Research Paper Medical Science

# Investigation of Single Nucleotide Polymorphisms of Gonadotropin-Releasing Hormone Receptor (GNRHR) Gene and Its Association with Litter Size in Iraqi Goats

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**ABSTRACT**

In the present study, the polymorphisms of gonadotropin-releasing hormone receptor (GnRHR) gene was analyzed as a genetic marker candidate for litter size in 40 Iraqi goats. Two alleles (A and C), two observed genotypes (CC and AC), and single nucleotide polymorphisms (SNPs) were detected in 27out 40delivered goats. The frequencies ofalleles C and A in Iraqi goat breeds were 0.85 and 0.15, respectively. In addition, comparisonsbetween the nucleotide sequences of CC and CA genotypes showed one mutation (A>T) atexon 2. The results showed that CC genotype was associated with better litter size in Iraqi goat's breeds. Therefore, these results suggest that GnRHRgene is a strong candidate gene thataffects litter size in goats.

**KEYWORDS** *GnRHR*, polymorphisms, litter size, Iraqi goat

## Introduction

Animal fertility is a measure of reproductive success.Low heritability and lack of information imply a slow genetic improvement of female fertility traits. The progress in development of molecular markers suggests their potential use for genetic improvement in livestock species.Detection of polymorphism in the genome of different trait populations can be used to develop the genetic markers that are responsible for specific traits. The presence of these genetic markers in animal genomes can also explain a significant proportion of the variation observed in the trait of interest (1).

The hypothalamic decapeptide gonadotropin-releasing hormone (GnRH) plays a critical role in reproductive development and function by regulating the biosynthesis and secretion of the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH).LH and FSH then stimulate sex steroid hormone synthesis and gametogenesis in the gonads to ensure reproductive competence.GnRH is released into the hypophysial portal circulation and transported to the anterior pituitary, where it binds to specific, high-affinity cell surface receptors (GnRHR) on gonadotropes, the pituitary cell type that produces gonadotropins(2).GnRH receptor it's a member of the rhodopsin-like G protein-coupled receptor (GPCR) family. The cow, sheep, and human receptors are 328 amino acids long, while the mouse and rat receptors are 327 amino acids (3). The sheep gene has been localized near the Booroola fecundity gene (FecB) (FecB increase ovulation rate and latter size in sheep) locus on the same chromosome 6 (4).The aim of this study was to search for SNPs of *GnRHR*gene and evaluate the associations between *GnRHR*polymorphisms and litter size.

## Materials and Methods

**Samples and DNA extraction**: Whole blood samples were obtained from 40 Iraqi healthy does range in age from (2-5) years old and weight (25±2.2) kg.The animals were housed semi opened, in animal place at animal farm of the College Vet. Med./Baghdad Universities.In total, 4ml whole blood was collected into a K3-EDTA tube for DNA extraction and transported in cool box to the laboratory of Molecular department of Biotechnology Research Centre /AL-Nahrain University. DNA was extracted from the samples by DNAextraction kit (*Accu-Prep*® Genomic DNA Extraction Kit Bioneer/ Korea) according to the manufacturer's protocol.

**Detection of Gene *GnRHR*by Using PCR:** Detection of *GnRHR*gene was conducted by using primers for amplification of for *exon 2 GnRHR*gene. A fragment 241bp of *GnRHR*was amplified using a forward primer (*GnRHR*F: **5-CCT ACA GTT ATA CAT CTT TGG GA-3'**) and a reverse primer (*GnRHR*R: **5-GAG AAA TAC ATA CTG TGG GGA T-3'**) (Primers set supplied by alpha DNA Company, Canada).The PCR amplification was performed in a total volume of 50µl containing 5µl DNA, 10µlprofitaq PCR PreMix KIT (Bioneer /Korea), 1.6µl of each primer (10 pmol) then the volume was completed with 50µl of nucleases free water. The thermal cycling conditions were done as follows: Denaturation at 95 °C for 4 min, followed by 35 cycles of 94 °C for 30s, 52°C for 30s and 72 °C for 30s with final incubation at 72 °C for 10 min using a thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). The PCR products were separated by 2% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302nm) after ethidium bromide staining.

**Sequencing and Sequence Alignment:** Sequencing of exon 2 of *GnRHR*gene was performed by Macro gen company, USA. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and BioEdit program. The results were compared with data obtained from Gene Bank published ExPASY program which is available at the NCBI online.

## Results and Discussion

*GnRHR*gene was successfully amplified using specific PCR primers for exon 2. Figure (1) showed PCR amplification of exon 2 of the *GnRHR*where a specific product at 241bp was observed. The sequencing alignmentto amplified productof exon2 GnRHRgene from 27 of does (only parturition animals) by BLAST and BioEditprogram, which was performed by national instrumentation center for environmental management (nicem) online at (http://nicem. Snu .ac. kr/main/ ?en _ skin

=index.html), biotechnology lab, machine is DNA sequencer 3730XL, Applied Biosystem), Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov).

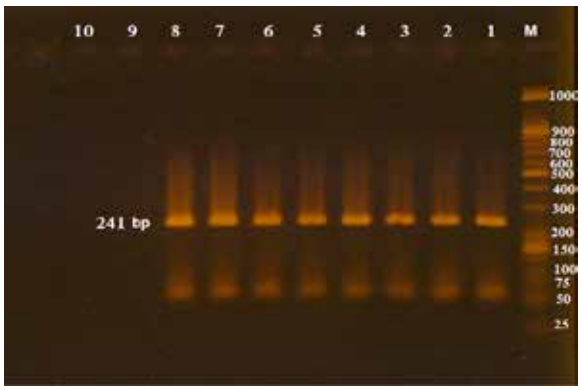


**Figure (4.4):** PCR product the band sized 241bp. The product was electrophoresis on 2% agarose gel at 5 volt/cm$^2$, 1x TBE buffer for 2 hours. M: DNA ladder (25-1000bp), lane (1-8) PCR product of band size 241bp. visualized under U.V light after staining with Ethidium Bromide.

However, the polymorphism of exon 2 of GnRHR genes from all of does was found after compare with the wild type sequences of gene bank, under number : gb|KC429680.1| from 23-210 number of nucleotide from Capra hircus breed Shaanbei gene of Gene Bank havefive mutations in DNA exon2 GnRHR gene were identified in the present study (Table 1). (177A>T) mutation is missense that changes the amino acid Arginine to Serine. This mutation was detected in all does produce single or twines kids. The second (64CC>AA) mutation is missense that change amino acid Proline to Lysine and come with (177A>T) mutation in five samples of single parturition, other missense mutation (201T>A) detected with (177A>T) mutation in one sample of single parturition.The last missense mutation (124C>T) come with (177A>T) and (123C>T) nonsense mutation in one sample have twine parturition.

**Table (1): Represent location, type of polymorphism, mutations and amino acid change in sense of GnRHR gene in Iraqi does**

| animal No and % | location of gene bank | Nucleotide change | No. of codon/ location | Amino acid change | Predicted effect | Type of mutation |
|---|---|---|---|---|---|---|
| 20 (74%) | A177T | AGA > AGT | 51/Sense | Arginine > Serine | Missense | Transversion |
| **5 (18.5%)** | CC64AA A177T | CCA >AAA AGA > AGT | 14/Sense 51 /Sense | Proline> Lysine Arginine > Serine | Missense Missense | Transversion **Transversion** |
| **1 (3.7%)** | **A177T** T201A | AGA> AGT AGT> AGA | 51/Sense 59/Sense | Arginine > Serine Serine > Arginine | Missense Missense | Transversion Transversion |
| **1 (3.7%)** | C123T C124T A177T | ATC > ATT CCT TCT AGA > AGT | 33/Sense 34/Sense 51/Sense | Isoleucine > Isoleucine Proline> Serine Arginine > Serine | Nonsense Nonsense Missense | Transition Transition Transversion |

**Genotype distribution of GnRHR gene in Iraqi does**

Initial genotyping data using the curve method revealed 19 (70.37%) clear C/C homozygotes, 9 (33.33%) out of them twining parturition and 8 (29.63%) clear C/A heterozygotes but only one from them have twine parturition, as we show in table (2) and Figure (2) shows sample curves represent each of the two genotypes.

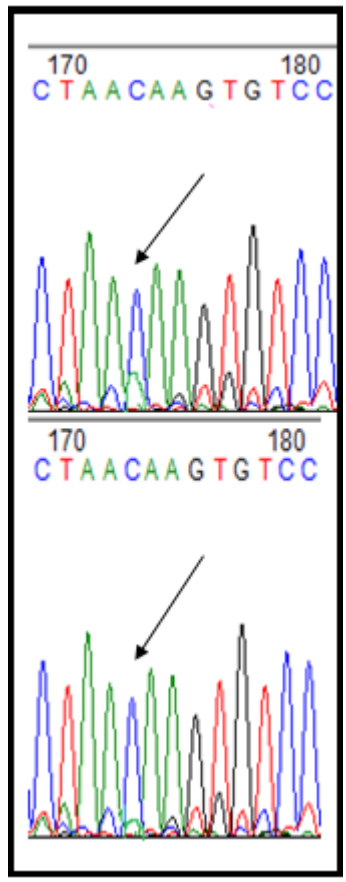


**Figure (2):**Nucleotide sequence chromatogram representing genotype of exon2 GnRHR gene genotype. The top does is a C/A heterozygote, and the next does down is a C/C homozygote

**Table (4.7): Distribution of genotype according of type of parturition (No. and %).**

| Genotype | No. of animal | | Percentage (%) | |
|---|---|---|---|---|
| | Single | Twine | | |
| CC | 19 | | 70.37 a | |
| | 10 | 9 | 37.04 | 33.33 |
| CA | 8 | | 29.63 b | |
| | 7 | 1 | 25.93 | 3.70 |
| AA | 0 | | 0.00 c | |
| Total | 27 | | 100 % | |
| Chi-square value ($\chi^2$) | --- | | 11.293 | |
| P-value | --- | | 0.0019 | |
| ** (P<0.01) | | | | |

In the present study, the homozygous A/A genotypes were not detected in the GnRHRgene, similar to a previous finding in another breed of goats were observed two type of genotype (AA and AC) (5) and (GG and AG) or (GG and GT) (6) and the same result in bovines (7). We conclusion, this genotypes (A/A) may have a negative effect on reproduction, so that goats with this genotype might have been eliminated through the process of artificial selection and breeding programmers or it is also possible that A/A genotype was not found in the study population due to its low allele frequency that results from low populations .

**Allele frequency for exon 2 GnRHR gene in Iraqi does**

Amounted of repeat alleles C and A that return to exone2 GnRHR gene in a present study on Iraqi does 0.85and 0.15, respectively (Table 4.8).

Li *et al*., (5) finding in his study on two different breed of does that A and C allele frequency were (0.78 and 0.22), (0.82 and 0.18) respectively.

**Table (4.8): Allele frequency of gene in sample study**

| Allele | Allele frequency |
|---|---|
| C | 0.85 |
| A | 0.15 |
| Total | 1 (100%) |

So we conclude in present study that Iraqi goat have single mutation (177A>T) of exone2 GnRHR with C allele and genotype C/C might be associated with better litter size in Iraqi goat. However, further analysis should be performed in order to validate both the association and the physiological significance of the mutation in the exon 2 of GnRHRgene

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