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Investigation of the polymorphism in FSHR gene associated with fertility in pregnant and non-pregnant Iraq buffaloes

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ABSTRACT

This study was conducted on 20 Iraqi buffaloes (13 pregnant and 7 non pregnant) and diagnosed by rectal palpation in AL-Thahib AL-Abiad village / Baghdad from July 2016 to July 2017, age of animals ranged from 4-7 years. Blood samples from jugular vein (5 ml) with anticoagulant to extraction DNA by intron-kit for PCR technique estimated the size of band of FSHR gene to PCR-RFLP to limited genotype of Iraqi buffaloes differentiated between pregnant and non pregnant animals and these analysis was done in biotechnology department / Al-Nahrin University. The results in this study was one homozygous GG genotype 193 and 63 bp of FSHR gene axon 10 as well SNP (single nucleotide polymorphism) A/G located in 62 and the allele frequency was 1.0 and 0.0 in G and C. Finally the GG genotype was recorded 76.9% compared with 28.5% in pregnant and non pregnant Iraqi buffaloes respectively (P<0.01). In conclusion of this study RFLP-PCR of FSHR gene only one homozygous GG genotype and the genotype allele frequency percentage in pregnant Iraqi buffaloes was highly significant P<0.01 compared with non pregnant.

Keywords: fertility, FSHR, polymorphisms, buffalo.

1. INTRODUCTION

The buffaloes have two species represented by swamp and river but the river characterized by economic value as they considered main source of meat and milk products , but the genetic evaluation of productive buffalo for the benefit of the concepts of genetic quantity to promote very enough and related with easy selection of buffaloes [1]. Follicular stimulation hormone (F.S.H) initiated and responded for the follicle maturation due to binding with specific receptor (F.S.H. R) on external surface of granulose cell in ovary [2]. This lead to the action of F.S.H R to modified gene [3]. The presence of allelic differentiations in F.S.H R gene was observed in bovine [4 and 5] and these variations in molecular structure cause desensitization of the F.S.H receptors [6]. Many recent studies using molecular biology experiments estimate various molecular aspects [7,8,9 and 10]. The pregnancy diagnosis in buffaloes usually by rectal palpation and sometime used ultrasonography methods [11], while in Iraq depended on rectal palpation mainly due to aggressive animals so that using ultrasound was very difficult due to as controlling these animals for long period was impossible [12 and 13].

2. MATERIALS AND METHODS

The genomic related with DNA in this study was prepared from blood samples of 15 healthy female buffaloes, pregnant and non-pregnant . Blood sample (5 ml) was gathered aseptically from jugular vein of buffaloes into tubes containing anticoagulants (0.5 M EDTA) for each animal. The total DNA produced by the standard protocol by intron kit (Korea) procedure. Two conserved primers, F.: 5-CTGCCTCCCTCAAGGTGCCCCTC-3 and R.: 5-AGTTCTTGGCTAAATGTCTTAGGGGG-3. Thermal cycling included: Denaturation at 95 °C for 3 min, after

that by 35 cycles of 94 °C for 1 minutes, 58°C for 1 min and 72 °C for 1 minutes with final incubation at 72 °C for 10 minutes. Polymerase chain reaction results were extracted using apparatus at 2% agarose-gel with the visualized by contact with ultra violet light. Sequence of nucleotides of F.S.H-R gene by using BioEdit program, Data were submitted to statistical analysis using chi-Square test, spss program [14].

3. RESULTS AND DISCUSSION

The outcomes in table 1 observed that the pregnant animals at different stage of gestation period was recorded 65% (13/20) with significant differences P<0.01 compared with non-pregnant animals was 35% (7/20), these finding were reported by many authors in Iraq [12 and 13].

Table 1: Non pregnant and pregnant Iraq buffaloes at different stage by using rectal palpation.

S. No.	No. of animals	Non pregnant		Pregnant animals at different stage	
		No	%	No	%
1	5	1	20	4	80
2	5	2	40	3	60
3	7	3	42.8	4	57.7
4	3	1	33.3	2	66
Total	20	7/20	35	13/20	65a

Different letters mean sig. differences (P<0.01)

The results in Fig. 1 observed the genetic polymorphisms of axon 10 to FSHR gene by PCR technique and they used in this study the size of band which recorded 306 bp fragment these finding agreement with houde etal [15] which reported the same gene in bovine [16] as well as marson etal [5] reported the result in buffaloes resembled to this study.

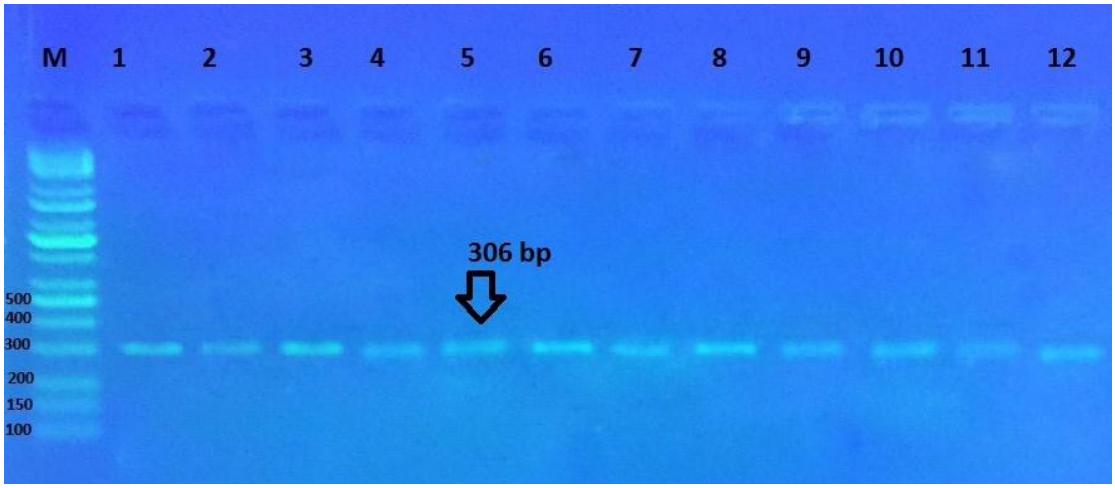

Figure 1: FSHR gene in Iraqi buffaloes by PCR technique which revealed size of band 360 bp with red stain safe.

But in fig 2 the outcomes which represented PCR-RFLP analysis by ALU1 enzyme recorded one genotype consisted of two digested fragment 193 and 63 bp (GG 1-10 pregnant and 11-12 non pregnant) Iraqi buffaloes respectively, these results represented the first trail in Iraq, and has no agreement with Marson et al [5] which reported two genotype consisted of GG and GC but in Egypt recorded three different genotype include GG, GC and CC [17].

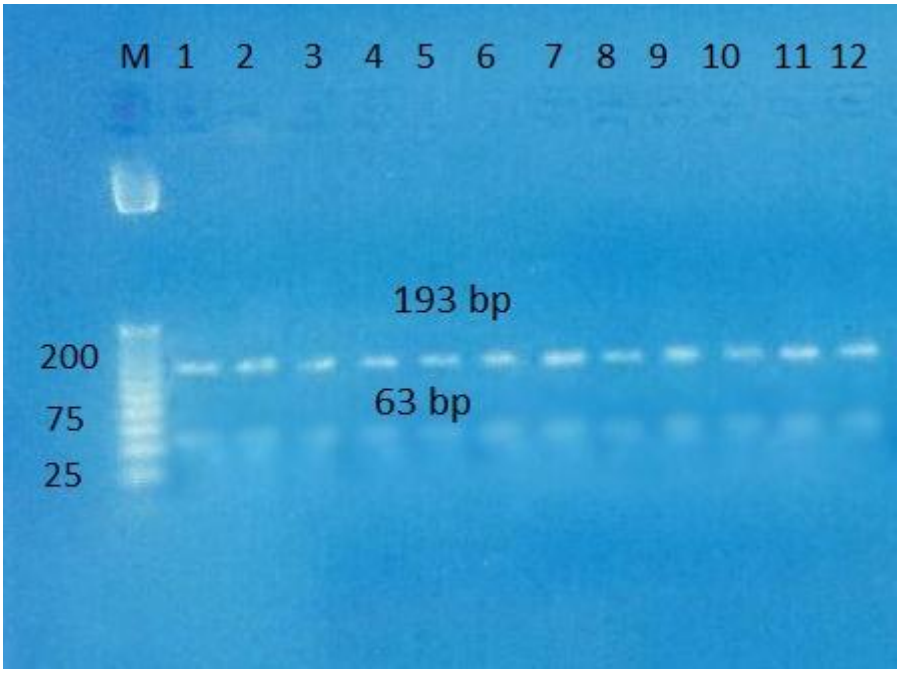

Figure 2: PCR-RFLP technique observed lines 1-12 homozygous GG genotype in Iraqi buffaloes.

The outcomes in table 2 and figures 3,4 observed analysis of nucleotides and protein of FSHR gene axon 10 and they recorded SNP adenine (A) to quinine (G) in located 62. And amino acid (aspartic acid to glycine) and these finding were in agreement with [17]. The nucleotide sequences of buffaloes FSHR gene were submitted in GenBank database and have accession numbers: ID: KY636355.1

Table 2: Type of SNP polymorphism and amino acid change in sense of FSHR gene in buffalo.

location of gene bank	Nucleotide change	Amino acid change	Predicted effect	Type of mutation
A62G	GAT>GGT	Aspartic acid>Glycine	Missense	Transition

Score	Expect	Identities	Gaps	Strand
379 bits(205)	1e-101	205/205(100%)	0/205(0%)	Plus/Plus

```
Query  1    TCCTCTATGCCATCTTCACCAAGAACTTCCGCAGGGATTTCTTCATTCTGCTGAGCAAGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TCCTCTATGCCATCTTCACCAAGAACTTCCGCAGGGATTTCTTCATTCTGCTGAGCAAGT  60

Query  61   TTGGCTGCTATGAAGTGCAAGCCCAGACCTATAGGTCAGAAACCTCATCCACTGCCCACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TTGGCTGCTATGAAGTGCAAGCCCAGACCTATAGGTCAGAAACCTCATCCACTGCCCACA  120

Query  121  ACTTTCATCCAAGGAATGGTCACTGCCCCCCAGCTCCCAGGGTTACCAGTGGTTCCAATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  ACTTTCATCCAAGGAATGGTCACTGCCCCCCAGCTCCCAGGGTTACCAGTGGTTCCAATT  180

Query  181  ACACACTTATCCCCCTAAGACATTT  205
            |||||||||||||||||||||||||
Sbjct  181  ACACACTTATCCCCCTAAGACATTT  205
```

Score	Expect	Identities	Gaps	Strand
425 bits(230)	2e-115	232/233(99%)	0/233(0%)	Plus/Plus

```
Query  1    TCAACTCCTGTGCCAACCCCTTCCTCTATGCCATCTTCACCAAGAACTTCCGCAGGGGTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5    TCAACTCCTGTGCCAACCCCTTCCTCTATGCCATCTTCACCAAGAACTTCCGCAGGGATT  64

Query  61   TCTTCATTCTGCTGAGCAAGTTTGGCTGCTATGAAGTGCAAGCCCAGACCTATAGGTCAG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  65   TCTTCATTCTGCTGAGCAAGTTTGGCTGCTATGAAGTGCAAGCCCAGACCTATAGGTCAG  124

Query  121  AAACCTCATCCACTGCCCACAACTTTCATCCAAGGAATGGTCACTGCCCCCCAGCTCCCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  125  AAACCTCATCCACTGCCCACAACTTTCATCCAAGGAATGGTCACTGCCCCCCAGCTCCCA  184

Query  181  GGGTTACCAGTGGTTCCAATTACACACTTATCCCCCTAAGACATTTAGCCAAG  233
            |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  185  GGGTTACCAGTGGTTCCAATTACACACTTATCCCCCTAAGACATTTAGCCAAG  237
```

Figure 3: Sequencing of *FSHR* gene in Iraqi buffalo in gene bank (Sequence ID: KY636355.1 and Sequence ID: KU043387.1

follicle stimulating hormone receptor, partial [Bubalus bubalis]
Sequence ID: AFO83999.1Length: 101Number of Matches: 1
Related Information
Range 1: 24 to 100GenPeptGraphicsNext MatchPrevious Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
160 bits(405)	1e-49	Compositional matrix adjust.	76/77(99%)	76/77(98%)	0/77(0%)	+3

```
Query  3    NSCANPFLYAIFTKNFRRGFFILLSKFGCYEVQAQTYRSETSSTAHNFHPRNGHCPPAPR  182
            NSCANPFLYAIFTKNFRR FFILLSKFGCYEVQAQTYRSETSSTAHNFHPRNGHCPPAPR
Sbjct  24   NSCANPFLYAIFTKNFRRDFFILLSKFGCYEVQAQTYRSETSSTAHNFHPRNGHCPPAPR  83

Query  183  VTSGSNYTLIPLRHLAK  233
            VTSGSNYTLIPLRHLAK
Sbjct  84   VTSGSNYTLIPLRHLAK  100
```

Figure 4: Amino acid sequence in Iraqi buffalo in gene bank (Sequence ID: KU043387.1)

The result in table 3 which observed allele fragment of FSHR gene and they recorded G (Guanine) 1 and C (cytosine) equal 0 but in table 4 they recorded genotype in pregnant and non pregnant Iraqi buffaloes was GG 76.9% (13/20) and 28.5% (7/20) in pregnant and non pregnant respectively and these results which represented the first trail in Iraqi but these finding disagree with Campagnani [18] which recorded GG 0.49% in buffaloes in brazil as well as Marison et al [4] recorded 0.3% of GG in Europe buffaloes.

Table 3: Allele frequency of FSHR gene in sample study.

Allele	frequency
G	1.0
C	0.0
Total	1 (100%)

Table 4: Distribution of genotype according of type of Pregnancy (No. and %).

Genotype	No. of animals 20		Percentage (%) 100%
	Pregnant 13	Non-pregnant 7	
CC	0	0	
GG	10 (76.9%)	2 (28.5%)	
CG	0	0	
Total	20		100 %

Finally, we conclude from this study RFLP PCR of FSHR gene, only one homozygous GG genotype and the genotype allele frequency percentage in pregnant was highly significantly $p<0.01$ compared with non pregnant Iraqi buffaloes.

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