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RELATIONSHIP OF CYP19 EXON4 POLYMORPHISM WITH GENITAL HORMONES IN LOCAL AWASSI EWES

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Abstract

The aim of this study was to evaluate the polymorphism relationship of CYP19 exon4 gene and its effect on the concentration of estrogen hormone and pregnancy in local Awassi ewes. 50 ewes of the local Awassi sheep were used in the Husayniyah threshold fields located on the Najaf, Karbala road, in Iraq, during a period from September of 2019 to January of 2020. Blood samples from Awassi sheep were used for DNA extraction and detection of CYP19 gene by PCR and serum samples were used for measurement concentration of estrogen and progesterone. The highest concentration in the hormone (16.33 pg/ml), the lowest concentration (9.38 pg/ml), the highest concentration in the hormone pregnancy (0.58 ng/ml) and the lowest concentration (0.13 ng/ml) were isolated . CYP19 gene exon 4 of the 105 bp gene and the identification of genotypes, depending 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. P<0.05) in TT and TC genotypes (15.57,15.46) respectively, compared to CC (14.23), while there was no significant difference between TT and TC genotypes. P<0.05 in genotypes TC (0.47) on TT and CC genotypes (0.32,0.24) while increased pregnancy hormone genetic model TT significant decrease compared to genetic Btrz TC and CC. 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 Exon4.

***Keywords*:** Exon4, CYP19 Gene, Local Awassi ewes, Hormonal Tonic and Pregnancy.

Introduction

Molecular biology has evolved in the past few years as some aspects of it have revolutionized the field of practical applications and finding modern technologies in this field, one of the most important of these applications is 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 (Teneva *et al*., 2007). 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 (Jones *et al*., 2000; Teneva *et al*., 2009) and in growth (Lamb *et al*., 2009). This enzyme belongs to the family of iron-containing proteins, and the derived from measurements Spectrophotometric wavelengths, the longest wave absorbed by this enzyme is 450 nm (Ana *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. And when the manufacture of estrogen is not sufficient, this works to accumulate the androgen produced in the cells of theca (ovarian follicles) in the ovarian follicles. This accumulation appears to inhibit the process of formation of ovarian follicles and then these vesicles will die (Serge *et al*., 2003), and the length of the gene in humans is estimated at 120kb A baseline pair and in sheep 1546 a baseline pair (Adiguzel *et al*., 2009) Given the lack of studies at the molecular level of the CYP19 gene, especially the study of genetic variation in Iraq and its relationship to the levels and concentrations of some reproductive hormones this study aimed to. Determination of the genetic polymorphism of the CYP19 gene, the fourth exon region in Awassi sheep strains, and its effect on hormonal concentration and pregnancy through diagnosis of alleles The averages in the study samples were at the probability level (0.05). Note that the hormonal and pregnancy hormone concentrations were at three replications per concentration.

Materials and Methods

The study carried out and collected blood samples from Awassi sheep from the jugular vein with a volume of (5 ml) for the purpose of separating (serum) and (2 ml) in (EDTA Tube) for the purpose of extracting DNA in the Husayniyah threshold fields located on the Najaf-Karbala road from September of 2019 to January of 2020, and this is with regard to the field side, while conducted Genetic analysis (laboratory part) in the Biotechnology Laboratory, Al-Qasim Green University where the DNA was extracted and the CYP19 gene was detected by PCR and the measurement of the estrogen and progesterone concentration in the laboratories of the Department of Life Sciences, College of Science, University of Babylon and a sequencing nitrogenous baseline examination was conducted (Sequencing)) Out of race.

Experimental animals

In this experiment, 50 ewes of the local Awassi sheep were used in the breed. 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 and its resulting concentration 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 (as shown in Table 1) was selected to perform molecular detection and phenotypic knowledge of the CYP19 gene.

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

Abbreviated gene	Sequence	Product size
EXONE4 OF CYP19	(F) 5-GCA CAG TCA CTA CAT ATC CCG A-3	105bp
	(R) 5-GCT TTC CAG AGT GCT GGA TTA T-3	

PCR sequence interaction of the studied gene

The materials in Table (2) were used for molecular detection using the chain reaction polymerase enzyme of the CYP19 gene with a size of 25. 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 (2).

Table 2: The materials used in the chain reaction of the CYP19 polymerase enzyme.

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

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

Table 3 : The followed conditions for beam multiplication of the CYP19 gene in the PCR reaction.

Number of cycles	Time	Temperatures	Steps	Number
1	5 minutes	94ºC	The first stage of mutant	1
35	30 seconds	94ºC	The Mutant	2
	30 seconds	55ºC	Crossover	3
	30 seconds	72ºC	Elongation	4
1	5 minutes	72ºC	The final elongation stage	5

Loading polymerase chain reaction and electrophoresis reaction:

I carried 10µL of volumetric evidence (DNA ladder) and 5µl of PCR products in a 1.5% concentration in the agarose 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 transilluminator 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 (Messer *et al*., 1997).

Statistical analysis

Statistical analysis of study samples was performed using SPSS version (25) and calculated mean hormonal

concentrations of pregnancy to find the relationship between the genotypes of the Exone4 gene for the CYP19 gene and hormonal concentrations. To know the significant differences between the averages in the study samples and on a level Possibility (0.05). Note that the concentration of the hormone pregnancy and pregnancy was at three replications per concentration.

Results and Discussion

DNA extraction and purification:

The results of DNA extraction and purification showed the presence of high purity and concentrations of DNA which were measured by (Nano drop) device and the results were between (13.9 to 42.9 µl / ng) and also the presence of DNA was confirmed by the method of electrical relay as shown in Figure (1).

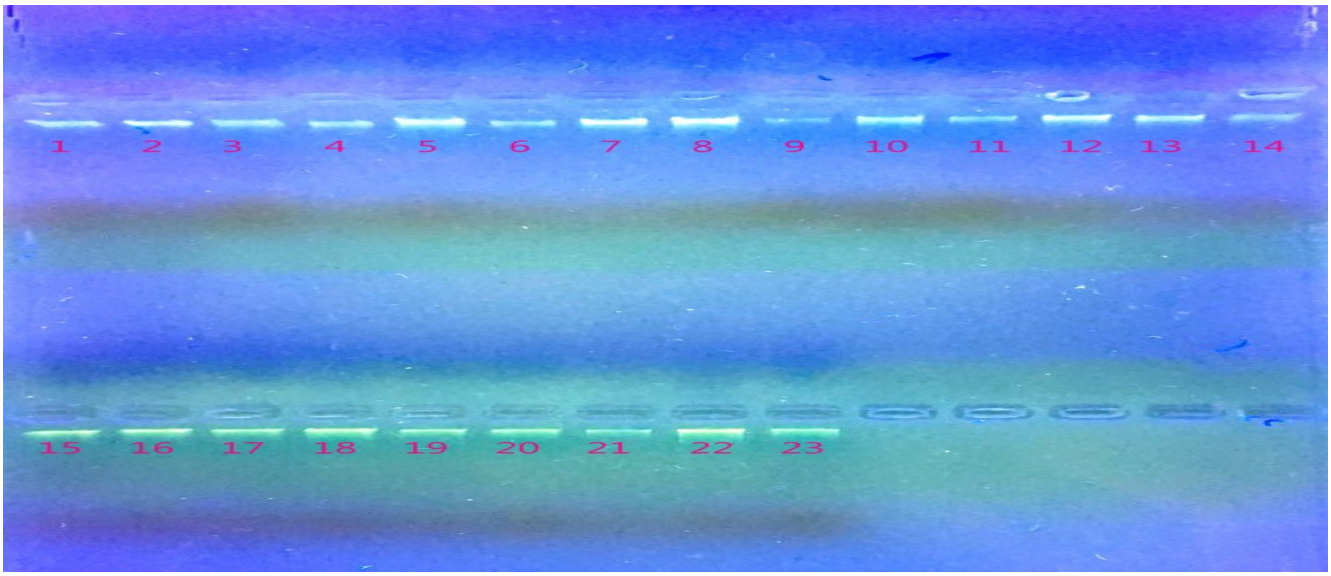

Fig. 1 : DNA bundles extracted from Awassi sheep blood samples using electrophoresis technique.

CYP19 gene detection using PCR technique

The results of the detection of the CYP19 gene using the initiator of (4 Exon) showed that 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 105 base pairs and as shown in Figure (2).

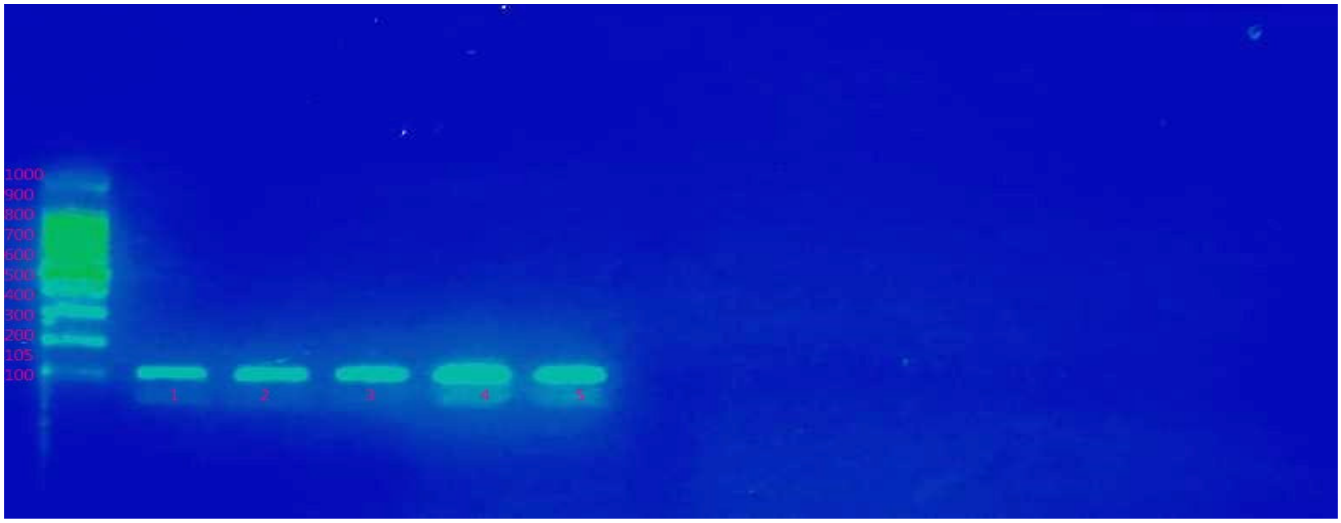

Fig. 2 : Electrophoresis of the CYP19 gene for the third exon region using 1.5% ac arose M. Standard DNA bundles, (16 samples) amplified gene output by PCR.

Study of the CYP19 (Exon4) nucleotide sequence:

A sequence of nitrogenous bases was used to find the series of nitrogenous bases forming the package (105 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, CC) for the samples. These genotypes were obtained using the results of the genetic analysis.

The percentages and number of genotypes of the CYP19 gene

The results shown in Table (4) showed the percentages of genotypes of the CYP19 gene in the fourth exon region in the studied samples, as 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%. (3) 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% At the time when the current study indicated a low rate of the genotype TT. A study conducted 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 (8).

* TT. The dominant genotype

* TC. A genotypic or asymmetric genotype

CC. Mutant genotype

Table 4 : Shows the percentages of Awassi sheep samples for the CYP19 gene in Exxon 4.

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

Genotype

The results showed that there was a difference in the sequence of nitrogenous bases for the bundle of 105 base pairs for the Exon4 (CYP19) gene. Three genotypes were obtained, as in Figure (3).

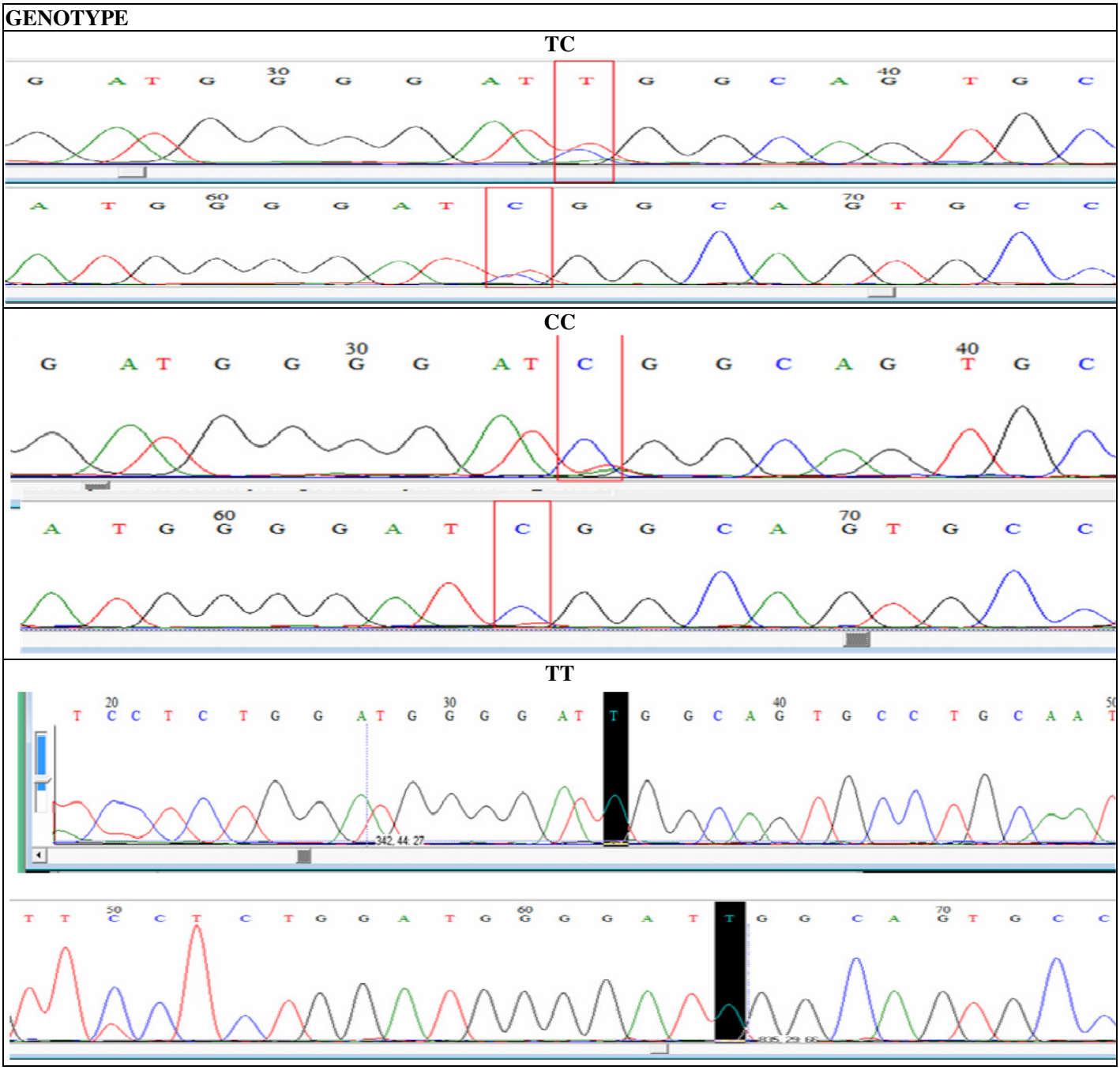

Fig. 3 : Genotypes of the CYP19 gene, depending on the NCBI location for the Sequencing alignment procedure and the use of the Bio edit program for the CYP19 gene.

Relationship of genotypes with the concentration of the hormone in the study samples:

The results in Table (5) showed a significant difference <0.05p between the genotypes produced as TC and CC genotypes were superior to 15.422 ± 3.06 pg / mL 15.350 ± 2.44 and picogram / milliliter compared to the concentration of the hormone of the genotype TT 14.678 ± 2.15 pg / mL. The results of the current study showed a 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; Lamb *et al*., 2009), 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 accumulate androgen produced in theca cells of the follicle ovarian 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 (Serge *et al*., 2003).

Table 5 : Averages of the hormone concentration in the samples Study 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 0.05p <and the different letters indicate a significant difference at the probability level p< 0.05.

The relationship of genotypes with the concentration of progesterone in the study samples:

The results in Table (6) show the mean pregnancy hormone concentrations that there are significant differences (p <0.05) between the resulting genotypes, as the TC hybrid model with a concentration of 0.398 ± 0.010 ng / ml exceeded the pure genotypes TT and CC with a concentration of ± 0.300 0 .011 ng / mL 0.298 ± 0.022 ng / mL in sequence The results of the current study show a clear effect of the genotypes of the CYP19 gene and its effect on the concentration of the pregnancy hormone through its effect on reproductive traits, it has an effect on the variation of the effects of nocturnal difference at a time when it exceeds the night Certain in a trait of a particular sheep breed was not the case of another breed (Serge *et al*., 2003) and that the genetic mutation has a clear effect on the hormone concentration of the hormone and the pregnancy hormone of the mutant genotype (TC) due to the fact that the mutation event results in a change in the genetic makeup of the organism Genotype, which leads to the appearance of patterns Phenotype and a section of mutations is harmful and some of them are beneficial and the effect of the mutation lies in a gradual change in the sequence of genetic information of genes or their number and then a change in the genetic makeup of the organism and causes a kind of development of the organism which leads to the emergence of behaviors Phenotype phenotypes make it able to adapt to environmental conditions and the variation in the research results of the current study and the different genotypes of the CYP19 gene result from the presence of a genetic mutation of the type (missense), which means the lost mutation and it encodes for a different amino acid as it happens. The basal substitution that causes the substitution of amino acid Another amino the change that occurs in the mutant gene may lead to a change in the expression of the gene and its ability to show its effect in a positive or relative way. Therefore, the genetic variation obtained is reflected in the action of the gene through the mechanism of hormonal secretion hormone estrogen and pregnancy hormone and increase their concentrations clearly in the mutant pattern and that Many researchers and studies have focused on the importance of genetics and finding and developing modern methods for genetic improvement by knowing the effect of genes, genetic parameters, and genotypes of sheep and animal breeds (Jones *et al*., 2000; Teneva *et al*., 2009).

Table 6 : The mean pregnancy hormone concentrations in study samples according to the genotypes of the CYP19 gene in Awassi sheep samples.

progesterone concentration, nanogram / ml	Genotype
0.300 ±0.011 A	TT
0.398 ±0.010 B	TC
0.298 ±0.022 A	CC

Similar letters indicate that there is no significant difference at the probability level 0.05p <and the different letters indicate a significant difference at the probability level p < 0.05.

Conclusion

The prevalence of genotypes 50% CC over dominant genotypes 20% TT and mutant mutant genotypes TC 30% of the CYP19 gene in local Awassi sheep. The polymorphism of the CYP19 gene in Exon4 has a significant effect on hormonal concentrations of hormonal and pregnancy. Persons carrying 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 mutant ranked second in importance after the TC hybrid genotype in terms of measuring concentrations Hormonal load and pregnancy. The TT and TC genotypes outperform the CC genotype in measuring the hormone.

References

Adiguzel, A.; Ozkan, H.; Baris, O.; Inan, K.; Gulluce, M. and Sahin, F. (2009). Identification and characterization of thermophilic bacteria isolated from hot springs in Turkey. J. Microbiol., 79: 321-328.

Ana, M.B.O.L.; Raimundo, N.B.L and Samuel, R.P. (2009). Aromatase gene and its effects on growth, reproductive and maternal ability traits in a multibreed sheep population from Brazil. Genetics and Molecular Biology. 32 (3): 484-490.

Azawi, M.S. (2015). Molecular Genetic Analysis of CYP19 and PRL Genes and Comet Test and Relation Some aspects of performance of local and Turkish Awassi sheep. Ph.D. thesis - College of Agriculture - University of Baghdad.

Heine, PA.; Taylor, JA.; Iwamoto, GA.; Lubahn, D.B. and Cooke, P.S. (2000). Increased adipose tissue in male

and female estrogen receptor-alpha knockout mice. Proc Natl Acad Sci USA97: 12729- 12734.

Jones, M.E.; Thorburn, AW.; Britt, K.L.; Hewitt, K.N.; Wreford, N.G.; Proietto, J.; Oz, O.K.; Leury, B.J.; Robertson, K.M. and Yao, S. (2000). Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. Proc Natl Acad Sci. USA, 97: 12735-12740.

Lamb, D.C.; Lei, L.; Warrilow, A.G.; Lepesheva, G.I.; Mullins, J.G.; Waterman, M.R. and Kelly, S.L. (2009). "The first virally encoded cytochrome P450 ". Journal of Virology, 83(16): 8266-9.

Messer, L.A.; Wang, L.; Tuggle, C.K.; Yerle, M.; Chardon, P.; Pomp, D.; Womack, J.E.; Barendse, W.; Crawford, A.M.; Notter, D.R. and Rothschild, M.F. (1997). Mapping of the melatonin receptor 1a(MTNR1A) gene in pigs, sheep and cattle. Mamm.Gen. 8: 368- 370.

Serge, C.; Sophie, L.; Christelle, D.; Isabelle, D.; Barbara, B. and Sonia, B. (2003). Aromatase expression and role of estrogens in male gonad: a review. Reproductive Biology and Endocrinology, 35: 1.

Teneva, A.E.; Dimitrova, I.; Georgiev, G.; Polihronoval, G. and Ivanova, K. (2009). Molecular characterization of Bulgarian livestock genetic resources and their optimized utilization for animal production. FAO / IAEA International Symposium on Sustainable Improvement of animal Production and Health, 8-11 June 2009, Vienna, Austria, Synopses -126.

Teneva, A.E.; Todorovska, N.; Tyufekchiev, A.; Stella, P. and Boettcher, D. (2007). Molecular characterization of Bulgarian livestock genetic resources. II. Microsatellite variation among Bulgarian cattle breeds. Biotechnology in Animal Husbandry, 23(5-6): 227-242.