone Receptor (FSHR) gene by using Restriction Fragment Length Polymorphism RFLP and direct sequence methods and to investigate their possible associations with infertility problems in Egyptian buffaloes.

2. MATERIAL AND METHODS

All of the experimental procedures were performed in the Central Laboratory of Faculty of Veterinary Medicine, Benha University, Egypt, and were approved by the

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Animal Ethical Committees of the Benha University with ethical approval number (BUFVTM01-07-2020).

2.1. Animal and sample preparations:

This study was performed on a total number of 50 Egyptian buffaloes at Mahallet Mousa experimental farm in Kafr El-sheikh province, Egypt. Heifers weighted 300 to 350 kg of body weight or aged 24 months were naturally served for the first time. Animals were assigned into three main groups: normal fertile (20 animals), infertile due to anestrum (15 animals) and infertile due to repeat breeding (15 animals). A total of 20 apparently healthy buffaloes kept under approximately the same managemental conditions and matched for age and body weight and showing normal estrus signs were included as normal fertile. Heifers with smooth ovaries (no palpable follicles) and lack of estrous signs were diagnosed as anestrum. While those having estrous signs and not conceived after three services were diagnosed as repeat breeders. Buffaloes were diagnosed as anestrum by rectal palpation and by the aid of ultrasonographic examination once a week for at least four successive weeks.

2.2. DNA extraction and amplification:

Blood samples were collected from the jugular veins of 50 animals in EDTA- containing vacutainer tubes. all samples were labeled, stored in an ice box and transferred to the laboratory and stored at -20 ºC till further processing .The genomic DNA was extracted from the blood using TIANamp genomic DNA extraction Kit following the manufacturer protocol (www.tiangen.com/en). TIANamp genomic DNA Kit is based on silica membrane technology. Centrifugation completely removes contaminants after adding special buffer system. Purified DNA is eluted in water and ready for use. The FSHR locus was amplified by using PCR forward primer 5'-ATCACGCTGGAAAGATGGCATACC-3' and reverse primer5'-GACATTGAGCACAAGGAGGGAC-3' (Yong et al., 2006). The PCR reactions were performed with a total volume of 25µl containing 3.0µl DNA template, 1.0µl of each forward and reverse primers (10 pmol), 12.5µl Master mix and 7.5 µl nuclease free water. PCR reaction was conducted as the following: PCR tubes containing the mixture were subjected to 5 min at 94 ºC for initial denaturation, 30 cycles of amplification (denaturation at 94 ºC for 1 min, annealing at 57 ºC for 2 min and extension at 72 ºC for 2 min) and final extension at 72 ºC for 10 min. The size and quality of PCR amplicons were detected by 2 % agarose gels stained with ethidium bromide (Gibco-BRL, Waltham, MA,USA) alongside a Gene Ruler TM 50 bp Ladder (Thermo Fisher Scientific,Waltham, MA, USA) as a molecular weight marker to confirm the length of the PCR products, and checked with UV light of Gel Documentation System.

2.3. Restriction Fragment Length Polymorphism (RFLP) and direct sequence methods:

Amplicon was digested by Hin1II (NlaIII) restriction enzyme by incubation for 15 min at 37 °C. Restriction Fragment Length Polymorphism (RFLP) was carried out in a reaction volume of 40 µl; 20 µl of PCR product, 5 µl of 10x G buffer , 14 µl of dH2O and 1 µl of restriction enzyme (Hin1II) Fast Digest (Fermentas, Vilnius, Lithuania). The restricted fragments were subjected to gel electrophoresis on 4 % Agarose gel which was stained with ethidium bromide stain and visualized under UV trans-illuminator.

After getting the purified PCR amplicons by using PCR purification Kit (Roche, Mannheim, Germany). The sequencing was done by using the same forward primer with Big Dye Terminator in 3500 xlGenetic Analyzers (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(Kumar et al., 2016) software were used for sequences alignment and detection of DNA polymorphism.

3. RESULTS

DNA fragments of 231-bp from FSHR exon 10 gene of Egyptian buffalo were amplified as shown in Fig 1. PCR-RFLP was performed to detect G/A non-synonymous SNP (Methionine/Isoleucine) at position 150 bp from the start of the amplified DNA fragment of FSHR exon 10 gene. In all tested samples there was one genotype in the three studied buffaloes' groups (normal fertile, anestrum and repeat breeder) as shown in Fig2. The obtained results were confirmed by sequencing two samples from each buffaloes' group. The all investigated samples were monomorphic and genotyped as A/A genotype as shown in Fig 3.

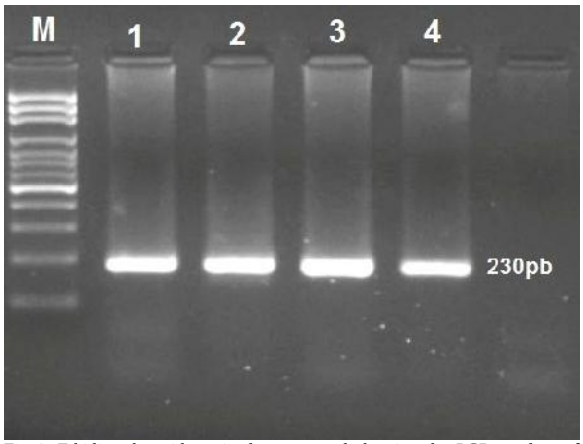

Fig 1. Ethidium bromide stained agarose gel showing the PCR product of buffalo FSHR gene in Egyptian water buffaloes. M: 100-bp ladder. Lanes 1-4: 231-bp PCR product of FSHR gene

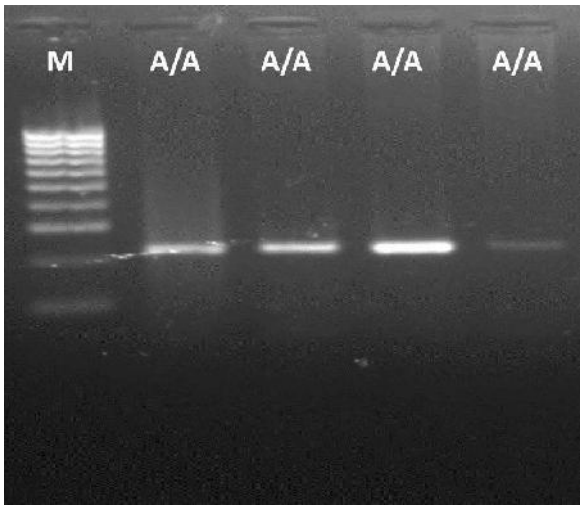

Fig 2. Gel electrophoresis showing the PCR-RFLP products of 231-bp of FSHR gene in the Egyptian buffaloes. The genotypes are indicated at the top of each lane. M is DNA molecular marker.

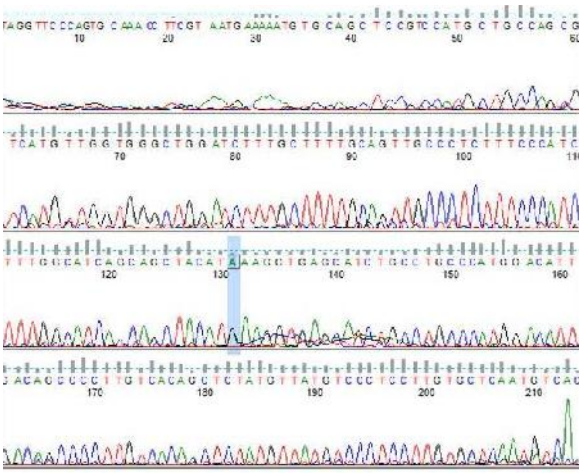

Fig 3. Sequence chromatogram of 231-bp fragment of FSHR exon 10 gene in Egyptian water buffaloes.

4. DISCUSSION

In the present study, we investigated the polymorphism of FSHR exon 10 gene and their possible association with infertility traits in Egyptian buffaloes. Our results showed that there was no polymorphism in the FSHR exon 10 gene.Our results come in agreement with that reported by Sosa et al. (2015) ;Othman and Abdel-Samad (2013) in Egyptian buffaloes, Kathiravan et al. (2019) in Murrah Buffaloes and Al-Hamedawi et al. (2017) in Iraqis buffaloes who reported no polymorphism in the amplified 306 bp fragment of FSHR exon 10 by applying RFLP technique. Moreover, Lan et al. (2006); Li et al., (2010)in Haimen goats and Huang et al. (2010)in Lezhi black goats reported no polymorphism at the same SNP after amplifying 230 bp of FSHR exon 10 gene in goats.

On the contrary, ShafiK et al. (2017) reported a non-synonymous G/ASNP causing a change of methionine into isoleucine amino acid after amplifying the the samefragment of our study in FSHR exon 10 gene by applying PCR-SSCP technique in Egyptian buffaloes. It seems that FSHR gene is highly conserved in the studied buffalo's population. This might be attributed to the high inbreeding coefficient of investigated population or due to the presence of some kind of relationship among the sampled animals. Moreover, the FSHR gene might not be exposed to any kind of selection. Although the great importance of FSHR gene on reproduction and fertility traits, we didn't find any polymorphism between the anestrum, repeat breeding and normal fertile buffaloes' groups for the studied locus. We recommend increasing the numbers of the investigated animals and searching for other loci within this important FSHR gene.

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CONFLICT OF INTERESTS

The authors declared that they have no conflict of interest

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