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DETECTION OF *POU1F1* GENE USING SEQUENCING ANALYSIS OF AWASSI SHEEP BREED (*OVIS ARIES*)

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Abstract

The study was carried out on 46 head of Awassi ewes (age 2-4 years old), at Karbala sheep station to determine changes in the nitrogen bases of POU1F1 gene and some molecular parameters, as well as calculation of the number of Hyplotypes and its frequencies, and detect the change in amino acids of the POU1F1 gene in the studied animals comparing with some Breeds from other countries. Six different Fragments of the gene were selected, including all the exons and parts of the introns. All the six studied fragments (P1, P2, P3, P4, P5, and P6) were successfully amplified; sizes of amplified products were 637bp, 789bp, 999bp, 868bp, 1190 bp, and 469bp, respectively, the results showed changes in 12 sites in the nitrogen bases at different sites of introns and exons which give 7 haplotypes. The value of Haplotype Diversity (HD) was (0.696), while nucleotide diversity (π) value was (0.00059). Frequencies of haplotypes H1, H2, H3, H4, H5, H6 and H7 were 0.487, 0.282, 0.108, 0.062, 0.021, 0.021 and 0.021, respectively. The amino acids of POU1F1 did not show any change in any site of POU1F1 protein among all studied animals, whereas comparing our results with some other breeds showed some changes in amino acids. The fragments of POU1F1 gene of Awassi sheep breed have been submitted at Gene Bank under the accession numbers (LC469323 to LC469349), the amino acids of the POU1F1 protein of which 291 amino acid for Awassi breed was also submitted under accession number BBJ06446.

Keywords: Awassi Sheep, POU1F1 gene, Haplotypes, amino acids sequence

Intoduction

The POU1F1 gene is known as the Pituitary Specific Transcription Factor-1 (Pit-1), it's one of the most important members of the POUs family, which are transcription factors with specialized and similar areas associated with DNA. These factors have many functions, such as control of hormone production, the development and differentiation of organs, the regulation of immunoglobulin and the influence of the nervous system responsible for hormone secretion (Assa-Munt *et al.,* 1993) and (Gold, 2014) and (Pfaffle and Blum (2000). The POU1F1 gene is located on the first chromosome for cattle, sheep, goats and pigs (Woollard *et al*., 2000), in humans it is located on the third chromosome (Romero *et al*., 2011). It regulates the genetic expression of many hormones such as growth hormone (GH), prolactin hormone (PRL) and thyroid stimulating hormone (TSH-β) (Sun *et al*., 2002) The gene expression of POU1F1 mainly occurs in the pituitary tissue and mammary gland tissue (Gil-Puig *et al*., 2002).

Many studies have been conducted on the functions of POU1F1 gene for different types of animals, Goat (Zhu *et al.,* 2019) and (Lan *et al.,* 2007) Cattle (Zhang *et al.,* 2013) and chicken (Qiu *et al.,* 2006). The POU1F1 gene is composed of 6 exons and 5 introns, the exons are encoded into a peptide series of 291 amino acids. Previous studies observed that the occurrence of mutations in this gene cause deterioration in the biological processes of growth in the early stages, shortness of stature, occurrence of mental retardation, and various congenital malformations in humans (Lee *et al.,* 2011). Bai *et al.* (2016) studied three breeds of Chinese sheep, changes of three sites (C355T, C71G and C330G) which known as a single nucleotide polymorphism (SNP) in the sixth exon of the POU1F1 gene.

Sadeghi, *et al.* (2014) showed a change of alanin amino acid to thironine amino acid in one site (c.105A> G) at the third exon. Due to the lack of studies on this gene on sheep in general and local sheep in particular, this study aimed to determine the formation of single-nucleotide polymorphism (SNPs) and some Genetic Parameter of the POU1F1 gene for the six exons and parts of the introns.

Materials and Methods

In this study, 46 Awassi ewes (2-4 years old) were used at Al-Kafeel sheep station- Karbala, about 150 km away from Baghdad. Blood specimens were taken from jugular vein for all ewes and preserved in 4ml EDTA treated tubes.

DNA has been extracted using Geneaid kit (Taiwan). Six fragments (P1, P2, P3, P4, P5 and P6) of the POU1F1 gene were amplified in the genetic engineering laboratory at the Faculty of Agriculture, University of Basrah. Required primers (P1-P5) were designed in Gene Bank based on some fragments with Accession numbers (AJ549204, AJ549205, AJ549206 and AJ549207).

Required primer for the Fragment (P6) was designed depended on (Ozmen *et al*., 2014). Table (2) showed primers' sequences, sizes and malting temperature (mT). PCR products have been confirmed by electrolyzed it using 2% agarose, and Diamond™ Nucleic Acid Dye (Promega USA), then sent to (Yang Ling Biotechnology Co; Itd China) for sequence analyzing.

Alignment of all samples' sequences of POU1F1 gene was conducted using Biomedit V.7.2.6 program. DnaSP V.6.12 Program was used for the detection of some molecular parameters [Hoplotypes, Haplotype Diversity (HD), nucleotide diversity (π), Tajima's D Statistic and Fu's Fs F Statistic] of the gene. Clustal Omega was used for the purpose of comparing the amino acids of the protein POU1F1 in this study with other studies.

Table 1 : Shows the used Primers of POU1F1 gene, their sizes and melting degrees

mT (C)	Product Size	Primers	]Fragment
60	637 bp	F: 5'- AGTGAGATCTGAAACGGCCC - 3' R: 5'- ACTATGAGGTGTACGGCATTT - 3'	P1
64	868 bp	F: 5' - AAAACTGGTCAGTCACGCCA- 3' R: 5' - GTATGGAGGCGGGCAATGAA - 3'	P2
62	780 bp	F: 5' - TTCCCAGCAGAGCACTTAACA -3' R: 5' – GTGCTTGTTAACAGCTGTGGGA - 3	P3
59	1190 bp	F: 5' – ACCAGGCAATTCTACACTGAG - 3' R: 5' – TCTCAATTGGCTCTATTCATTTTCA -3'	P4
62	999 bp	F: 5'- TCCCTCGGTTGAATTTGTGCTA -3' R: 5'- TCCAAAGCCTGCAGAGCAAA -3'	P5
59	469 bp	F: 5'- GTATTGCTGCTAAAGACGCC -3' R: 5'- GAGGGAAAGATATAGTGAAAGGG -3'	P6*

*Ozmen *et al.* (2014)

Results & Discussion

The results of PCR technique showed a successful amplification process of selected fragments of the POU1F1 gene in all six exons and parts of the introns. PCR products were electrolyzed with 2% agarose gel, the amplified fragments P1, P2, P3, P4, P5 and P6 were sized (637bp, 868bp, 789bp, 1190bp, 999bp and 469bp), Respectively figure (1).

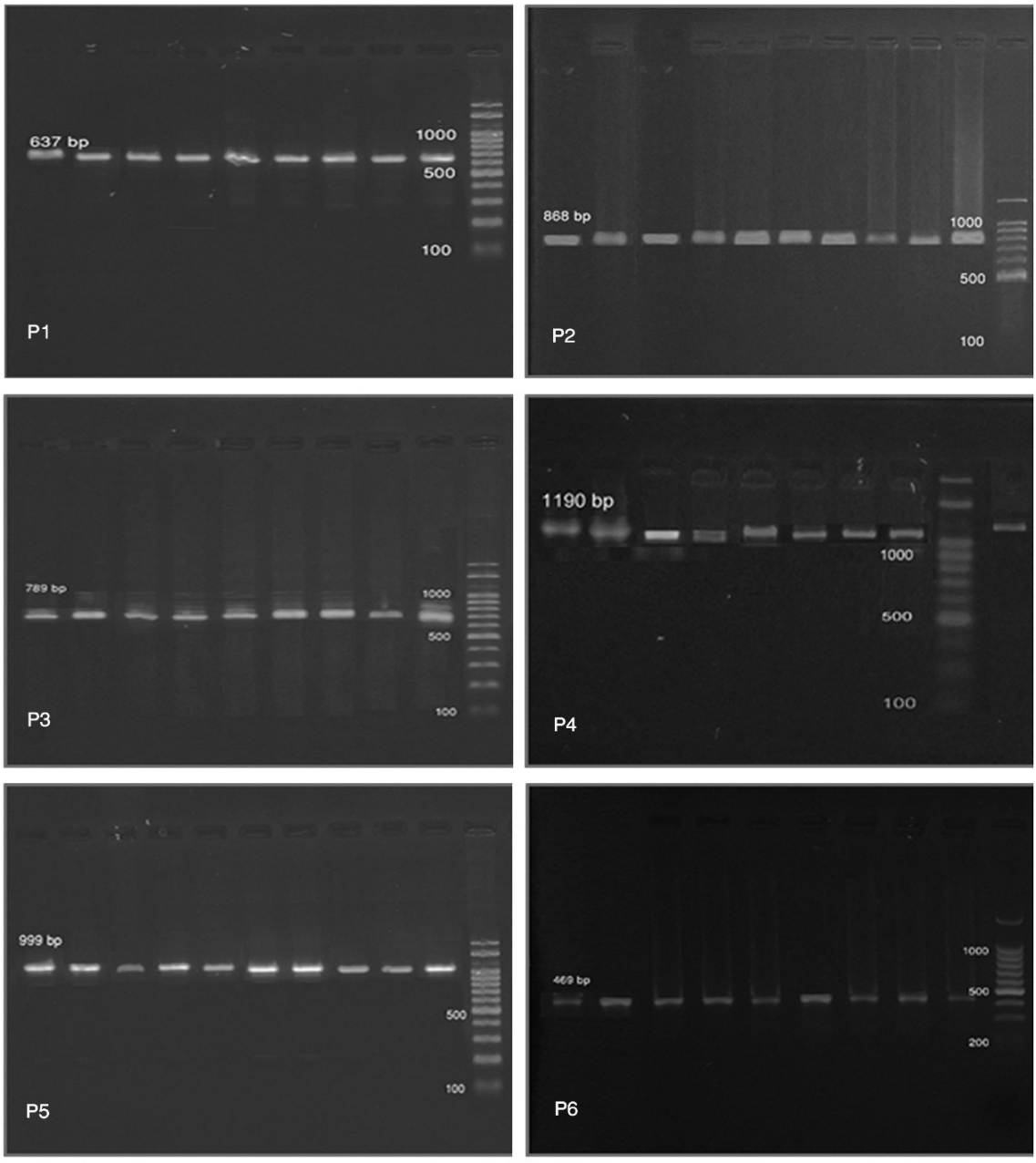

Fig. 1 : Shows the amplification results of the studied Fragments of POU1F1 gene

Molecular analysis of the current study were based on the six studied fragments of the POU1F1 gene. The total nitrogen bases were (4982)bp, the total net nitrogen bases after alignment were (4010)bp. The number of Haplotypes are seven, Figure (2) and Table (2), which resulted from a changes in 12 different sites, Figure. (3). The frequencies of Haplotypes H1, H2, H3, H4, H5, H6 and H7 were 0.478, 0.282, 0.108, 0.065, 0.022, 0.022 and 0.022 respectively.

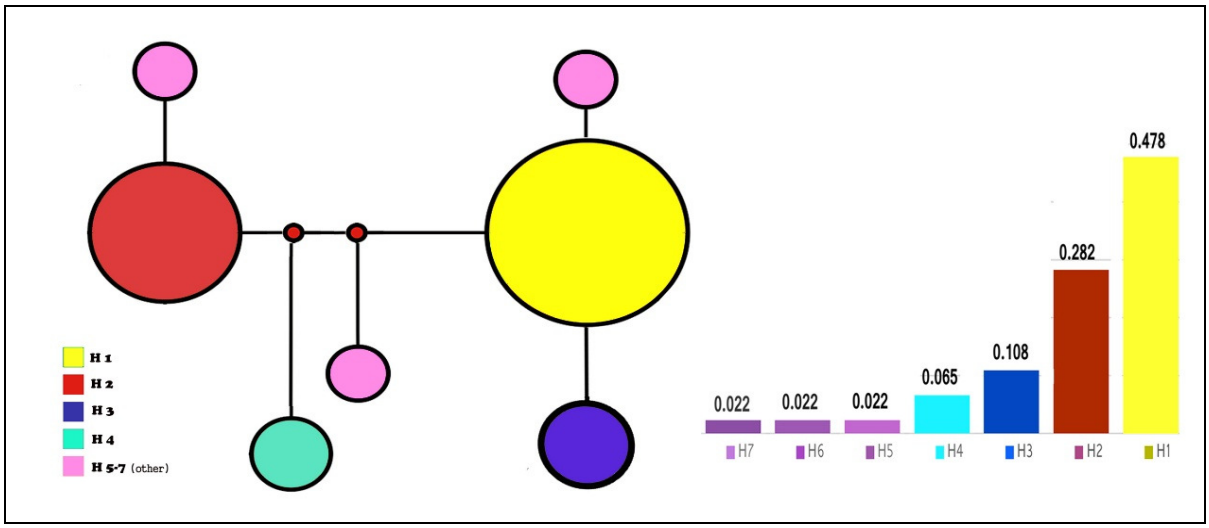

Fig. 2 : Explains the network of Haplotypes and its frequencies of the studied fragments for POU1F1 gene.

Table 2 : Shows the Haplotypes Frequency and Changes of nitrogen bases

Bases Haplotypes	Haplotypes Frequency	No. of animals	Haplotypes
CGGCAAGAGTAA	0.48	22	H1
CGGCACGAATAG	0.28	13	H2
CGGCCAGAATAG	0.11	5	H3
CGGCCAAGGTAA	0.07	3	H4
CGGCAAGAAGAA	0.02	1	H5
CGGCACGAGGTG	0.02	1	H6
TACGAAGAGTAA	0.02	1	H7
	1	46	Total

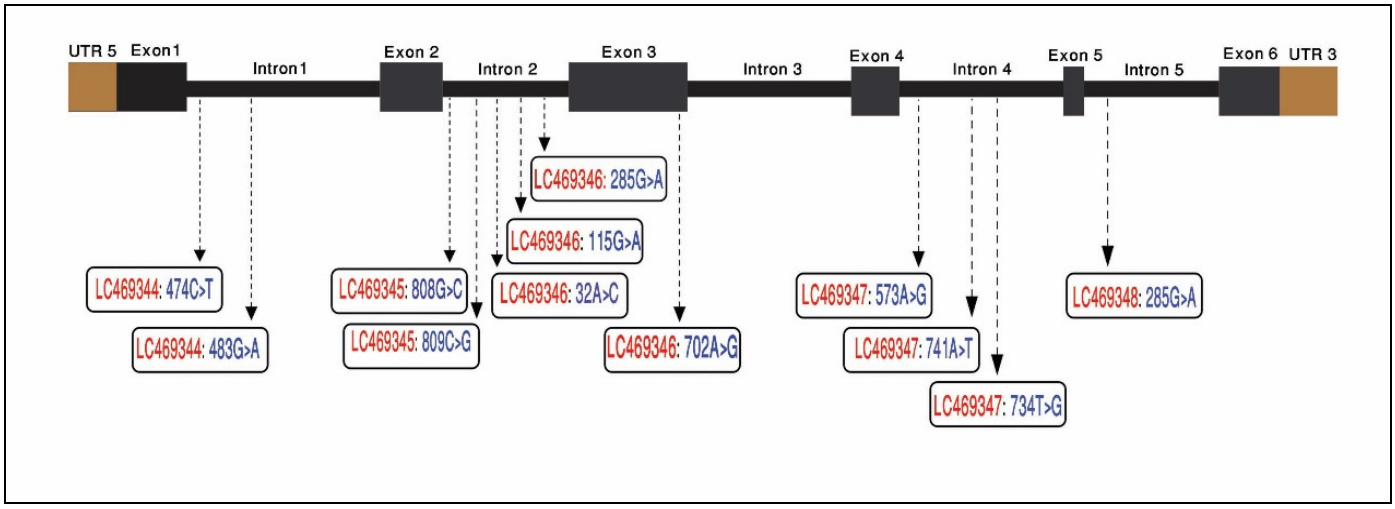

Fig. 3 : Showed the changes obtained from analyzing sequences of six different fragments of the gene.

Haplotype diversity (HD) recorded is (0.696), Nucleotide Diversity (π) is (0.00059), the value of (Tajima's D Statistic) was (-0.38128), this value is close to zero (Table 4), (Aris-Brosun and Excoffier 1996) point out that Tajima's D Statistic values near zero mean Population stability and were not under any of the various evolutionary pressures. Fu's Fs test (F Statistic) was (-0.23568), Fu (1997) found that the value of this test, which approaches zero, is less than expected from Haplotypes , as the Population was also stable and not subject to large expansion.

Taple 3 : Haplotype Diversity (HD)and Nucleotide Diversity (π)

Gene	Number of Sequences (N)	Number of Polymorphic (NH)	Haplotype Diversity (HD)	Nucleotide Diversity (π)	Tajima's D Statistic	Fu's Fs FStatistic
POU1F1	46	12	0.696	0.00059	-0.38128	-0.23568

In the first intron there was a change in two sites LC469344.483G>A and LC469344.474C>T, in the second intron there were change a five sites (LC469345. 32A>C, LC469345.115G>A, LC469345.285G>A, LC469345.808G >C and LC469345.809C>G). While in first and second exon there no found change in any site.

But in the third Exon there was a change in one site LC469346.702A>G which is included in the code of arginine amino acid in the peptide series of POU1F1 protein at site (c.arg142arg), and this change did not influence the amino acid. In the fourth intron, three changes were found: LC469347.573A>G, LC469347.734T>G and LC469347.741 A>T, In the fifth intron, one change was found in the site LC469348.285G> A. The results of the molecular analysis showed no change in any site of nitrogen bases in the first, second, fourth, fifth, and sixth exons.

The fragments of POU1F1 gene of Awassi sheep breed of this study have been submitted at Gene Bank under the accession numbers (LC469323 to LC469349). The amino acids of the POU1F1 protein of which 291 amino acid for Awassi breed was also submitted under accession number (BBJ06446) Table(4).

Table 4 : Shows the fragments' sequences submitted to Gene Bank (NCBI).

Fragments	Gene Name	Local on the gene	Size of fragment	Accession Numbers
P1	POU1F1	Exon-1 & part of Intron-1	520 bp	LC469344, LC480423, LC480424
P2	POU1F1	Exon-2 & part of Intron-2	818 bp	LC469345, LC480425, LC480426
P3	POU1F1	part of Intron2 & Exon-3	718 bp	LC469346, LC480427, LC480428, LC480429, LC480430
P4	POU1F1	Exon-4 & part of Intron-4	813 bp	LC469347, LC480431, LC480432, LC480433, LC480434
P5	POU1F1	part of Intron-4 & Exon-5	679 bp	LC469348, LC480435, LC480436
P6	POU1F1	Exon-6 & part of UTR,3	462 bp	LC469349

comparing with a similar study on Portuguese sheep (Pastos *et al.,* 2006), which aimed to detect the genetic Polymorphism of the POU1F1 gene for 100 heads of Portuguese sheep for breed (Churra da Terra). Sequence analysis explains a number of changes in the sites distributed in different fragments of the POU1F1 gene.

A change was found in the site 58G>A of the second exon which change cysteine amino acid to tyrosine amino acid. In the third exon, two changes were found, one in the site 89G>A (which changes Glycine to Asparagine) and the second was in thesite105A>G (which changes Alanin to thereonin), as well as a changes in some sites in the fourth intron of the gene. In another study for (Mura *et al.,* 2012) on the Sarda sheep in Poland, the results detected changes in six sites, one site for each of the second exon, third exon, fourth intron and UTR5, in addition to two changes in the UTR3 region. Bai *et al.* (2016) noted that changes in three sites (71.C>G, 355C.T and 355C>T) which known as the single nucleotide polymorphisms (SNPs) in the sixth exon of the POU1F1 gene.

The total number of amino acids encoded POU1F1 gene is (291 amino acids). Most of the Changes of nitrogen bases were found in the introns of POU1F1 gene, in addition to one change in one site of the third exon, which caused changing the adenin to Guanine in the site 702 of the third exon, for this why After the analyzing of amino acid for all animals of our study, there were no changes observed in peptide series for POU1F1 protein for animal of this study.

Table (4) comparing the amino acids of the POU1F1 protein in the current study with the amino acids recorded in the Bank Gene (NCBI) of some breeds for different countries, some differences were found in the amino acids forming the POU1F1 protein between the different Breeds, India's Madgyal breed was different in 11 amino acids, the Portuguese Churra da Terra breed differed in only one amino acid (methionine) in site (86) of the POU1F1 protein.

Table 5 : Amino acids of the POU1F1 gene in the Iraqi Awassi sheep and some other breeds

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1-Madgyal (India)**      MSCQPFTSTDTFIPLNSESSATLPLIMHPSAAECLPVSNHATNVMSTATGLHYSVPSCHY  60
2-Awassi (Iraqi)         MSCQPFTSTDTFIPLNSESSATLPLIMHPSAAECLPVSNHATNVMSTATGLHYSVPSCHY  60
3-Rambouillet (spain)    MSCQPFTSTDTFIPLNSESSATLPLIMHPSAAECLPVSNHATNVMSTATGLHYSVPSCHY  60
4-Churra da Terra (Portugal MSCQPFTSTDTFIPLNSESSATLPLIMHPSAAECLPVSNHATNVMSTATGLHYSVPSCHY  60
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1-Madgyal (India)        GNQSSTYGVMAGSLTPCLYKFPDHTLSHGFPPMHHRLLSEDPSAADFKQELRRKSKLIGK  120
2-Awassi (Iraqi)         GNQSSTYGVMAGSLTPCLYKFPDHTLSHGFPPMHQPLLSEDPTAADFKQELRRKSKLIEE  120
3-Rambouillet (spain)    GNQSSTYGVMAGSLTPCLYKFPDHTLSHGFPPMHQPLLSEDPTAADFKQELRRKSKLIEE  120
4-Churra da Terra (Portugal ) GNQSSTYGVMAGSLTPCLYKFPDHTMSHGFPPMHQPLLSEDPTAADFKQELRRKSKLIEE
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1-Madgyal (India)        PRYWESPEIQELEKFANEFKVKRIKLGYTQTNVGEALAAVHGSEFSQTTICRFENLQLSF  180
2-Awassi (Iraqi)         PIDMDSPEIRELEKFANEFKVRRIKLGYTQTNVGEALAAVHGSEFSQTTICRFENLQLSF  180
3-Rambouillet (spain)    PIDMDSPEIRELEKFANEFKVRRIKLGYTQTNVGEALAAVHGSEFSQTTICRFENLQLSF  180
4-Churra da Terra (Portugal ) PIDMDSPEIRELEKFANEFKVRRIKLGYTQTNVGEALAAVHGSEFSQTTICRFENLQLSF  180
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1-Madgyal (India)        KNACKLKAILSKWLEEAEQVGALYNEKVGANERKRKRKTTISIAAKDALERHFGEQNKPS  240
2-Awassi (Iraqi)         KNACKLKAILSKWLEEAEQVGALYNEKVGANERKRKRRTTISIAAKDALERHFGEQNKPS  240
3-Rambouillet (spain)    KNACKLKAILSKWLEEAEQVGALYNEKVGANERKRKRRTTISIAAKDALERHFGEQNKPS  240
4-Churra da Terra (Portugal ) KNACKLKAILSKWLEEAEQVGALYNEKVGANERKRKRRTTISIAAKDALERHFGEQNKPS  240
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1-Madgyal (India)        SQEILRMAEELNLEKEVVRVWFCNRRQREKRVKTSLNQSLFPISKEHLECR  291
2-Awassi (Iraqi)         SQEILRMAEELNLEKEVVRVWFCNRRQREKRVKTSLNQSLFPISKEHLECR  291
3-Rambouillet (spain)    SQEILRMAEELNLEKEVVRVWFCNRRQREKRVKTSLNQSLFPISKEHLECR  291
4-Churra da Terra (Portugal ) SQEILRMAEELNLEKEVVRVWFCNRRQREKRVKTSLNQSLFPISKEHLECR  291
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*G(Glycine), A(Alanine), V(Valine), L(Leucine), I(Isoleucine), P(Proline), F(Phenylalanine), Y(Tyrosine), W(Tryptophan), S(Serine), T(Threonine), C(Cystine), M(Methionine), N(Asparagine), Q(Glutamine), D(Aspartate), E(Glutamine), K(Lysine), R(Arginine) and H(Histidine)

** Accession Numbers- Awassi (Iraqi): BBJ06446, Madgyal (India):QAT78108, Rambouillet (spain) :XP_027824081 and Churra da Terra (Portugal): ABD39233

Conclusions

The results showed that the rate of change in the nitrogen bases in the POU1F1 gene for the Awassi breed sheep is low and that the change in the encoded regions (exons) is almost non-existent for different animals. Also, most of the amino acids of POU1F1 protein are similar among most International Breeds

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