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# Genetic Polymorphisms of SCD1 gene and its relationship with some reproductive traits in Holstein cows in Iraq

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SCD1 Gene; Reproduction traits; Holstein cows

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## ABSTRACT

The research was conducted at the Khalis cow station in Al-Khalis district - DIYALA Governorate and the central laboratory of the College of Agriculture - University of Tikrit for the period from December 13, 2020, to February 1, 2022, a sample of 63 Holstein cows, with the aim of extracting the genetic material and determining the genetic polymorphism of the SCD1 gene and its relationship to reproductive traits in Holstein cows, studying the percentage distribution of genetic structures in the studied cows, the allele frequency and calculating the value of chi-square ($\chi 2$) was determined. The percentage distribution of the SCD1 gene in the studied cows samples was 47.62, 46.03 and 6.35 % for the AA, AV and VV genotypes respectively. The value of the chi-square ($\chi 2$) was highly significant ($P < 0.01$). The frequency of the allele A and V was 0.71 and 0.29, respectively. Some reproductive traits have been significantly affected by the different genotypes of the SCD1 gene. Cows with the AV genotype showed the shortest open days (92.82 ± 5.89 days), and AV genotype cows achieved the shortest calving interval (374.27 ± 5.96 days). Based on the study of genetic polymorphism of the SCD1 gene it can be concluded that it is possible to improve reproductive traits in cows. We also recommend applying the study to a larger sample of several different gene seasons and locations to give more accurate results to apply selection and elimination strategies.

# تعدد الأشكال الجينية لجين SCD1 وعلاقته ببعض الصفات التناسلية في أبقار هولشتاين في العراق

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**الخلاصة**

أجري البحث في محطة أبقار الخالص الكبرى في قضاء الخالص – محافظة ديالى والمختبر المركزي لكلية الزراعة – جامعة تكريت للمدة من كانون الاول 2020/13 لغاية شباط 2022/1, على عينة مكونة من 63 بقرة هولشتاين (حلوب), بهدف فصل المادة الوراثية وتحديد التراكيب الوراثية لجين ( *SCD1* ) وعلاقة هذه التراكيب بالصفات التناسلية في ابقار الهولشتاين, فضلاً عن دراسة النسبة المئوية لتوزيع التراكيب الجينية في الابقار المدروسة وتكرار الأليلات المتحصل عليها وحساب قيمة مربع كاي **($\chi$2)**. حيث بلغت نسبة توزيع التراكيب الوراثية لجين SCD1 في عينة الابقار التي تمت دراستها 47.62 و 46.03 و 6.35 للتراكيب الوراثية AA و AV و VV على التوالي وكانت قيمة مربع كاي **($\chi$2)** عالية المعنوية ($P<0.01$) وبالتالي لا تخضع لتوازن هاري وينبرك, بلغ تكرار الاليل A هو 0.71 في حين كان تكرار الاليل V هو 0.29 على وفق تحليل جين SCD1 في الدراسة الحالية. تأثرت بعض الصفات التناسلية معنوياً باختلاف التراكيب الوراثية لجين SCD1, إذ حققت الابقار ذات التركيب الوراثي الهجين AV اقل المتوسطات في المدة من الولادة الى التلقيح المثمر(المدة المفتوحة) اذ بلغت (92.82 ± 5.89). كما حققت الابقار ذات التركيب الوراثي AV اقصر مدة في الفترة بين الولادتين إذ بلغت (374.27 ± 5.96). وبناءً على دراسة تعدد الاشكال لجين SCD1 يمكن الاستنتاج انه من الممكن تحسين الصفات التناسلية في الابقار, كما نوصي تطبيق الدراسة على عينة اكبر ولعدة مواسم ومواقع مختلفة من الجين مع دراسة عدد اكثر من الصفات الاقتصادية لإعطاء نتائج اكثر دقة في تطبيق استراتيجية الانتخاب والاستبعاد.

**الكلمات المفتاحية:** جين SCD1, الصفات التناسلية, ابقار الهولشتاين.

## INTRODUCTION

Cows are among the important farm animals in the production of milk, and they produce nearly 90% of the total milk production, and one of the reasons for raising dairy cattle is the increase in milk production (FAO, 2009, Sejian et al, 2016). As the economic feasibility of cow breeders depends primarily on the productive performance and productive longevity of cows (Dash *et al.*, 2018). The increase in the amount of milk produced by dairy cows was accompanied by a decrease in reproductive efficiency (Lopez-Gatius *et al.*, 2003), as a result of a negative correlation between reproductive and productive performance in milk cows (Chagas *et al.*, 2007).

Most of the research in recent years focused on discovering candidate genes for traits Production, reproduction, and mission in selection and breeding programs, and the most prominent of these genes is the Desaturase1 CoA Stearol (SCD1) gene (Carvajal *et al.*, 2016). The SCD gene was first discovered in rat liver (Strittmatter *et al.*, 1974). In cow, the SCD1 gene is located on chromosome 26 (Bouwman *et al.*, 2011), located on the long arm of chromosome 26 in cattle (Bernard *et al.*, 2001). It contains 6 exons and 5 introns (Kovalchuk *et al.*, 2020). The SCD1 gene encodes a protein of 359 amino acids (Kęsek *et al.*, 2017). The SCD1 gene is primary expressed in adipose tissue and mammary gland in farm animals (Li *et al.*, 2020). The SCD1 gene encodes an enzyme

involved in the biosynthesis of fatty acids, primarily oleic acid (Bernard *et al.,* 2013). The fatty acid-forming protein SCD1 belongs to the desaturase family and is an integral membrane protein located in the endoplasmic reticulum (Bernard *et al.,* 2013, Kulig *et al.,* 2013).

SCD1 is an enzyme responsible for the conversion of saturated fatty acids into monounsaturated fatty acids (MUFA) (Taniguchi *et al.,* 2004, Li *et al.,* 2020). The desaturation process is carried out by inserting a double bond between carbon atoms 9 and 10 from the carboxylic end along a chain of fatty acids greater than 12 carbons to convert 16 or 18 carbon-saturated fatty acids into monounsaturated fatty acids of the same length (Li *et al.,* 2020). The study aim of extracting the genetic material and determining the genetic polymorphism of the SCD1 gene and its relationship to reproductive traits in Holstein cows.

## MATERIALS AND METHODS

The fieldwork was conducted at the Khalis cow station in DIYALA Governorate, and molecular analyzes were conducted in the central laboratory of the College of Agriculture - Tikrit University for the period from 1/12/2020 to 1/2/2022 for the purpose of determining the genotypes of the SCD1 gene and the relationship of these structures to reproductive characteristics. Reproductive traits (Services per conception, days open , Gestation period, Dry period, calving interval) were calculated through the station's own records. For the purpose of extracting the genetic material and conducting molecular analysis, 4 ml of blood was withdrawn by sterile syringes of 5 ml jugular vein from 63 Holstein cows and the blood was discharged into tubes containing anticoagulant K2EDTA with a volume of 2 ml.

## DNA EXTRACTION

The DNA was extracted using the chemical extraction method (buffered solutions) in which chemicals (sucrose, MgCl2, Tris HCL, Triton X-10, SDS, Na2EDTA, sodium citrate, Ammonium acetate, Nacl, absolute Ethanol and 70% Ethano) were used in the preparation of solutions (Red blood cell lysis buffer and Nucleic lysis buffer). The extracted samples were electrophoresed in an agarose gel at a concentration of 1% with a voltage difference (80 V - 70 mA for 60 minutes) as in (Figure 1).

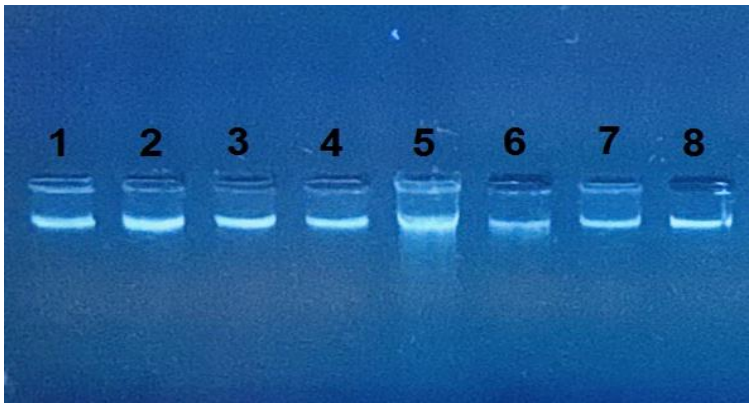


**Figure 1.** The extracted genetic material.

**PCR AND RFLP**

Genotypes were determined from the extracted genetic material by amplifying the target region in the Exon3 region of the SCD1 gene by PCR using a mixture of Master Mix manufactured in the American Promega company and the primer prepared from the Korean Macrogen company (F-5`-CCCATTCGCTCTTGTTCTGT-3`). And (R- 5`-CGTGGTCTTGCTGTGGACT-3`) according to the following program: the initial deformation stage at a temperature of 94 °C for 5 minutes for one cycle, then the smashing stage at a temperature of 94 °C for a period of 45 seconds and fusion at a temperature of 56.7 C° for 30 seconds and elongation at 72°C for 45 seconds with 35 cycles, then the final elongation stage at 72°C and final elongation at 10°C for one cycle. Then, the amplified samples were electrophoresed in agarose gel at a concentration of 2% with a voltage difference (80 volts - 70 mA for 60 minutes). The PCR output was a band of (400) bp, as in (Figure 2).

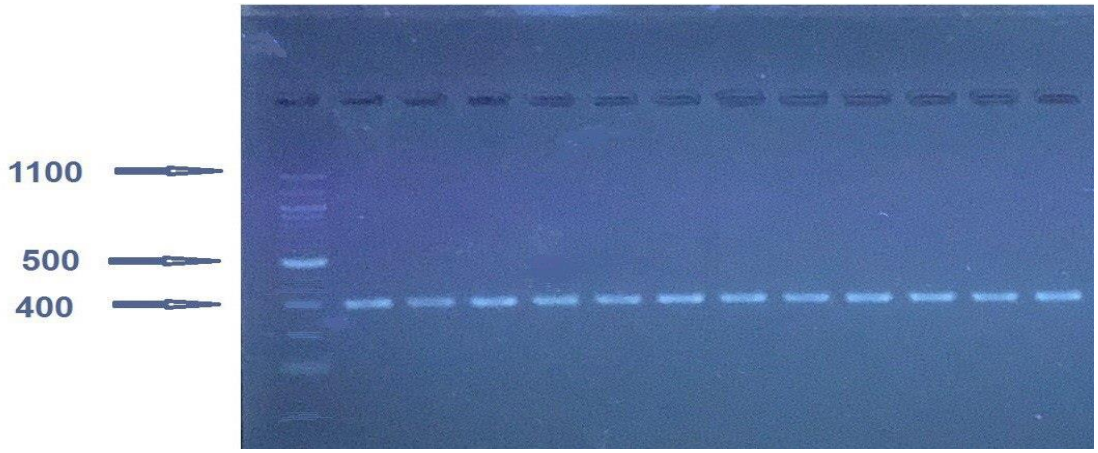


**Figure 2.** Samples on an agarose gel after electrophoresis.

After that, the Restriction Fragment Length Polymorphism (RFLP) technology was used by cutting the PCR product with the NcoI restriction enzyme (provided by BioLabs New England in England) to cut within the (C*CATGG) sequence, and this was done by making the following mixture: taking 5 µL of PCR product, then taking 0.3 µl of NcoI diluted at a concentration of 10 IU per µl, then adding to the mixture 1 µl of buffer solution (10X Buffer), then adding 3.7 µL of ion-free aqueous solution for a total mixture of 10 µl, then incubating the mixture at a temperature of 37 ° C for 3 hours, and then the cutted samples were electrophoresed in agarose gel at a concentration of 2.5% with a Voltage difference (80V – 70mA for 60min) with the use of DNA Ladder (1500-100)bp.

**Statistical analysis**

The data were analyzed statistically using the SAS (2018) statistical analysis system software to study the effect of genetic polymorphism of the FADS2 gene on the traits and components of milk production in Holstein cows, significant differences between the averages were extracted using the Duncan (1955) polynomial test by applying the (Least Square Means) Method.

Mathematical model: The relationship of the SCD1 gene genotypes with the studied traits:

$Yijklm = \mu + SCD1i + Pj + Sk + Xl + eijklm$

Yijklm: observed value m for genotype i, Parity j, birth season k, and sex newborn l.

µ: the overall mean of the trait.

SCD1i: influence of phenotypes of the gene (SCD1 AA, AV, and VV).

Pj: influence of Parity (second, third and fourth).

Sk: influence of birth season (winter, spring, summer and autumn).
Xl: the influence of the sex of the newborn (male, female).
eijklm: the random error that is normally distributed with a mean of zero and a variance of 2eσ.

## RESULT AND DISCUSSION

The RFLP technique revealed the existence of a SNP by substituting the nitrogenous base A to V in the Exon5 region of the gene, and after using the NcoI enzyme, the genotypes appeared on the basis of bundles in the agarose gel, and the result was: AA when there is one band size of 200 bp, AV when there are two bp band size (400,200), VV when there is one band size 400 bp as in (Figure 3).

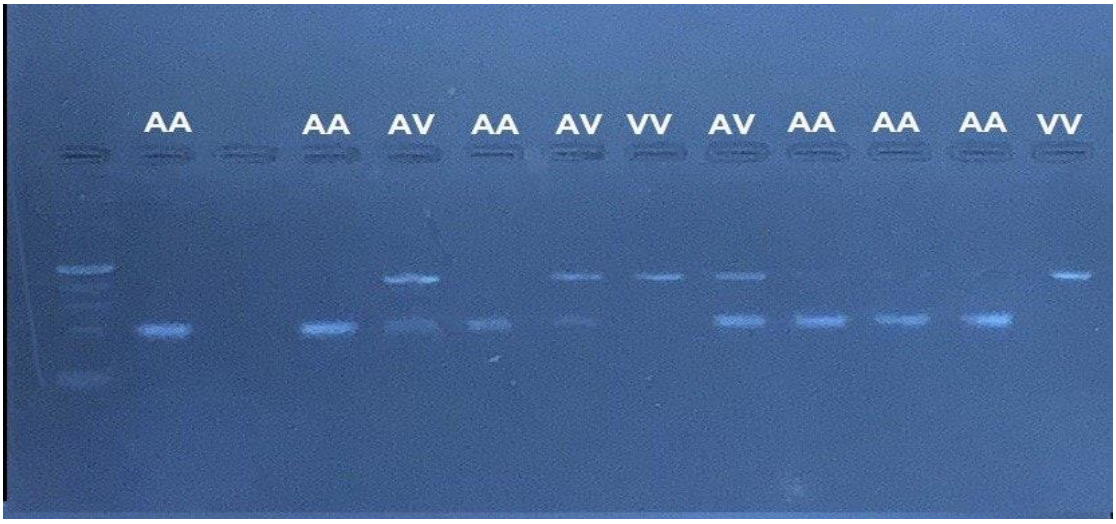


**Figure 3.** Shows the types of genetic polymorphism of the SCD1 gene in cows.

Through Table (1), high significant differences (P≤0.01) were observed between the observed and expected numbers ratio, which amounted to 47.62 and 46.03 for the AA and AV genotypes, respectively, In the studied samples of Holstein cows there is a clear prevalence in the percentage of genotypes (AV, AA) with a decrease in the percentage of the VV genotype. The percentage of genetic polymorphism was Compatible across studies (Mashhadi et al., 2012; Kisk et al., 2017; Alwan et al., 2020; and Kesic-Wozniak et al., 2020). The frequency of alleles A (0.71) and V (0.29) were also computed and matched what was found (Clark et al., 2010) since they were not subject to Hardy Weinberg equilibrium and therefore estimated using the value of the chi-square (2).

Through the results of the analysis of the current study in Table (2), it was found that there was a significant differences (P<0.05) in the Days Open between cows with different genotypes. The AA, was the highest( 106.70 ± 5.84 days), while cows with genotype AV have the shortest period ( 92.82 ± 5.89 days), The outcomes were different from what was discovered (Nanaei et al., 2014), which showed that cows with the genotype VV gave a considerably higher in comparison to the AA genotype in the Days open.

**Table 1:** Number, percentages and allele frequency of the SCD1 gene of Holstein cows.

| Genotype (SCD1) | Number | Percentage % |
|---|---|---|
| AA | 30 | 47.62 |
| AV | 29 | 46.03 |
| VV | 4 | 6.35 |
| Total | 63 | 100% |
| chi-square value ($\chi 2$) | ---- | 21.857 ** |
| Allele | Allelic frequency | |
| A | 0.71 | |
| V | 0.29 | |
| ** P<0.01 | | |

The results indicated that there was a significant differences (P < 0.05) in the calving interval between cows of genotype AA, which showed the highest ( 389.77 ± 5.90 days), and the shortest calving interval was among individuals carrying the hybrid genotype AV, ( 374.27 ± 5.96 days ) ,with a difference Significant amounted to 15.5 days, Which contradicted the findings of research (Demeter et al., 2009; Nanaei et al., 2014) that discovered no appreciable variations across the Polymorphism of the SCD1 gene.

**Table 2:** Relationship of SCD1 genotypes to reproductive traits in Holstein cows.

| Genotype SCD1 | N | Least Squares Means ± standard error | | | | |
|---|---|---|---|---|---|---|
| | | S/C | D.O | G.P | D.P | C.I |
| AA | 30 | 2.47 ± 0.20 | 106.70 ± 5.84 a | 283.12 ± 3.09 | 100.11 ± 18.20 | 389.77 ± 5.90 a |
| AV | 29 | 2.08 ± 0.20 | 92.82 ± 5.89 b | 286.59 ± 3.12 | 89.84 ± 18.37 | 374.27 ± 5.96 b |
| VV | 4 | 2.56 ± 0.48 | 105.93 ± 14.25 ab | 278.82 ± 7.55 | 93.83 ± 44.41 | 386.75 ± 14.39 ab |
| Significant | | NS | * | NS | NS | * |
| The means that carry different letters within the same column differ significantly among themselves | | | | | | |

N: Number, S/C: Services per conception, D.O: Days open, G.P: Gestation period, D.P: Dry period, C.I: Calving interval, *: (P<0.05), NS: Non significant

## CONCLUSION

Amplification of the target region in Exon 5 of the SCD1 gene and the use of the NcoI enzyme led to the emergence of three genotypes (AA, AV, VV), and the numbers of cows genotypes is not subject to Hardy Weinberg balance. The AA genotype also achieved the advantage in open-day and Calving interval compared to other genotypes with a large difference (P < 0.05),

And here can be said that there is a positive relationship between most reproductive traits and allele A when we neglect the data of cows of the genotype VV, due to their small number.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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