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Polymorphism Relationship of Fourth Cyp19 Exon8 to Genital Hormones in Local Awassi Ewes

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Abstract: This study was conducted for the purpose of studying the polymorphism relationship of CYP19 exon8 gene and its effect on the concentration of hormonal hormone and pregnancy in local Awassi ewes. The highest and lowest concentration in the hormone (16.33and 9.38pg/ml) and the highest and lowest concentration in the pregnancy hormone (0.58 and 0.13 ng/ml) were isolated. CYP19 gene exon 8 of the 105 bp gene identification of genotypes depend on the neo-sequence. The distribution of CYP19 genotypes in local Awassi ewes specimens was 20, 30 and 50% for TT, TC and CC genotypes respectively. The hormone concentration in TT and TC genotypes (15.57 and 15.46) was more as compared to CC (14.23) while there was no significant difference between TT and TC genotypes. The current study revealed that the TC genotype of CYP19 gene was prevalent on the TT and CC genotypes of polymorphism of the CYP19 gene in Exon8 .

Keywords: Exon8, CYP19 Gene, Local Awassi ewes, Hormonal tonic, Pregnancy

Molecular biology has revolutionized the field of practical applications and finding modern technologies in this field, one of the most important of the technology of polymerase chain reaction (PCR) that can be used to study any part of DNA> the main goal of using DNA markers is to locate important quantitative traits in the application of genetic selection programs and to improve the productive traits of farm animals For the purpose of identifying genes related to economic traits, the CYP19 gene is Cured gene The aromatase enzyme is important for the manufacture of the estrogen hormone by converting the androgen into estrogen, which plays a regulatory role in male and female reproduction, as well as the deposition of fats (Teneva et al 2007) and in growth (Jones et al 2000). This enzyme belongs to the family of iron-containing proteins, and longest wave absorbed by this enzyme is 450 nm (Lamb et al 2009). The aromatase enzyme produced in granular cells (Granulosa) is essential in the formation of the ovarian follicle and the quality of the egg, as well as its relationship to stimulation of estrus and the development of the mammary glands through its role in converting androgen into estrogen. The manufacture of estrogen is not sufficient and this works to accumulate the androgen produced in the cells of theca in the ovarian follicles. This accumulation appears to inhibit the process of formation of ovarian follicles and then these vesicles will die (Ana-Maria et al 2009).

MATERIAL AND METHODS

Experimental animals: In this experiment, 50 ewes of the local Awassi sheep were used. They gave birth to a single, milk-producing (non-dry) healthy state. The breeding system is closed, and the barns are closed to feed in the barn and taken out for grazing in the morning.

Collect blood samples: Collect 5 ml of blood from the Jugular vein from each animal in the blood collection tube (Test Tub for the purpose of obtaining a serum) and collect 2 ml of blood in a collection tube on an EDTA tube of type K3 EDTA transported in a box Chilled to the laboratory for freezing at -18 ° C until DNA extraction time.

Measuring the estrogen and progesterone concentration with the ELISA device

DNA extraction: DNA was extracted from the ewe blood samples to perform a molecular examination of the gene under study (CYP19) as follows:

Method of DNA extraction (Protocol of DNA Isolation): DNA was extracted from the frozen blood according to the instructions provided by the USA Geneaid company. USA took 5 ml of sheep blood samples and put it in a container tube on an anticoagulant for the purpose of separating (serum) and the tubes were placed in the centrifuge at a speed of 14000 for a period of 5 minutes, the serum is withdrawn from the cells by the micro pipette and placed in the abndrov tube. Then, the hormone concentration and pregnancy are estimated using the enzyme-linked immunoassay (ELISA) and according to the manufacturer of the Elabscience Detection Kit (China)

Measuring the DNA purity using the Nano drop

apparatus: Blood DNA Genomic Screening Using Nano drop (THERMO. USA)

Electrophoresis of DNA: Electrophoresis procedure to determine the DNA segments after the extraction process and to detect the presence of DNA to know the size of the resulting beam.

Gene detection (CYP19) using PCR

Choose the initiator: A primer (Table 1) was selected to perform molecular detection and phenotypic knowledge of the CYP19 gene.

PCR sequence interaction of the studied gene: The samples were placed in the reaction apparatus according to the reaction conditions of each duplicate gene segment. After the reaction was completed, the polymerization reaction result was carried over to ensure the duplication of the required piece. After that, these materials were mixed with the mixer device (Vortex), then the tubes were transferred to the polymerization reaction device and the conditions of the chain polymerase reaction were set as shown in the table below.

The conditions used to detect the gene (CYP19) in the PCR device

Loading polymerase chain reaction and electrophoresis reaction: 10µL of volumetric evidence (DNA ladder) and 5µl of PCR products in a 1.5% concentration in the acarose gel, as the migration was carried out with a difference of voltage of 100 volts / cm and a current of 65 milliamps for one hour, and the packages were seen by the UV light (transillminator and then photographed using the photo documentation system).

Sequencing of the nitrogenous bases for the target bundle: After extracting the genetic material and multiplying the target bundle by PCR technology, the size of which is 105 base pairs, the package was sent to the Korean company Macrogen to find out the sequence of nitrogenous bases for each experimental sample and then analyzed the results.

Analysis of the results of the CYP19 gene nitrogen sequence: Sequencing results were analyzed using NCBI to conduct alignment sequencing using Bio edit and Mega7 to detect the presence of SNP and the evolution of the CYP19 gene (Adiguzel et al 2009).

Statistical analysis: Statistical analysis of study samples was performed using SPSS version 25 and calculated mean hormonal concentrations of pregnancy and pregnancy، to find the relationship between the genotypes of the Exone4 gene for the CYP19 gene and hormonal concentrations of pregnancy and was used.

Table 1. Sequence equipped (Primer) equipped by IDT Integrated DNA (Technologies, Canada) CYP19

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

RESULTS AND DISCUSSION

DNA extraction and purification: The DNA extraction and purification showed the presence of high purity and concentrations of DNA between 14.9 to 41.9 µl ng^{-1}) and also the presence of DNA was confirmed by the method of electrical relay (Fig. 1)

CYP19 gene detection using PCR technique: All study samples contain this exon, and when the results of the electrical relay showed the presence of the package for this exon, the package size is 101 base pairs and as shown in (Fig. 2).

CYP19 (Exon8) nucleotide sequence: A sequence of nitrogenous bases was used to find the series of nitrogenous bases forming the package (101 base pairs) for the CYP19 gene in the fourth exon region. The results showed the presence of a sample bearing the genetic makeup (TT, TC, and CC) for the samples. These genotypes were obtained using the results of the genetic analysis.

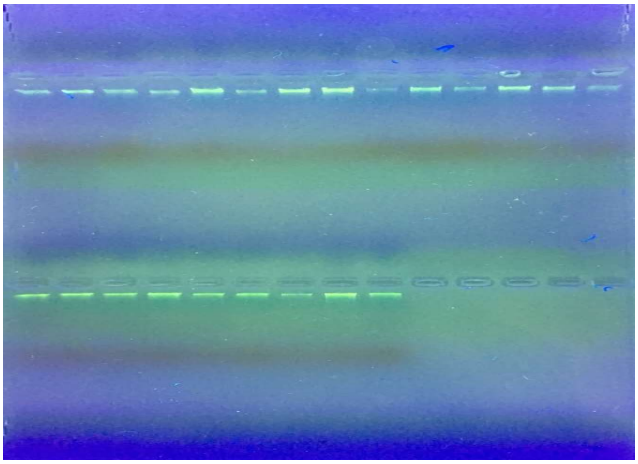

Fig. 1. DNA bundles extracted from Awassi sheep blood samples using electrophoresis technique

Fig. 2. Electrophoresis of the CYP19 gene for the third exon region using 1.5% ac arose M. Standard DNA bundles, amplified gene output by PCR

Percentages and number of genotypes of the CYP19 gene: The highest percentage due to genotyping (CC) was 50% and the lowest percentage due to genotype (TT) was 20%, while the genotype ratio was (TC) 30%. Lamb et al (2009) showed the genotypes of the CYP19 gene in the third exon region and that the pure predominant AA genotype appeared at the lowest percentage of 8.75%, and this is consistent with the findings of the current study, whereas the genotype AB58.75% and the genotype BB32.50% current study indicated a low rate of the genotype TT. Study in Brazil showed that this composition was non-existent in the strains 1/2 Dorper, Poll Dorset, Santa Inês and Brazilian Somali as the genotypes of the genotypes AB and BB reached 0.64 and 0.36 respectively, and this is due to a decrease in the frequency of the allele A and the rams were all AB (Ana-Maria et al 2009).

* TT. The dominant genotype
* TC. A genotypic or asymmetric genotype

CC. Mutant genotype

Genotype: There was a difference in the sequence of nitrogenous bases for the bundle of 101 base pairs for the Exon8 (CYP19) gene (Fig. 3).

Relationship of genotypes with the concentration of the hormone: There were significant difference between the genotypes produced as TC and CC genotypes (15.422 and 15.350 pg /mL) were superior to the genotype TT (14.678 pg /mL). The results showed clear effect of the different genotypes of the CYP19 gene on the concentration of the hormone, as this gene is responsible for the production of the aromatase enzyme, which is responsible for the manufacture of the hormone of the through the conversion of androgen to the modifier, which is important not only in regulating the reproductive effectiveness of males and females, but it is also important In the deposition of fats (Heine et al 2000, Jones et al 2000). The CYP19 gene has a relationship in stimulation of estrus and development of the mammary glands through its role in converting androgens into the hormone, and when the synthesis of the hormone is insufficient, this works to

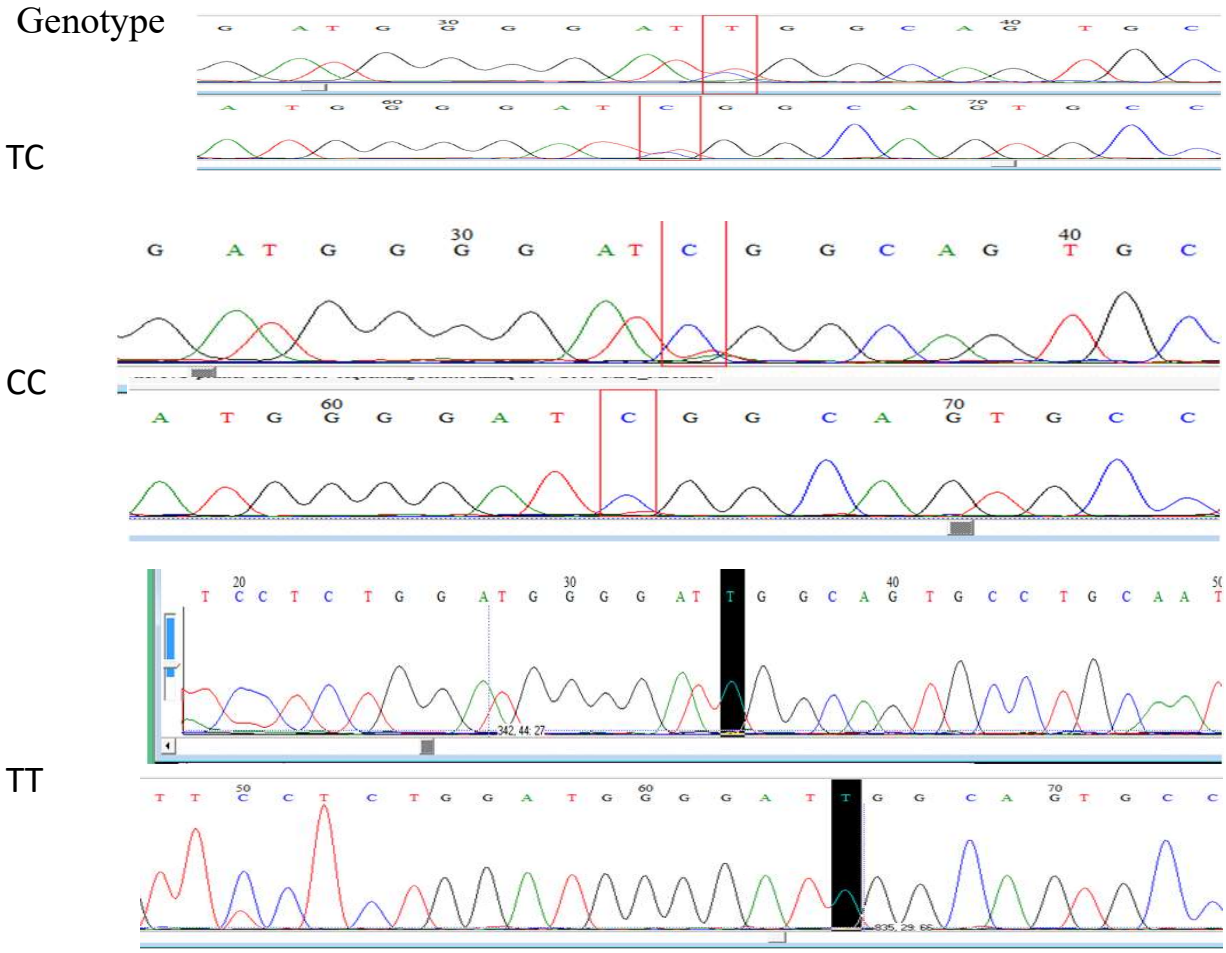

Fig. 3. Genotypes of the CYP19 gene, depending on the NCBI location for the Sequencing alignment procedure and the use of the Bio edit program for the CYP19 gene

accumulate Androgen produced in theca cells of the ovarian follicle and consequently inhibit the process of formation of ovarian follicles and then their death, and studies have shown that mutations that have this gene have reproductive traits (Ana-Maria et al 2009)

Table 2. Conditions for beam multiplication of the CYP19 gene in the PCR reaction

Number of cycles	Time	Temperatures	Steps
1	5 Minutes	94°C	The first stage of mutant
35	30 Seconds	94°C	The Mutant
	30 Seconds	58°C	Crossover
	30 Seconds	72°C	Elongation
1	5 Minutes	72°C	The final elongation stage

(U.S, 2014)

Table 3. Percentages of Awassi sheep samples for the CYP19 gene in Exon 8

Genotype	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)		

Table 4. Averages of the hormone concentration in the samples according to the genotypes of the CYP19 gene in Awassi sheep samples

Estrogen concentration (Picogram/ml)	Genotype
14.678± 2.15 A	TT
15.422± 3.06 B	TC
15.350± 2.44 B	CC

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

Table 5. Mean pregnancy hormone concentrations in study samples according to the genotypes of the CYP19 gene in Awassi sheep samples

Progesterone concentration (Nano gram ml^{-1})	Genotype
0.300± 0.011 A	TT
0.398± 0.010 B	TC
0.298± 0.022 A	CC

Relationship of genotypes with the concentration of progesterone in the study samples: The TC hybrid model at a concentration of 0.398 ng/ml exceeded the pure genotypes TT and CC at a concentration of 0.300 and 0.298 ng/ml sequentially. This affects reproductive traits, and has an effect on the variability of the effects of the night change at a time when the night exceeds a specific trait in a particular trait, the sheep breed was not the case of another breed (Ana-Maria et al 2009). The genetic mutation has a clear effect on the LH concentration and pregnancy hormone for the mutated gene type (TC) due to the fact that the mutation event results in a change in the genetic makeup of the genotype of the organism that leads to the emergence of phenotypes and some mutations are harmful and some are beneficial. The genetic makeup of an organism causes a type of development of the organism which leads to the emergence of phenotype behaviors that make it able to adapt to environmental conditions (2014. US) Variation in results that the change indicates the presence of a missense genetic mutation, which means that the mutation is missing and encodes to a different amino acid when it occurs. Therefore, the genetic variation obtained is reflected in the work of the gene through the mechanism of secretion of pregnancy hormone and their concentrations increase clearly in the mutant.

CONCLUSION

There was prevalence of genotypes 50% CC over dominant genotypes 20% TT and mutant genotypes TC 30% of the CYP19 gene in local Awassi sheep. The polymorphism of the CYP19 gene in Exon8 has a significant effect on hormonal concentrations and pregnancy. The mutant genotype TC genotype of the CYP19 gene outperformed the genotypes in all tests conducted in the current study, which included measuring hormonal concentrations of modulator and pregnancy. Individuals carrying the mutant genotype CC t ranked second in importance after the TC hybrid genotype in terms of concentrations hormonal load and pregnancy. The TT and TC genotypes outperform the CC genotype in concentration of hormone.

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