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IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN EXON 2 OF BONE MORPHOGENETIC PROTEIN 15 GENE IN EGYPTIAN BUFFALOES

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ABSTRACT

Bone Morphogenetic Protein 15 (BMP 15) is a member of transforming growth factor-β (TGF- β) superfamily which plays an important role in ovarian follicular development and ovulation rate. In this study a partial sequence of this gene was screened for polymorphisms. PCR amplification of DNA isolated from blood of 100 Egyptian buffaloes hiefers (50 infertile and 50 normal). Revealed two loci, BMP15.1 (222 bp) and BMP15.2 (222 bp) which includes exon 2 of BMP 15 gene. Polymorphisms in these two loci were detected by using direct sequencing and single strand conformational polymorphism (SSCP) technique. A single pattern of SSCP was detected. This was confirmed by nucleotide sequencing which revealed absence of single nucleotides polymorphisms (SNPs) or any other type of polymorphisms in the two loci. The sequencing and PCR-SSCP patterns revealed no polymorphism in all studied animals. Further studies must be done on another loci on BMP 15 gene or other genes related to infertility in Egyptian buffaloes.

Keywords: *BMP15 gene, polymorphism, Egyptian buffalo, infertility, PCR-SSCP.*

INTRODUCTION

Buffaloes belongs to Bovidae family and Ruminantia suborder. Egyptian buffalo had classified geographically and some phenotypic differences into Beheri, Baladi, Menoufi and Saidi ***(FAODAD-IS, 2013).*** The domestic water buffalo has a great economic importance for small farmers in developing countries like Egypt ***(Michelizzi et al., 2010).***

Infertility defined as the loss of ability to conceive after one year of regular mating or insemination, although, in many cases, the diagnosis is unexplained, several causes such as decreased ovulation rate, mechanical stoppage, deficiencies of sperm and parental age. Repeat breeder is the main source of economic loss in dairy farms. Infertility genetically may result from gene disorders, abnormalities in chromosomes, and phenotypes with multifactorial inheritance ***(Sah et al., 2003).***

Transforming growth factor was firstly identified as a secreted factor which control cell proliferation ***(Roberts and Sporn, 1990).*** Based on cell signaling pathways, the proteins

can be classified into two major groups, the Bone Morphogenetic Proteins/Growth Differentiation Factors, and activins/Transforming Growth Factors ***(Chang et al., 2002).*** Several BMP family members, including BMP -2, 3, 4, 5, 6, 7, and 15 are expressed in uterus ***(Tanwar and McFarlane, 2011),*** ovary ***(Sun et al., 2010)*** and in the granulosa cells and oocyte ***(Otsuka et al., 2001)***. They perform great biological activities as regulators in development of ovarian follicles, female reproductive tract differentiation, blastocyst implantation in the uterus, and organogenesis and morphogenesis during embryo development ***(Kishigami and Mishina, 2005).***

The aim of the present study was to search for polymorphisms in *BMP15 gene* in infertile and normal buffalo heifers using PCR –SSCP and DNA sequencing.

MATERIAL AND METHODS

All experiments were conducted in the Biotechnology Lab, Department of Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University, Egypt.

2.1. Animals and sample collection

The experiments were performed in 2014–2015 from a buffalo nucleus herd kept in Nataff-Kadeem Station and Mahalet-Mousa Farm, Animal Production Research Institute (APRI). A total of 100 heifers (50 repeat breeders and 50 normal) were genotyped for the *BMP15* gene. Blood samples were obtained from jugular vein into sterilized tubes containing EDTA as an anticoagulant and stored at −20°C until use.

2.2. Genomic DNA extraction

Total genomic DNA was extracted using DNAeasy Blood & Tissue Kit **(Fermentas, Thermo scientific, #K0721)** according to the manufacturer's protocol.

2.3. PCR amplification

Two loci of *BMP15* gene were amplified by PCR, *BMP15.1* and *BMP15.2* which includes in exon 2. The PCR reactions were performed with a total volume 20 µL containing 2 µL DNA template, 1 µL from each primer, 10 µL master mix (Thermo Scientific, #K1071) and 6 µL nuclease free water. This mix was put in thermal cycler and condition was optimized as shown in **table 1**. PCR products were electrophoresed on 1.5% agarose gels. Fragment size was determined using Gene Ruler™ 100bp Ladder (Fermentas).

2.4. Genotyping using PCR- SSCP technique

PCR-SSCP was performed as previously described by ***El-Magd et al,*** **(2014)** with minor modification.

2.5. DNA sequencing

The purified PCR products were sequenced by automated sequencer (ABI 310, Applied Biosystem, USA). The Sequences were analyzed using geneious 4.8.4 software. The obtained sequences were edited manually using CLC Main Workbench 7 to identify nucleotide substitutions.

RESULTS

The amplified product of the *BMP 15.1* locus was 222 bp **(Fig 1)** and that of *BMP 15.2* locus was 222 bp **(Fig 2)**. Analysis of SNPs in Exon 2 of BMP15 gene using PCR-SSCP revealed no SNPs were detected in either *BMP15.1* locus **(Fig 3)** or *BMP15.2* locus **(Fig 4).** Nucleotide sequencing confirmed the results and showed no SNPs in the two loci of BMP15 gene **(Figs. 5, 6).**

To detect polymorphic sites/SNPs, all sequenced amplicons of *BMP15* were aligned and compared using Clustal Omega and CLC Main Workbench7 software. The results revealed that there are no differences between all studied (infertile and normal animals) in all sequences of BMP15.1 locus and BMP15.2 locus **(Fig 7, 8)**.

Table (1): Primer sequences and PCR condition of *BMP15* gene.

Locus	primer	PCR condition
BMP15.1	F 5́ GAGTGTTCAGAAGACCAAACCTC3́ R 5́ TGGGGAGCAATGATCCAGTGATCC3́	94 °C /3 min, 35 cycles (94 °C for 30 s ,60°C for 40 s, 72 °C for 1 min) and final extension at 72 °C for 5 min.
BMP15.2	F 5́ CTACTGTAAGGGAGTATGTCCTCG3́ R 5́ CTGCATGTGCAGGACTGGGCAA3́	

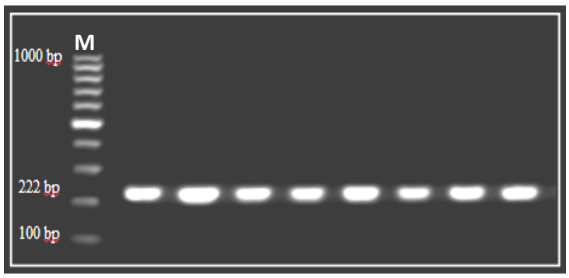

Figure (1): Ehidium bromide stained agarose gel of PCR product representing amplification of *BMP15.1* (222 bp) from normal and infertile buffalo heifers. M: 100 bp ladder.

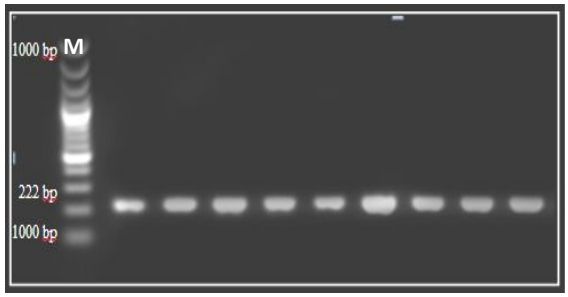

Figure (2): Ehidium bromide stained agarose gel of PCR product representing amplification of *BMP 15.2* (222 bp) from normal and infertile buffalo heifers. M: 100 bp ladder.

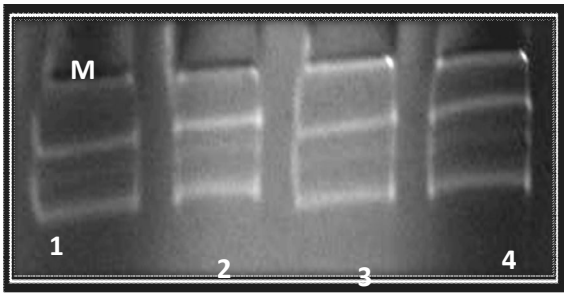

Figure (3): PCR-SSCP patterns of *BMP 15.1* locus in Egyptian buffalo show one trimorphic SSCP pattern was detected.

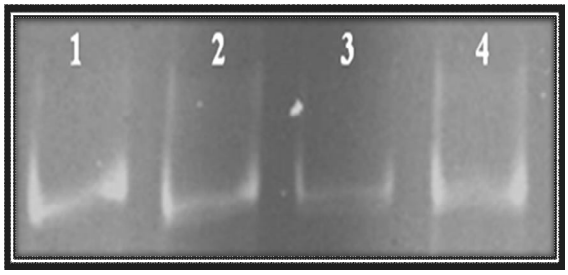

Figure (4): PCR-SSCP patterns of *BMP 15. 2* locus in Egyptian buffalo show one dimorphic SSCP pattern.

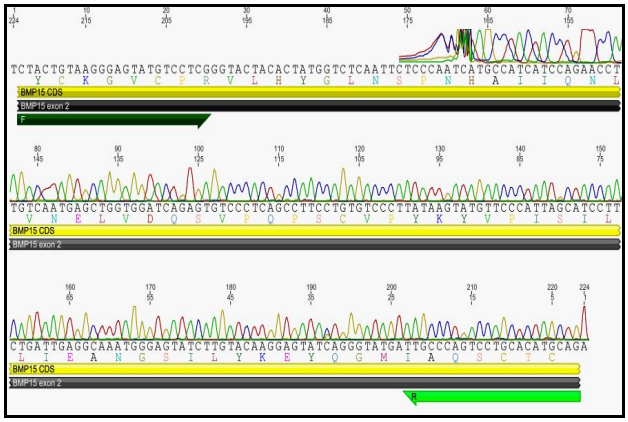

Fig (5). Nucleotide sequences of *BMP15.1 locus* taken as representative example.

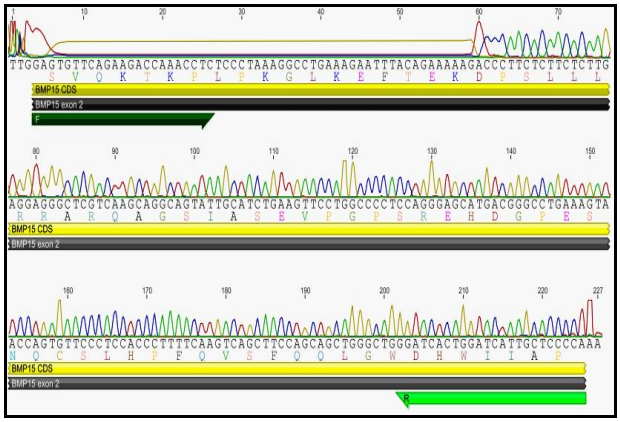

Fig (6). Nucleotide sequences of BMP15.2 locus taken as representative example.

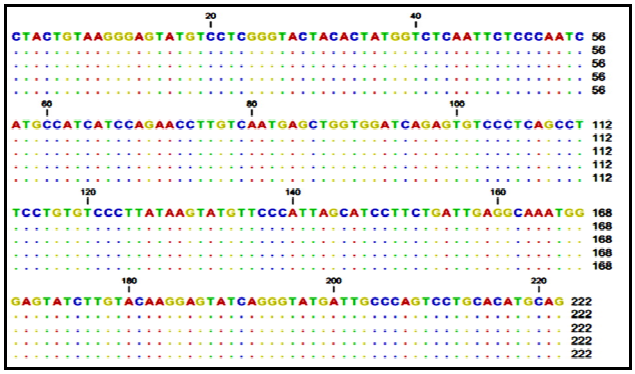

Fig (7). DNA sequence alignment of BMP 15.1 locus.

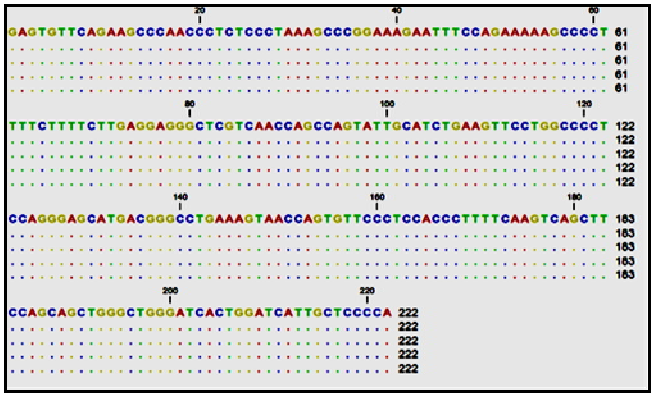

Fig (8). DNA sequence alignment of BMP 15.2 locus

DISCUSSION

The discovery of molecular biology and DNA based markers created new methods to study livestock genetics and animal breeding. Selection which depends on genotype can enhance productivity of farm animals, also enhance the adaptation to environment and keep on genetic diversity ***(Hayes, 2007)***. Molecular markers considered one of the most vital tools for the genome analysis and enable the association of heritable traits with underlying genomic variation. Also it can studies of candidate genes and their effect on the phenotypic traits are the basis for Marker assisted selection ***(Kulibaba and PodStreshnyi, 2012)***.

Previous studies have revealed that spontaneous mutations in the ovine and murine *BMP15* (FecX) gene may alter fertility and ovulatory rate in these species. Infertile animals with arrested folliculogenesis at primary stage results from deletion of the BMP15 ***(Hanrahan et al., 2004)***.

This agree with the finding of ***Gholibeikifard et al., (2014)*** whose used PCR-SSCP technique and showed no evidence of mutation was observed in BMP15 gene in Iranian Bluchi Sheep Breed, all of which were monomorphic for exon 2 BMP-15 gene.

Zhang et al., (2009) calculated the allele and genotype frequencies of the BMP15 gene in 8 breeds of cattles are presented two genotypes (AA and AB) were detected, while only one genotype (AA) was detected in Menggolian, Holstein and Simmental, this result disagrees with our result.

Some mutations in exon 2 of BMP15 gene have been associated with prolificacy in various breeds of sheep. Polymorphism of BMP15 gene exon 2 was studied in 100 Mehraban and 100 Lori ewes, using PCR-SSCP and DNA sequencing methods. A new point mutation (G→A) was found at position 57 of the amplified fragment ***(Zamani et al., 2015).***

CONCLUSION

PCR-SSCP failed to produce patterns capable of discriminating between the exon 2 of BMP15 gene in infertile and normal heifers so further studies should be done on other loci on BMP15 or on another gene related to infertility in Egyptian buffalo.

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الملخص العربي

تحديد الطفرات الموضعية فى الاكسون الثانى للجين BMP15 وعلاقته بالعقم فى الجاموس المصرى

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أجريت هذه الدراسة على الجين BMP15 والذى يعتبر عاملا مهما من عوامل النمو التى تلعب دورا كبيرا فى نمو البويضات وتنشيط عملية التبويض. تمت هذه الدراسة على بعض المواقع الجينية التى تمثل الاكسون الثانى حيث تم اخذ عينات دم من ١٠٠ حيوان من الجاموس المصرى (٥٠ حيوان طبيعى و٥٠ حيوان يعانى من عدم الخصوبة) وتم استخلاص الحامض النووى DNA وتضخيمه باستخدام تفاعل البلمرة المتسلسل PCR ثم تحديد الطفرات الموضعية باستخدام الشريط الفردىSSCP وتاكيده بعمل تتابع نيوكليوتيدى sequencing. اظهرت النتائج عدم وجود طفرات موضعية فى المواقع تحت الدراسة.