



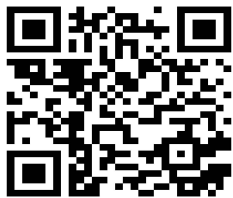
A Study on the Association Between CYP19 Exon 3 Variants and the Hormone Estrogen in Local Female Goats

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Abstract:

The goal of this study was to determine how the genetic polymorphism of the CYP19 gene exon 3 affected the levels of the estrogen hormone in female goats. In Babil Governorate/AlQaism district, Blood samples from female goats were collected from their fields. To measure the estrogen levels, the Enzyme Linked Immuno Sorbent Assay was utilized (ELISA). The CYP19 gene's third axon, measuring 140 bp in length, was removed, and the genotypes of this axon were identified in accordance with the different nucleotide sequences and their correlation with

the levels of the estrogen hormone in the samples. In samples of local female goats, the genotype distributions of the CYP19 gene were analyzed by percentages. 40 %, 26.66 %, and 33.34 %, for TG, GG and TT genotypes respectively. We conclude that the TG genotype is superior to CYP19 gene 3 exon in domestic goats from Iraq. This outperformed the pure genotypes TT and GG because its estrogen hormone content was much higher ($P \leq 0.01$). These findings made it abundantly evident to us that girls with to ensure that a chosen generation possesses desirable qualities, we advise choosing genotype TG moms for their offspring.

Keywords: local female goats, Exon3, CYP19 gene, estrogen hormone.

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Introduction

Most Arab nations have paid little attention to the breeding and reproduction of goats, which are nonetheless retained on the periphery of agriculture and are frequently used for their twins in Asian and African nations (1). and meat supplies, in addition to their higher milk output than sheep and their capacity to gain an advantage over cows from

poorer forage sources of shrubs and plants and their ability to tolerate different environment (2). It is necessary to go towards breeding animals that are more suitable for difficult conditions, as goats are characterized by their high ability to live in various environmental conditions due to their adaptation to drought, lack of feed materials and low quality and

difficulty in obtaining them, as they are considered to be animals in arid regions, in which it is difficult for the rest of the domesticated animals to live in them (3) . Goats play a vital social and economic role in many rural areas of the world. economic role in helping to achieve food security. (4), and the selection on the basis of genetic makeup has become an important tool in the process of genetic improvement of farm animals (5). To determine the genes related to economic characteristics, this requires studying these genes and their relationship to milk production and its characteristics, so it can be used as a genetic indicator to conduct selection for this characteristic (MAS, Marker Assist Selection) and among these genes is the estrogen gene because of its important role in the development and growth of developing follicles in goats and sheep (6). In order to accelerate the process of developing the productivity of local goats and raise their reproductive efficiency in terms of the quantity of milk produced, the rate of twins, and the use of relevant modern biotechnologies such as estrus synchronization, which is one of the technologies that reduce the time and effort required to detect estrus, As well as the use of genetic selection by detecting genetic polymorphisms of the CYP19 gene and measuring the concentration of estrogen, which plays an effective role in stimulating the developing follicles in female goats. This study aimed Study on the Effect of CYP19 Gene Third Exon Polymorphism on Estrogen Concentration in Local Female Goats.

Materials and methods:

For the purpose of DNA extraction, blood samples in volumes of 3 ml were obtained in EDTA tubes from the jugular vein of native female goats. The location where the genetic material (DNA) is extracted, the Biochemical Laboratory at Qadisiyah City's Laboratory Section, is where genetic analysis is carried out.. ELISA is used to measure the levels of estrogen in the body (ELISA), detection of exon 3 CYP19 gene by PCR, and sequencing of nitrogenous bases outside Iraq.

Nutrition

The goats are reared in semiopen pens to give a proper climate and environment, particularly during the breeding season, gestation, and farrowing, as well as medical and veterinary care, and a healthy diet.. Concentrate, vegetables and roughage must be provided and this amount must be increased during mating and gestation.

Experimental animals:

In this experiment, 30 native goat samples were used. They are born single, milk-producing, and in good health. They fed in the barn and went out to graze in the morning.

Sample gathering

Each animal's jugular vein was used to collect blood samples (3 ml), which were then put in collection tubes with (K3 EDTA. Tubes) anticoagulant (EDTA tubes), and shipped in a freezer to the laboratory to freeze until DNA and estrogen concentrations were extracted.

Extraction of DNA

For molecular identification DNA of the CYP19 gene was extracted. from local goat blood samples in the manner described below.

Procedure for obtaining DNA

According to the directions in the kit provided by Geneaid, USA and the test kit's manufacturer, Blood was used to extract the DNA..

Use a Nanodrop instrument to determine the DNA concentration.

Nanodrop technique was used to extract the genomic DNA that was extracted was examined (THERMO, USA).

Gel Electrophoresis

After the extraction procedure, the size of the CYP19 exon 3 gene in the resultant bundles was determined by electrophoresis and the presence of DNA was identified..

Measurement of estrogen concentrations using ELIZA

5 ml of fair game intimate was unperturbed and placed in tubes containing anticoagulant , placed in

a centrifuge at a rise of (14000) for 5 for a few moments, fit, impediment was abstracted by micropipette and placed in an Eppendorf pipeline, and the estrogen distinction was verge on profit the Enzyme consanguineous immuno sorbent assay (ELISA) according to the detection kit's manufacturer.

Primer selection

The manual as shown in Feed 1 was choose for the molecular origination of the third exon of the CYP19 gene.

Table (1): Grammar book bind information provided by DNA Integrated Technologies IDT.

Abbreviated Gene	Sequence	Product Size	reference
CYP19 EXON3	(F) 5-CCA GCT ACT TTC TGG GAA TT-3	140 bp	(7)
	(R) 5-AAT AAG GGT TTC CTC TCC ACA-3		

Gene-specific (PCR)

The text in advisers aboard (2) was used as a template for a 20 microliter PCR of the CYP19 gene for molecular disclosure..

Table 2: Matter worn in the (pcr) for the CYP19 gene.

Materials	volum (µl)
DNA extracted	2
Primer	F : 1 R: 1
D.W	16
final size	20

Table 3: Conditions for CYP19 gene exon 3 bundle duplication in PCR.

No.	Steps	Temperatures	Time (minute)	Cycles
1	Initial denaturation	94 °C	5	1
2	denaturation	94 °C	30	35
3	Annealing	55 °C	30	
4	extension	72 °C	30	
5	Final extension stage	72 °C	5	1

Loading the electrophoresis and per product:

Loading of PCR products with electrophoresis: A 1.5% concentration agarose gel was loaded with 10 L of PCR product. A UV (light transilminator) inspection of the bundles was followed by photographic documentation of the process. The migration was carried out for one hour at a voltage of 100 V/cm and a current of 65 mA.

Examine the bundle's nitrogen base sequence

Each experimental sample was delivered to The results were then reviewed. The order of nitrogenous bases was determined by the Korean Macrogen Company.

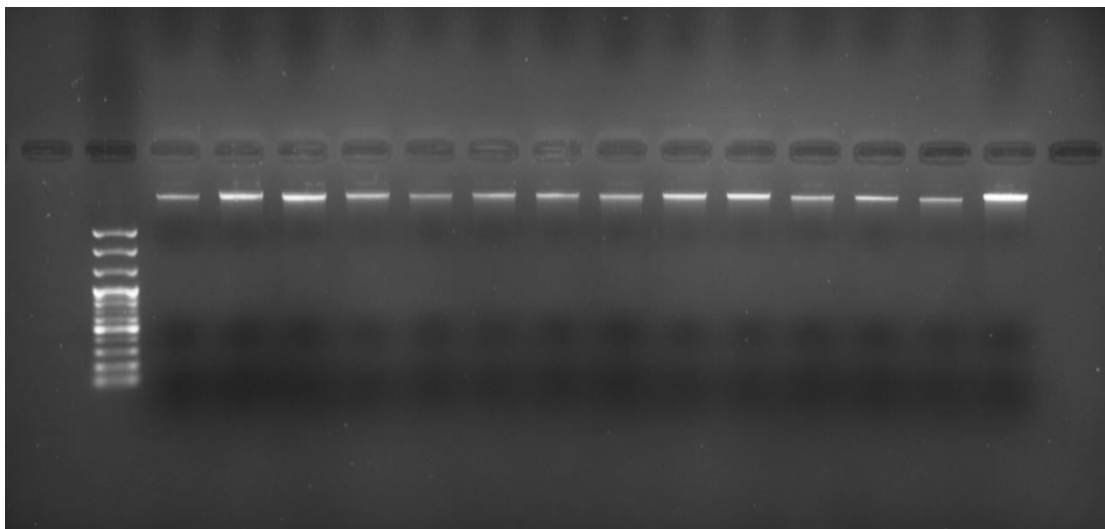
Analyzing the CYP19 gene's sequencing findings

The CYP19 gene's genotype was determined through analysis of the sequencing data using the NCBI (8).

Discussion:

DNA extraction:

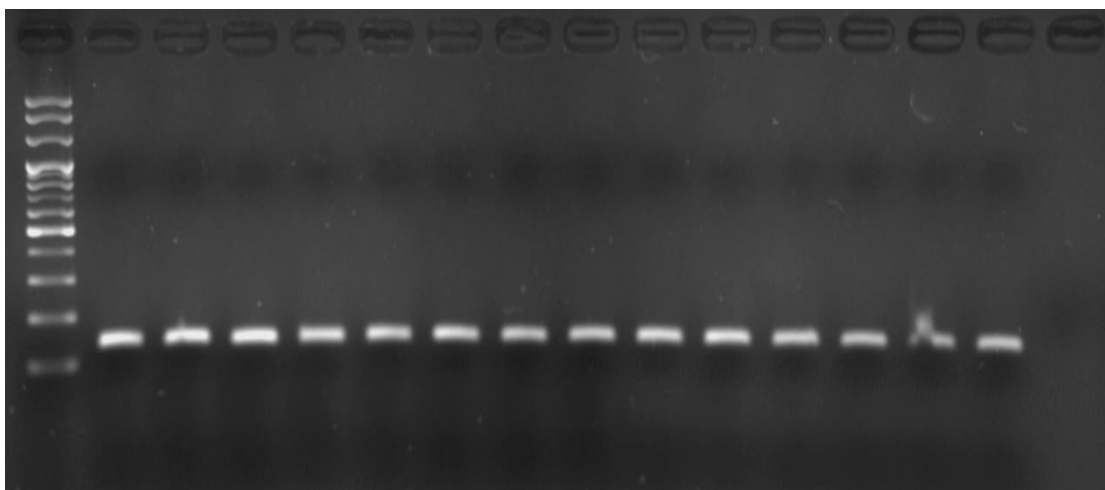
One popular strategy is to validate the technique of electrophoresis to detect DNA after confirming its presence via electrophoresis in the samples being examined. Figure 1) (9) Using the electrophoresis approach, DNA packages were recovered from local goat samples in Figure 1.



PCR-based CYP19 gene detection

According to the outcomes Exon 3 was present in all study samples when the CYP19 gene was detected using a specific primer (Exon 3), and

electrophoresis showed that a bundle of this exon with a size of 140 base pairs was present. (Fig. 2). The CYP19 gene's third exon region was electrophoresed using the PCR method to amplify a gene product, as shown in Figure 2.



Identification of the CYP19(Exon3) gene genotypes

Using a base detecting the sequence of nitrogenous bases, the bundle (140 base pairs) of nitrogenous bases that make up the third exon site of the CYP19 gene were located (Exon3). The findings of the genetic investigation using the (Bio edit) program and NCBI website revealed three genotypes, including (TT, TG, and GG). In Table (4), the genotype percentages for the CYP19 gene in the investigated samples were listed, with the greatest percentage

TT: dominant genotype.

TG: heterozygous mutant genotype.

GG: homozygous mutant genotype.

proportion being genotype (TG) at 40%, with genotype being the lowest (GG) at 26.66, while genotype (TT) was at 33.34.

The percentages of CYP19 exon3 genotypes in samples from adjacent goats are displayed in Table (4).

Table (4). The percentages of CYP19 exon3 genotypes in samples from adjacent goats.

Genotype	Number	Percentage
TT	10	33.34 %
GG	8	26.66 %
TG	12	40 %
Total	30	100 %

Genotypes and their relationship to estrogen hormone

The results in Table 3 revealed that the levels of estrogen in female goats with the TG genotype were significantly higher than those in those with the TT and GG genotypes, reaching 40%, 33.34%, and 26.66%, respectively. These findings were consistent with (10) which found substantial variations ($P \leq 0.01$) between the percentages of the various genotypes, which came to 8.75, 58.75, and 32.50% for the combinations AA, AB, and BB, respectively. This research focused on the third exon of the CYP19 gene in Turkish Awassi sheep. It explains the hybrid individuals' (AB) considerable superiority to the two pure genotypes, particularly the AA genotype. Yet, this study's findings were better than those discovered by (11) through their investigation on sheep, which revealed

revealed the genotypes TT and TC did not differ significantly from each other, and that the genotypes TT and TC scored significantly higher on the est

rogen concentration measurement (15.5745, 15.4617) than the genotype CC, which came in at 14.2362 ($P < 0.05$). Moreover, these outcomes were not as strong as (12) which observed significant superiority ($P < 0.01$) for the genotypes TC and CC, which amounted to 15,422 and 15,350 than the TT genotype of (14,678) when studied on the CYP19 gene, the eighth exon on Iraqi sheep. Within the same framework, genes associated with economic features can be found by analysing the cyp19 gene, which encodes the aromatase enzyme. This enzyme is essential for the generation of oestrogen, a hormone that regulates both male and female reproduction, fat deposition (14), growth (15) and also their effective in the formation of ovarian follicles and stimulates development of the mammary gland and estrus gland through (16).

Conclusion

The advantage of indigenous goats' TG CYP19 gene exon 3 genotype from Iraq, which outperformed the TT and GG pure genotypes due to its considera

bly higher ($P \leq 0.01$) estrogen hormone concentration. Using these findings, females with the TG genotype were chosen as the mothers of the progeny in order to produce a generation that had been chosen for having desired features.

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