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DETECTION OF SNPS IN EXON7 LOCUS OF *CYP19* GENE AND THEIR ASSOCIATION WITH ANESTRUM IN EGYPTIAN BUFFALOES (*BUBALUS BUBALIS*)

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ABSTRACT

CYP19 is a member of cytochrome P450 aromatase family, which regulates estrogen biosynthesis in animals so it can be used as a candidate gene for marker-assisted selection strategies for fertility in buffaloes. The sequence of this gene in buffalo was recently published but association study has not conducted yet. Therefore, the objective of this study was to detect polymorphisms in a *CYP19* locus containing exon 7, and to determine associations between these polymorphisms and anestrum in Egyptian buffalo. A PCR product of 241bp, containing exon 7 of *CYP19* gene was successfully amplified using PCR and subsequently, subjected to single strand conformation polymorphism (SSCP) followed by sequence analysis to identify its different allelic patterns. There was no any SNP in all examined Egyptian buffaloes as shown by one SSCP pattern and nucleotide sequencing. Consequently, these results indicated that this locus does not influence anestrum in Egyptian buffalo and further studies should be conducted to detect other SNPs associated with fertility in Egyptian buffalo.

Keywords: *CYP19*, buffalo, PCR, SSCP, sequencing, SNPs, anestrum.

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1. INTRODUCTION

River buffalo is a species of great economic potential, especially in developing countries; it is the main source of meat and milk in Egypt (Othman, 2013). According to the last estimate, there are about 195 million buffalo in the world today, 97% in Asia, 2% in Africa mainly in Egypt, and 0.2% in Europe mainly in Italy (FAO, 2013). The limited productivity of Egyptian buffalo is due to many reasons including inbreeding, feeding and health care. However, the major problem seems to be infertility that is much higher than that in cattle. Anestrum (in form of silent heat and long calving interval) have been recognized as major cause of infertility (Ahmed et al., 2010; Derar et al., 2012; Fooda et al., 2011), the animal is non pregnant with smooth ovaries giving no palpable evidence of either follicular or luteal activity on rectal examination. One of the physiological causes for true anestrum is a low level of ovarian estrogens (Hafez and Hafez, 2000). This disease is not only lower animal productivity and fertility but also bring economic loss which may be due to extension of the dry period as well as longer calving interval during the life time of the animals (Kumar et al., 2011). Genetic evaluation of animal reproductive performance depends on molecular technology for identifying genes and analysis of the polymorphism of these marker genes whose products are key enzymes in the metabolic pathways of important physiological processes and are related to phenotypes (Beuzen et al., 2000). The key enzyme in estrogen biosynthesis is cytochrome P450 aromatase, the protein product of CYP19 gene. The role of aromatase is the conversion of androgens to estrogens, and is essential for physiology of reproduction (Jędrzejczak et al., 2011). The bovine CYP19 gene have been mapped to the long arm of chromosome 10, band 2.6 in cattle (Goldammer et al., 1994), chromosome 11 in buffalo (Iannuzzi et al., 2001) and range from 56 kb to 120 kb in different species. It consists of 10 exons. The coding region includes exon II–X with translation start site in exon II (Simpson and Davis, 2001). To detect mutations and single nucleotide polymorphisms, the candidate gene should be amplified in small fragments and these fragment should then be analyzed by rapid technique as single strand conformation polymorphism (SSCP) to locate and identify the nature of mutation or polymorphism (Lakhotia and Somasundaram, 2003); (Orita et al., 1989). It seems that, no study was conducted to identify the CYP19 polymorphisms and their association with reproductive performance in Egyptian buffaloes. Therefore, the aim of this study was to isolate CYP19 gene locus containing exon7 and to detect any polymorphisms associated with anestrum in Egyptian buffalo cows.

2. MATERIAL AND METHODS

2.1. Animals source and grouping

The current study was conducted on 180 buffalo cows and heifers selected from Buffalo Nucleus Herd, Animal Production Research Institute, Ministry of Agriculture. Heifers were naturally served for the first time when they reach 300 to 350 kg of body weight and/or 24 months of age. Buffalo cows should be dried off two months before the expected calving date, and they were served not before two months after calving. Animals were assigned in two main groups: normal fertile and infertile due to anestrum. Buffalo cows with 5 successive calving was grouped as normal fertile, heifers which didn’t show oestrus over two years of age and buffaloes which didn’t ovulate over sex months after calving was grouped as anoestrus.

2.2. Total DNA extraction

Blood samples were collected in EDTA-containing vacutainer tubes (kept in ice box) from jugular veins of 40 animals in each group. The genomic DNA was extracted from the leucocytes using Gene JET genomic DNA purification kit following the manufacturer protocol (Fermentas, #K0721). The concentration of total extracted DNA was spectrophotometrically determined at 230 and 260 nm using Nanodrop then stored at -20ºC.

2.3. Polymerase chain reaction (PCR)

The *CYP19* locus was amplified by PCR using primers (Table 1) designed by Primer 3.0 software based on the published sequences of Indian buffalo (GenBank accession number, EF126034). The PCR was carried out in a reaction volume of 50 µL, containing 4.0 µL DNA template (approximately 100 ng), 10 µl Dream Taq Green PCR master mix 5x (Fermentas, #K1071, European Union), 2.0 µL (10 µmol/L) forward primer, 2.0 µL (10µmol/L) reverse primer, and 32 µL nuclease free water. PCR was performed by employing a PCR program as follows: Initial denaturation step at 94ºC for 2 min, then tubes were subjected to 35 cycles of 94ºC for 1 min, 60ºC for 1 min and 72ºC for 1 min, followed by a final extension step at 72ºC for 10 min. As a negative control, tubes were prepared with water instead of DNA template. Then PCR products were resolved by electrophoresis on 2% agarose gel in 1X TAE, stained with ethidium bromide and visualized with UV light of Gel Documentation System (Biometra Biomedizinische Analytik, GmbH).

2.4. Single Strand Conformation Polymorphism (SSCP)

The procedure was followed according to kumar et al (2009) with some modifications. The PCR products (5 µl) were mixed with 5 µl of SSCP dye (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue) in 0.2 ml PCR tubes, then incubated at 95 ° C for 10 min for denaturation and plunged in ice for 5 min to form single strand conformers then electrophoresed in a 12% polyacrylamide gel (ratio of acrylamide to bis-acrylamide was 39:1 [0.5 gram of bis-acrylamide and 19.5 gram of acrylamide was added to 50 ml distilled water and mixed well in water bath at 37ºC till complete solubility, 5 ml 10% TBE (contained 108g Tris, 55g boric acid +40 ml 0.5M EDTA [37.22g EDTA in 150 ml of distilled water] and up to 1000 ml deionized water and mixed well), 12 ml of 20 % acrylamide to bis-acrylamide mix were added to 8 ml deionized water, 20 µl of TEMED (tetra methylene diamine) and finally 200 µl of 10% Ammonium persulfate was added and mixed well. The optimal polymerization time was about 4 h. The gel was pre electrophoresed at 160 V for 30 min with 0.5x TBE as electrode buffer. The conformers of PCR products were separated in the gel at 14–16 ° C using constant voltage of 160 V for 4 h. The DNA fragments in the gel were detected by 500ml of Ethidium bromide 0.5 µg/ml in 1x TBE for 10-30 minutes on a rocking platform for proper fixing of SSCP conformers in the gel. Then the gel was transferred gently in 500ml of sterile distilled water for de-staining. The fragment patterns were visualized on the UV Trans-illuminator and photographed by gel documentation system (UVDI Major Science, USA).

2.5. DNA Sequencing

After getting purified PCR products (clones) with expected sizes , the clone was purified using PCR purification kit following the manufacturer protocol (Jena Bioscience # pp-201×s) to remove primer dimmers, primers, nucleotides, proteins, salt, agarose, ethidium bromide and other impurities. The PCR products were sequenced in automated sequencer (Applied Biosystem, USA) using *CYP19* primers. The Sequences were analyzed using the Chromas Lite 2.1 program (http://technelysium.com.au/?page_id=13) and the identity of the sequenced PCR product was examined using Blast search against Genbank database of Indian buffalo and cattle (*Bos Taurus*) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).
The alignments, annotations and assembly of the sequences were performed using Geneious 4.8.4 software http://www.geneious.com/web/geneious/home.

3. RESULTS

The PCR product with the expected size (241bp) was shown by 2% agarose gel electrophoresis (Figure 1). Then, the resulting PCR products were purified using gel purification technique. No polymorphism was detected in *CYP19* locus as revealed by presence of only one SSCP banding (monomorphic) pattern (Figure 2). Sequencing was conducted to verify the results of SSCP. The sequences of this *CYP19* locus in Egyptian buffaloes (submitted to GenBank with accession number KF957995) showed no polymorphism (Fig. 3). Nucleotide sequences alignment of this *CYP19* locus showed 100% identity with Indian water buffaloes (EF178281) (Fig.4) and 98% identity with *Bos Taurus* (Z69247) (Fig.5).

4. DISCUSSION

Genetic evaluation of animal reproductive performance depends on molecular technology for identifying genes of reproductive efficiency and analysis of the polymorphism of these marker genes (Beuzen et al., 2000). Taking into account various functions and influences of estrogens on the regulation of reproductive processes, it is not surprising that genes, such as CYP19 involved in their synthesis and function are regarded as candidates for the markers of reproductive traits.

Table 1: Forward and reverse primers sequence for *CYP19* locus, annealing temperatures (Ta), size of PCR amplicon (bp) and its localization in the gene.

locus	Primers		Ta (°C)	Size (bp)	Localization
	Forward (5-3)	Reverse(5-3)			
CYP19	GCAGCAAGGACTTGAAAGATG	GAACCTGGTGGGCTACAGTC	61	241	I6, E7, I7

I = Intron E = Exon

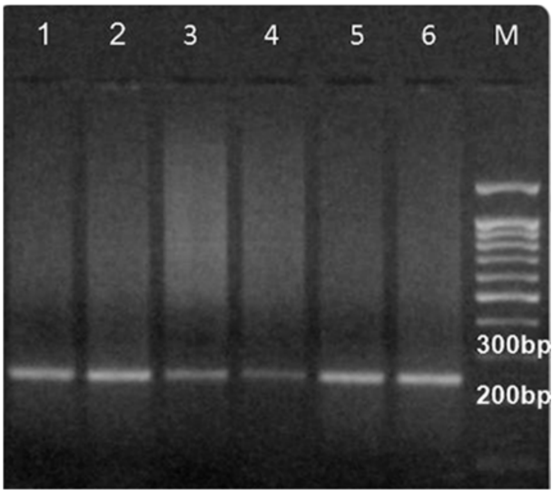


Fig.1. Ethidium bromide stained 2% agarose gel of unpurified PCR products of *CYP 19* locus (with an average size of 241 bp) before excision from gel (lanes 1-6). M represents 100 bp ladder.

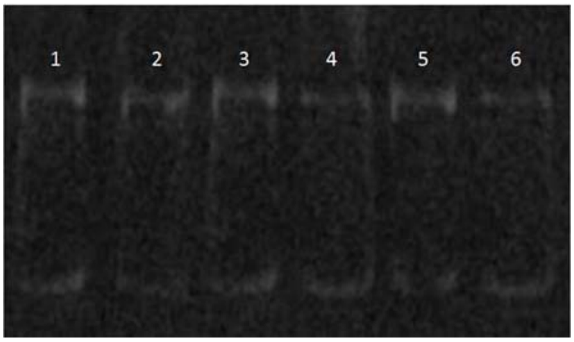


Fig.2. PCR-SSCP patterns of *CYP 19* locus in Egyptian buffalo show one SSCP pattern in this locus.

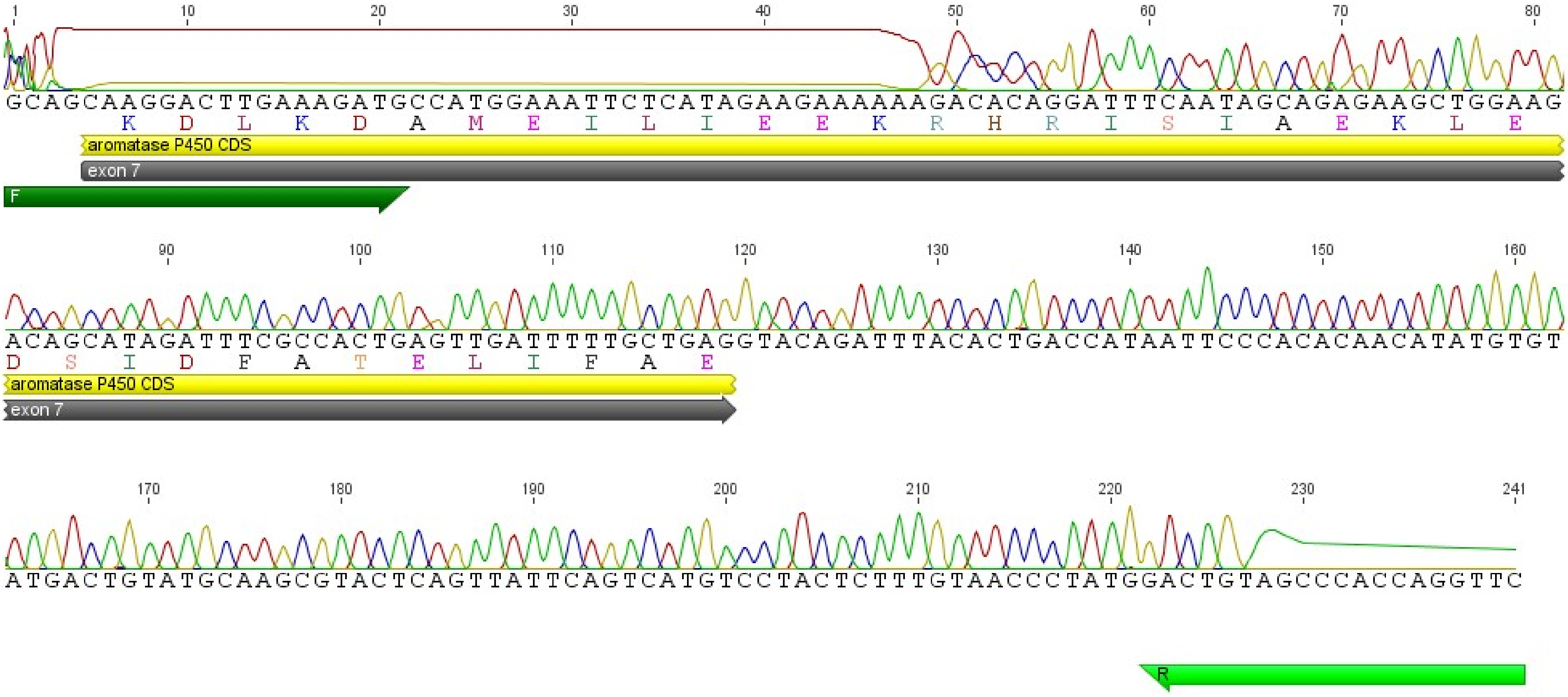
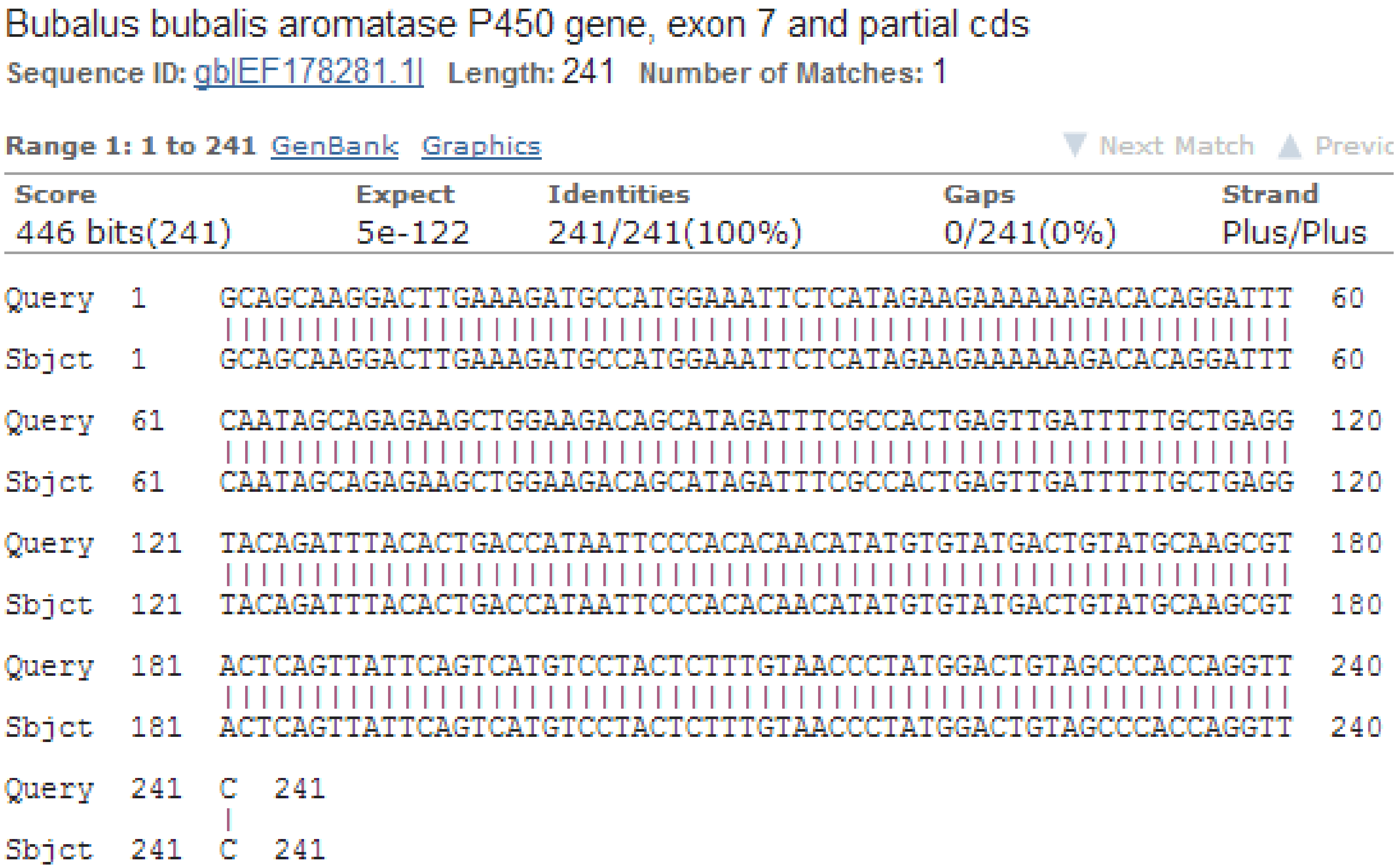


Fig.3. Nucleotide sequences of *CYP19* locus and the corresponding amino acid sequences were shown below nucleotide sequences of exon 7.



Bubalus bubalis aromatase P450 gene, exon 7 and partial cds

Sequence ID: gb|EF178281.1| **Length:** 241 **Number of Matches:** 1

Range 1: 1 to 241 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
446 bits(241)	5e-122	241/241(100%)	0/241(0%)	Plus/Plus

```
Query  1    GCAGCAAGGACTTGAAAGATGCCATGGAAATTCTCATAGAAGAAAAAAGACACAGGATTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GCAGCAAGGACTTGAAAGATGCCATGGAAATTCTCATAGAAGAAAAAAGACACAGGATTT  60

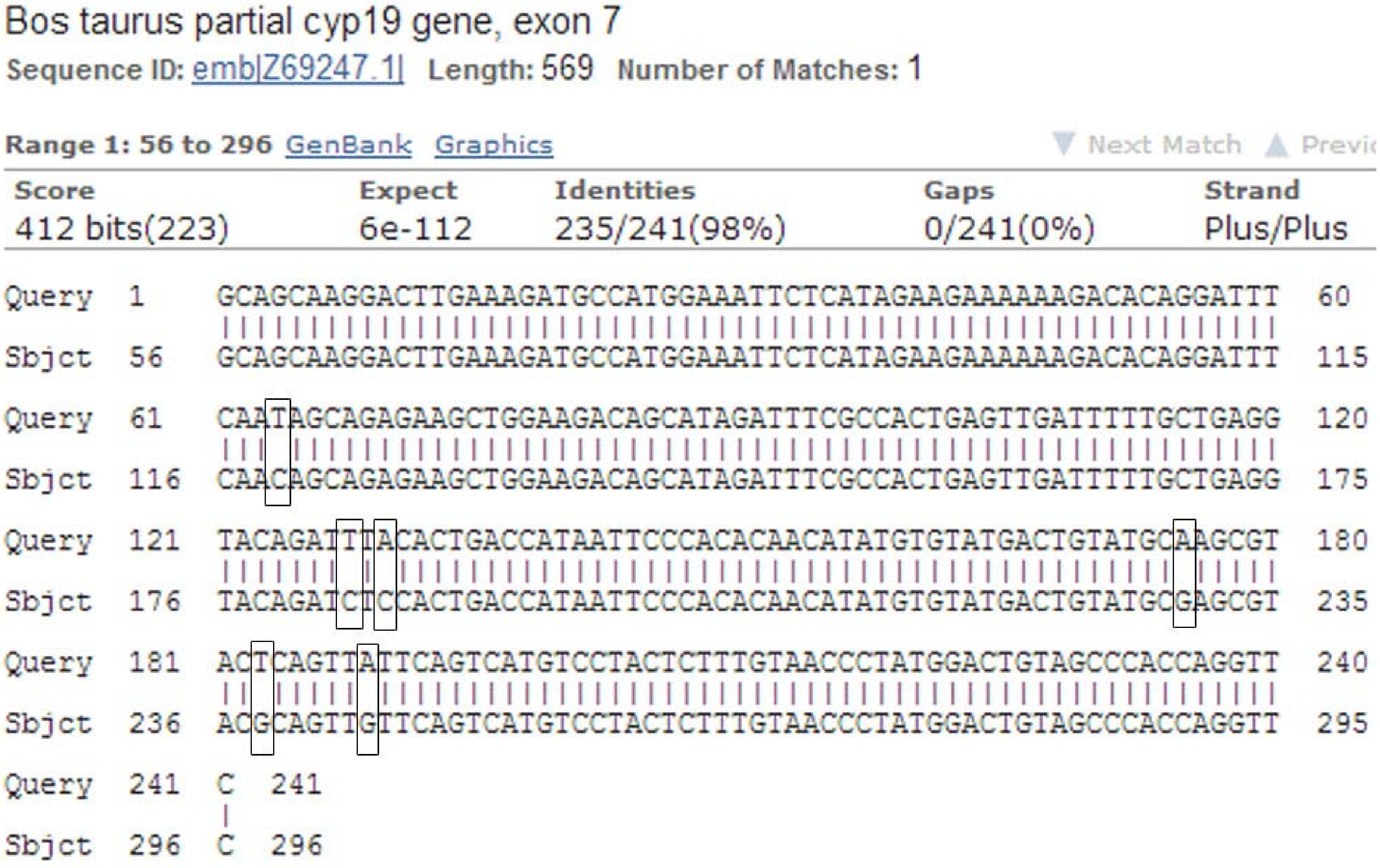
Query  61   CAATAGCAGAGAAGCTGGAAGACAGCATAGATTTCGCCACTGAGTTGATTTTTGCTGAGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CAATAGCAGAGAAGCTGGAAGACAGCATAGATTTCGCCACTGAGTTGATTTTTGCTGAGG  120

Query  121  TACAGATTTACACTGACCATAATTCCCACACAACATATGTGTATGACTGTATGCAAGCGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TACAGATTTACACTGACCATAATTCCCACACAACATATGTGTATGACTGTATGCAAGCGT  180

Query  181  ACTCAGTTATTCAGTCATGTCCTACTCTTTGTAACCCTATGGACTGTAGCCCACCAGGTT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  ACTCAGTTATTCAGTCATGTCCTACTCTTTGTAACCCTATGGACTGTAGCCCACCAGGTT  240

Query  241  C  241
            |
Sbjct  241  C  241
```

Fig.4. Nucleotide sequences alignment of *CYP19* locus with Indian water buffaloes (EF178281) using BLAST showed 100% identity.



```
Bos taurus partial cyp19 gene, exon 7
Sequence ID: emb|Z69247.1|  Length: 569  Number of Matches: 1

Range 1: 56 to 296 GenBank  Graphics                       ▼ Next Match  ▲ Previ

Score             Expect      Identities         Gaps           Strand
412 bits(223)     6e-112      235/241(98%)       0/241(0%)      Plus/Plus

Query  1    GCAGCAAGGACTTGAAAGATGCCATGGAAATTCTCATAGAAGAAAAAAGACACAGGATTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  56   GCAGCAAGGACTTGAAAGATGCCATGGAAATTCTCATAGAAGAAAAAAGACACAGGATTT  115

Query  61   CAATAGCAGAGAAGCTGGAAGACAGCATAGATTTCGCCACTGAGTTGATTTTTGCTGAGG  120
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  116  CAACAGCAGAGAAGCTGGAAGACAGCATAGATTTCGCCACTGAGTTGATTTTTGCTGAGG  175

Query  121  TACAGATTTACACTGACCATAATTCCCACACAACATATGTGTATGACTGTATGCAAGCGT  180
            ||||||| | |||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  176  TACAGATCTCCACTGACCATAATTCCCACACAACATATGTGTATGACTGTATGCGAGCGT  235

Query  181  ACTCAGTTATTCAGTCATGTCCTACTCTTTGTAACCCTATGGACTGTAGCCCACCAGGTT  240
            || ||||| |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  236  ACGCAGTTGTTCAGTCATGTCCTACTCTTTGTAACCCTATGGACTGTAGCCCACCAGGTT  295

Query  241  C  241
            |
Sbjct  296  C  296
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Fig.5. Nucleotide sequences alignment of *CYP19.7* locus with *Bos Taurus* (Z69247.1) using BLAST showed 98% identity.

Numerous studies have investigated the association between CYP19 and reproduction in cow; however only scanty studies were performed on such gene in buffalo. Therefore, this study was conducted to isolate a locus of this gene and to study their association with reproductive disorders in Egyptian buffalo.

Detection of single nucleotide polymorphisms (SNPs) can be achieved simply by many techniques including; single strand conformation polymorphism (SSCP) (Orita et al., 1989), RFLP (Cotton et al., 1988; Cotton, 1993; Ganguly and Prockop, 1990; Novack et al., 1986; Youil et al., 1995) and denaturing gradient gel electrophoresis (Myers et al., 1987; Sheffield et al., 1989). Among these techniques, SSCP is very commonly used, cheaper method. For instance a variation C286T in exon7 of the CYP19 gene was observed by SSCP and sequencing in humans (Means et al., 1989; Watanabe et al., 1997). In addition, the search for SSCP polymorphisms in candidate genes associated with genetic variation in traits of economic importance, could lead to the finding of genetic markers useful for improved selection of agricultural populations. Anestrum was the most common single cause of infertility in buffaloes (Ashturkar et al., 1995; Singh and Sahni, 1995), which occurs mainly due to low level of ovarian estrogens (Hafez and Hafez, 2000). The key enzyme in estrogen biosynthesis is cytochrome P450 aromatase, the protein product of CYP19 gene. Therefore, CYP19 gene can play a role in the determination of anestrous animal. Kumar et al. (2009) found a G72C SNP in intron7 of CYP19 in 10% of true anestrous animals (GC) as compared to control animals (GG). This SNP was in the intronic region (non-coding region), and so it is synonymous mutation and does not lead to change in amino acid sequence. In contrast, we did not find such SNP or any other SNP in this locus in all examined Egyptian buffaloes (normal cyclic or anestrous). This means that this SNP is not conserved in Bubalus bubalis. In cattle CYP19 gene, several SNPs were found, which were located mainly in the promoter regions. The polymorphic site in the promoter (P) 1.1 region of the bovine CYP19 gene was located at 1044 nt (Jędrzejczak et al., 2011; Komisarek and Dorynek, 2002; Vanselow et al., 1999). The P1.1 region also contains 3 SNPs detectable by PCR-RFLP method using restriction enzymes, namely PvuII, Cfr13I, and BseNI (Kowalewska-łuczak, 2010), whilst the P1.2 region contains 2 SNPs detectable by enzymes, namely BseNI and TaiI (Vanselow et al., 1999; Vanselow et al., 2000). Alignment of nucleotide sequences of CYP19 locus in Egyptian buffaloes showed 100% identity with Indian water buffaloes (EF178281) and 98% identity with Bos Taurus (Z69247). This result indicates that the sequence of CYP19 is highly conserved between cattle and buffalo. This is a preliminary study that provides the researchers with raw data, which could be used as a basis for further studies to associate other SNPs in CYP19 with fertility in buffalo.

5. REFERENCES

Ahmed, W.M., Hanafi, E.M., Ali, A.H., Shalaby, S.A., 2010. Clinical Perspective of Repeat Breeding Syndrome in Buffaloes. J. of Americ. Sci., 6(11): 661-666.

Ashturkar, R.W., Aher, V.D., Bhokre, A.P., 1995. Studies on infertility problems in non-descript buffaloes and cows. Indian Vet. J. , 72: 1050–1052.

Beuzen, N.D., Stear, M.J., Chang, K.C., 2000. Molecular markers and their use in animal breeding. The Vet J., 160(1): 42-52.

Cotton, R.G., Rodrigues, N.R., Campbell, R.D., 1988. Reactivity of cytosine and thymine in single-base-pair mismatches with hydroxylamine and osmium tetroxide and its application to the study of mutations. Proc Natl Acad Sci U S A, 85(12): 4397-401.

Cotton, R.G.H., 1993. Current methods in mutation detection. Mutation Res., 285: 125–144.

Derar, R., Hussein, H.A., Fahmy, S., Megahed, G., 2012. The effect of parity on the efficacy of ovulation synchronization (Ovsynch) protocol in buffalo (Bubalus bubalis). Anim. Reprod., 9(1).

FAO, 2013. Food and Agriculture Organization, faostat.fao.org/site/573/ default.aspx.

Fooda, T.A., Elbeltagy, A.R., Hassan, L.R., Awad, S.-h.S., 2011. Evaluated of Egyptian buffaloes crossing with Italian buffaloes for reproductive traits. J. of American Sci., 7(7): 209-213.

Ganguly, A., Prockop, D.J., 1990. Detection of single-base mutations by reaction of DNA heteroduplexes with a water-soluble carbodiimide followed by primer extension: application to products from the polymerase chain reaction. Nucleic Acids Res, 18(13): 3933-9.

Goldammer, T., Guérin, G., Brunner, R.M., Vanselow, J., Fürbass, R., Schwerin, M., 1994. Chromosomal mapping of the bovine aromatase gene (CYP19) and an aromatase pseudogene to Chromosome 10 and syntenic group U5. Mamm Genome, 5: 822-823.

Hafez, E.S.E., Hafez, B., 2000. Reproduction in farm animals. seventh ed, Lippincott Williams and Wilkins publications, PP 261–263.

Iannuzzi, L., Di Meo, G.P., Perucatti, A., Schibler, L., Incarnato, D., Cribiu, E.P., 2001. Comparative FISH-mapping in river buffalo and sheep chromosomes: assignment of forty autosomal type I loci from sixteen human chromosomes. Cytogenetics and cell genetics 94: 43–48.

Jędrzejczak, M., Grzesiak, W., Szatkowska, I., Dybus, A., Muszyńska, M., Zaborski, D., 2011. Association between polymorphisms of CYP19, CYP21, and ER1 genes and milk production traits in Black-and-White cattle. Turk. J. Vet. Anim. Sci., 35(1): 41-49.

Komisarek, J., Dorynek, Z., 2002. Polymorphism analysis of BTN, GHR, and CYP19 genes in cattle. Zesz. Nauk. Przeg. Hod., 62: 295-302.

Kowalewska-łuczak, I., 2010. Polymorphism of the CYP19 gene and milk production traits of dairy cattle. Turk. J. Vet. Anim. Sci., 34(6): 493-496.

Kumar, R., Kumar, D., Roy, B., 2011. Studies on repeat breeding of buffaloes. Buffalo Bulletin, 30(3): 177-187.

Lakhotia, S., Somasundaram, K., 2003. Conformation-Sensitive Gel Electrophoresis for Detecting BRCA1 Mutations. In: El-Deiry, W. (Ed.), Tumor Suppressor Genes. Methods in Molecular Biology™. Humana Press, pp. 403-412.

Means, G.D., Mahendroo, M.S., Corbin, C.J., Mathis, J.M., Powell, F.E., Mendelson, C.R., Simpson, E.R., 1989. Structural analysis of the gene encoding human aromatase cytochrome P-450, the enzyme responsible for estrogen biosynthesis. J Biol Chem, 264(32): 19385-91.

Myers, R.M., Maniatis, T., Lerman, L.S., 1987. Detection and localization of single base changes by denaturing gradient gel electrophoresis. Methods Enzymol, 155: 501-27.

Novack, D.F., Casna, N.J., Fischer, S.G., Ford, J.P., 1986. Detection of single base-pair mismatches in DNA by chemical modification followed by electrophoresis in 15% polyacrylamide gel. Proc Natl Acad Sci U S A, 83(3): 586-90.

Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K., Sekiya, T., 1989. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Natl Acad Sci U S A, 86(8): 2766-70.

Othman, O.E., 2013. RFL polymorphism of three fertility genes in Egyptian buffalo. J. of Appl. Biol. Sci., 7(2): 94-101.

Sheffield, V.C., Cox, D.R., Lerman, L.S., Myers, R.M., 1989. Attachment of a 40-basepair G rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. Proc. Natl Acad. Sci. USA 86: 232–236.

Simpson, E.R., Davis, S.R., 2001. Minireview: aromatase and the regulation of estrogen biosynthesis--some new perspectives. Endocrinol, 142(11): 4589-94.

Singh, B., Sahni, K.L., 1995. Causes of infertility in cattle and buffaloes under field conditions. Indian J of Anim Sci, 65: 1119–1121.

Vanselow, J., Kühn, C., Fürbass, R., Schwerin, M., 1999. Three PCR/RFLPs identified in the promoter region 1.1 of the bovine aromatase gene (CYP19). Anim. Genet, 30: 232-233.

Vanselow, J., Kühn, C., Fürbass, R., Schwerin, M., 2000. Isolation of the bovine CYP10 promoter 1.2 and identification of genetic variants. Anim. Genet, 31: 337-338.

Watanabe, J., Harada, N., Suemasu, K., Higashi, Y., Gotoh, O., Kawajiri, K., 1997. Arginine-cysteine polymorphism at codon 264 of the human CYP19 gene does not affect aromatase activity. Pharmacogenet., 7(5): 419-24.

Youil, R., Kemper, B.W., Cotton, R.G., 1995. Screening for mutations by enzyme mismatch cleavage with T4 endonuclease VII. Proc Natl Acad Sci U S A, 92(1): 87-91.

التعرف علي الطفرات في الإكسون السابع لجين *CYP19* وعلاقته بالخصوبة في الجاموس المصري

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الملخص العربي

يعد جين *CYP19* واحد من أعضاء عائلة سيتوكروم ب 450التي تقوم بتنظيم تخليق الأستروجين في الحيوانات لذلك فإنها يمكن أن تستخدم كجينات مساعدة لخطط الانتخاب للخصوبة في الجاموس. لقد تم نشر التتابع النيوكليوتيدي لهذا الجين في الجاموس حديثا ولكن لم يتم عمل دراسات عليه حتى الأن. لذلك كان الهدف من هذه الدراسة تحدد الطفرات الموجودة في جزء من جين ال*CYP19* والمشتمل على الإكسون السابع والتعرف علي مدي الارتباط بين هذه الطفرات والخصوبة في الجاموس. تم عزل جزء من جين ال*CYP19* يصل حجمه إلى 241 زوج من القواعد النيتروجينية يحتوي على الإكسون السابع باستخدام التفاعل البلمري المتسلسل وطريقة SSCP متبوعا بتحليل التسلسل الجيني للتعرف على الطرز المختلفة. ولكن لم نلاحظ أي طفرات في كل الحيوانات التي تم فحصها ولقد تحقق ذلك بظهور طرز أحادي الشكل وتتابع نيوكليوتيدي واحد في الحيوانات الطبيعية والتي لم يتم فيها الشياع. وبالتالي أوضحت النتائج أن هذا الموقع من الجين ليس له تأثير على الخصوبة في الجاموس المصري لذلك لا يمكن أن يستخدم في البرنامج الانتخابي لتحسين الخصوبة في الجاموس المصري.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(1):151-160, مارس 2014)