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The Relationship between the Fourth Exon 8 Polymorphism of the CYP19 Gene and Estrogen in Local Awassi Ewes

Razzaq Nasser Abed

Department of Medical Biotechnology, College of Biotechnology – Al-Qasim Green University, Iraq

Abstract:

The objective of this study was to examine the effect of exon 8 CYP19 gene polymorphisms on Local Awassi ewes' oestrogen level. The highest level of the hormone was 16.33 pg/ml and the lowest level of the hormone was 9.38 pg/ml. When exon 8 of CYP19 gene was observed to be 105 base pairs the genotypes were also established. Distribution of CYP19 genotypes in local Awassi ewes were TT, TC and CC 20%, 30% and 50%, respectively. The results of this study showed that the TT and TC genotypes (15.57, 15.46) were significantly higher than CC (14.23) ($P < 0.05$), but there was no significant different between TT and TC group. In the present study, TC genotype had a higher susceptibility compare to TT and CC genotypes of the polymorphisms in the exon 8 of CYP19 gene.

Keywords: Exon8, CYP19 Gene, Local Awassi ewes, Hormone estrogen

Corresponding Author: Razzaq Nasser Abed†, Department of Medical Biotechnology, College of Biotechnology – Al-Qasim Green University, Iraq

Introduction

Molecular biology has revolutionalised the methods of the contemporary growth and the application of this particular field. Among them the most important is the polymerase chain reaction (PCR) which allows to analyse any region of the DNA. In this respect, leading goals in using DNA markers of farm animals involve searching for such quantitative traits which are significant for organization of the genetic selection and improvement of various production traits. The genes concerned need to be studied in order to distinguish common genes that are related with the production features among the members of the examinees. Aromatase enzyme, which is encoded with Gene name CYP19, is believed to play major roles in synthesis of oestrogens by conversion of androgens to oestrogens. It also controls growth (Jones et al 2000), adiposity (Teneva et al 2007 & 2009) and male and female reproduction. The maximum wavelength of light

that the enzyme can absorb is 450 nm and it is in the group of iron-protein heme proteins (Lai et ah 2009). Care must be taken when describing the small stuff that has to do with growth of follicles and the calibre of the eggs secreted by the granulosa cells and that this is owing to an enzyme. It is also involved in the conversion of androgens into oestrogens that in turn are involved in the promotion of oestrus cycles and the development of the odour associated with the mammary glands. The androgens which are produced by the follicular cells are produced in large quantities due to the inability of the follicular cells to synthesize oestrogen needed for the maturation of ovarian follicles. These structures appear to block the formation of the ovarian follicles that they ultimately cause to degenerate. (Maria Ana and others, 2009). This study aimed at determining the allelic diagnostics of genetic variations of the CYP19 gene and exon 8 area in Awasi sheep, and association with oestrogen level.

2- Materials and Methods

2-1 - Experimental Animals

Fifty ewes of the native Awassi sheep breed were used in this experiment. A single, healthy, milk-producing child was born to each ewe. The ewes stay in the barn to feed and are taken out for grazing in the mornings, but the breeding system is closed.

2-2: Collect Blood Samples

To extract serum, draw five millilitres of blood from each animal's jugular vein and place it in a blood collection tube. Additionally, fill a K3 EDTA collection tube with 2 ml of blood. Bring these samples to the lab in a cold box, where they will be kept at -18°C until the time for DNA extraction comes.

2-3 Estimating the level of estrogen with the help of the ELISA device:

2-4 DNA extraction

In order to do a molecular analysis of the gene under investigation (CYP19), DNA was extracted from the ewe blood samples using the subsequent protocol.

2-4-1 Protocol of DNA Isolation

Using the method supplied by the US-based company Geneaid, DNA was recovered from the frozen blood. To make serum separation easier, 5 ml of sheep blood samples were first drawn and put in a container tube with an anticoagulant. After that, the tubes were centrifuged for five minutes at 14,000 RPM. After centrifugation, a micropipette was used to carefully remove the serum from the cellular material and transfer it to an Eppendorf tube. Then, using the Elabscience Detection Kit from China, the enzyme-linked immunosorbent test (ELISA) was used to determine the hormone concentration and pregnancy status.

2-4-2: Electrophoresis of DNA

After the extraction process, the electrophoresis method is used to detect DNA segments and determine whether DNA is present, which aids in calculating the size of the resultant bands. PCR is used to detect genes, particularly CYP19.

2-5: Choose the initiator

A primer was selected for the CYP19 gene's molecular detection and phenotypic investigation, as shown in Table 1.

Table 1. lists the sequence of the primer provided by Integrated DNA Technologies (IDT), Canada, for the CYP19 study.

abbreviated gene	Sequence	Product size
EXONE8 OF CYP19	(F) ACC AGT GCA TAT TGG AAA TGCTG	101bp
	(R) CTC TTC AAC CTG GGG ATG CT	

2-6 Interaction of the Examined Gene with PCR sequences

The samples were put into the reaction equipment in accordance with the particular circumstances for every section of duplicate genes. Following the completion of the process, the polymerization result was guaranteed to replicate the required component. These materials were then mixed using a Vortex mixer. These setting were made with the chain polymerase reaction as indicated in the table below after which all the tubes were transferred to the polymerization reaction apparatus.

Table 2. Materials used in the chain reaction of the CYP19 polymerase enzyme.

Ingredients size in microliter	Component
5	Master Mix
2	DNA
F: 1 R: 1	Primer
16	Distill Water
25	The final size

The conditions applied to detect the CYP19 gene using the PCR device are detailed below. Refer to.

Table 3. Certain Parameters Applied during the PCR process to amplify the CYP19 gene.

number of cycles	Time	temperatures	Steps	
1	5 minutes	94°C	The first stage of mutant	1
35	30 seconds	94°C	The Mutant	2
	30 seconds	58°C	Crossover	3
	30 seconds	72°C	Elongation	4
1	5 minutes	72°C	The final elongation stage	5

(U.S,2014)

2-6-1 Polymerase Chain Reaction and Electrophoresis Loading:

In a 1.5% agarose gel, load 10µL of DNA ladder and 5µL of PCR products. Conduct the electrophoresis at 100 volts/cm and 65 milliamps for one hour. Visualize the bands using UV light (transilluminator) and capture images with a photo documentation system.

2-6-2 Sequencing of Nitrogenous Bases for the Target Segment:

After isolating the genetic material and amplifying the target segment (105 base pairs) through PCR, send the samples to the Korean company Macrogen for nitrogenous base sequencing analysis.

2.6.3 Analysis of CYP19 Gene Sequence:

To find SNPs and evaluate the evolution of the CYP19 gene, analyze the sequencing findings using NCBI tools and align the sequences using BioEdit and Mega7 (Adiguzel et al., 2009)..

2-7 Statistical Analysis:

Use SPSS version 25 to do statistical analysis on the study samples. This involves figuring out average pregnancy-related hormone levels to investigate the connection between CYP19 gene Exone4 gene genotypes and hormone levels.

3. Results and Discussion

3-1 DNA extraction and purification:

The DNA was extracted using the gel electrophoresis method, as illustrated in Figure(1)

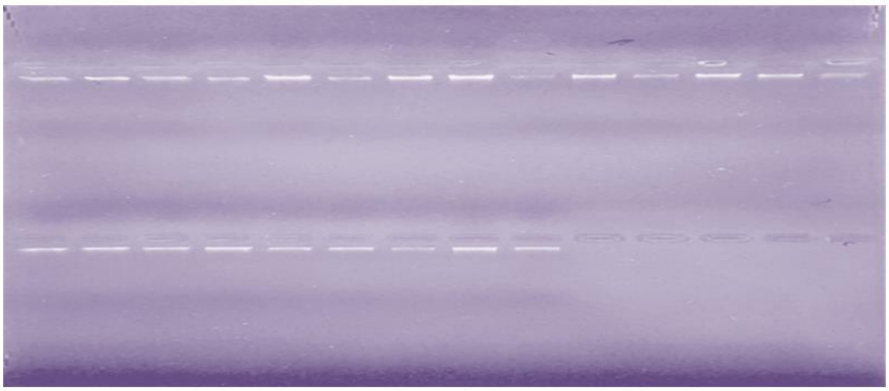

Figure 1. DNA bundles extracted from Awassi sheep blood samples using electrophoresis technique.

3-2 Detection of the CYP19 Gene Using PCR Technique

The presence of this exon was examined in the study samples. A product size of 101 base pairs was found when the electrophoresis findings verified the existence of this exon, as shown in Figure 2.

Figure 2. Displays the electrophoresis results of the CYP19 gene's third exon region using a 1.5% agarose gel. It includes standard DNA markers and the PCR-amplified gene products.

3-2-1 The Nucleotide Sequence for CYP19 Exon8:

Identifying the sequence of nitrogenous bases by concentrating on the 101 base pairs that make up this exon region. The results showed that the samples had the genotypes TT, TC, and CC. The results of genetic analysis were used to determine these genotypes.

3- 3 The final genotype percent and the number reference to the CYP19 gene.

According to the results of genotyping there were a highest percentage of 50 % for CC genotype for the controls and TT genotype percentage was least with 20%. The AL-MAL TC genotype contributed to 30% of the study population. Gazal et al. (2018) reported that the pure AA genotype was observed in the third exon region of the CYP19 gene located at a low frequency of 8.75% which concordant with the current study. Current study has revealed AB and BB genotype frequency to be 58.75% and 32.50% respectively, TT genotype was rarely observed in current study. This pattern was missing in the breeds of 1/2 Dorper, Poll Dorset, Santa Inês, as well as the Brazilian Somali where AB/BB only presented the ratio of 0.64 and 0.36 respectively. This was due to a lower representation of the A allele, as all the observed rams were AB, as indicated by (Ana Maria et al. in 2009).

Table 4. The percentages and number of genotypes associated with the CYP19 gene.

Genotype	The number	Percentage
TT	10	0.20
TC	15	0.30
CC	25	0.50
Total	50	100%
Chi square	----------	7.0758*
Allele		
T		0.35
C		0.65
*(P<0.05)		

3-4 Genotype

For the Exon8 (CYP19) gene, there was a variation in the nitrogenous base sequence for the 101 base pair bundle. Figure (3).

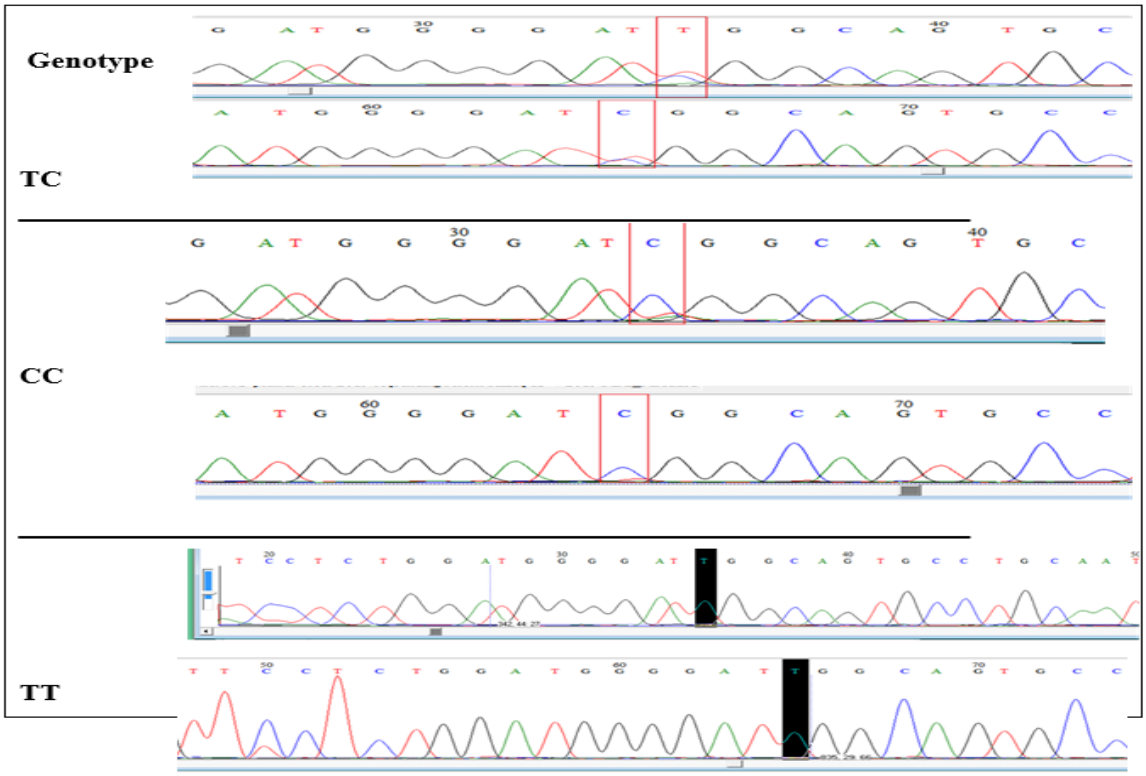

Figure 3. Illustrates the genotypes of the CYP19 gene, based on the NCBI location utilized for sequencing alignment. The BioEdit program was employed to analyze the CYP19 gene.

3-5 Relationship of Genotypes with Hormone Concentration

The TC and CC genotypes had better hormone concentrations of 15.422 ± 3.06 pg/mL and 15.350 pg/mL, respectively, according to the research, which showed a significant difference between the genotypes. In comparison, the concentration of the TT genotype was 14.678 pg/mL. The results demonstrate how various CYP19 gene genotypes affect hormone levels. Encoding the aromatase enzyme, which transforms androgens into oestrogens, is a critical function of this gene. This process is essential for controlling fat deposition as well as reproductive efficiency in both sexes (Heine et al., 2000; Jones et al., 2000). Because the CYP19 gene converts androgens to oestrogens, it is also associated with mammary gland growth and oestrus stimulation. The buildup of testosterone in theca cells of ovarian follicles due to inadequate oestrogen production can prevent follicle development and encourage follicle death. According to research, reproductive features may be impacted by mutations in this gene (Ana Maria et al., 2009).

Table 5. Displays the average hormone concentrations in Awassi sheep samples according to the genotypes of the CYP19 gene under study.

Genotype	Estrogen concentration, picogram / ml
TT	14.678 ±2.15 A
TC	15.422 ±3.06 B
CC	15.350 ±2.44 B

* Similar letters indicate that there is no significant difference at the probability level.

4. Conclusion

These results demonstrate that there is statistically a 50% expression of the CC genotype compared to the 20% TT and 30% TC genotypes of the CYP19 gene of the local Awassi sheep. Variances in Exon 8 of the gene largely determines estrogen levels. Individuals with the TC mutant genotype performed the best for all measures but especially for measuring the concentration of estrogen. The CC mutant genotype ranged second to the TC hybrid genotype. Once more, as far as estrogen measurement was concerned, both TT and TC genotypes fared much better than CC genotype.

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