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Investigation the Single Nucleotide Polymorphism of KISS 1 gene Associated with Pubertal of Iraq Goat.

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ABSTRACT

In this study, we have reviewed progress regarding the relationship between kiss peptin and puberty. This study was conducted on 25 Iraqi goat (prepubertal and pubertal) provide from January 2016 to January 2017 age of animals ranged from 4 month to the one years. Blood samples from jugular vein (5 ml) with anticoagulant to extraction DNA by intron-kit for PCR technique to estimated the size of band of Kiss-1 gene to PCR-RFLP to limited genotype of Iraqi goat for differentiated between prepubertal and pubertaland these analysis was doing in biotechnology department / Al-Nahrain University. The results in this study was homozygous CC377bp,TT with fragments digested at 256 and 121 bp and TC377, 256 and 121 bpgenotype of intron 1 of *KISS1* geneas well as single nucleotide polymorphismT/C in located 98 and the Finallythe result show high significant in FSH and estrogen hormone when compared prepubertal Iraqi Goat with pubertal(P<0.0001) . In conclusion of this study RFLP-PCR of KISS 1prepubertalIraqi Goat was highly significant P<0.01 compared with pubertal

Keyword: Pubertal, Fertility, KISS 1, Single nucleotide polymorphisms.

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INTRODUCTION

The onset of estrous cycles in goat is the increase in luteinizing hormone (LH) release frequency during the period of prepubertal (Huffman et al., 1987;Eblinget al., 1990)**.**In puberty can be characterized by release of GnRH into the hypothalamic frequent pulsatile, which in turn stimulates synthesis and release of gonadotropins from the pituitary (Ojeda and Skinner, 2006;Plant andWitchel, 2006)**.** Nutrition has been demonstrated to have a major effect on timing the onset of puberty in mammals(KrasnowaandSteinera, 2006)**.**In addition, the onset of puberty is largely influenced by the availability of food, as undernutrition delays the pubertal increase in the frequency of GnRH/LH release (Anson et al., 2000)**.***KISS1* of puberty onset is a regulator (Teles et al., 2010; Nimri et al., 2011) .The expression of the *Kiss1* gene is regulated by estrogen(Franceschiniet al., 2006).Also, kisspeptins are reported as regulators for luteinizing hormone (L.H) and folliclestimulating hormone (F.S.H) secretion in different species of mammals (Gottschet al., 2004).

MATERIAL AND METHODS

This study was conducted out on 25 healthy goat cross breed their ages ranged from 4 month to one years and weighted about (10-25 kg). The same animals divided into two groups, prepubertal and pubertal (beginning of the cycle).One blood sample was collected weekly for about 12 months from the jugular vein and the serum harvested was stored at 20C until assayed. use Sheep E2 estradiol (E2) Elisa kit (Catalog Number: MBS742826) and Sheep Follicle Stimulating Hormone (FSH) ELISA Kit (Cat.No: MBS014375), a serum separator tube and allow samples to (incubates) clot for 2 hours at room temperature. Centrifuge at approximately (3000 rpm) for 15 minutes. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C.The whole blood samples were collected from 25animals goat. Plasma was collected and stored at -20o C until processed for determining concentrations of estrogen and FSH. Genomic DNA was extracted from the whole blood according to the method of intron catkit. The primers *KISS1* gene (An et al., 2013a) F: CCC GCT GTA ACT AGA GAA AG; R: CAT CCA GGG TGA GTG ATA CT were lyophilized, they dissolved in the free ddH2O to give a final concentration of 100 pmol/µl, investigated by IDT (Integrated DNA Technologies company, Canada). The PCR products were separated by 2% agarose gel electrophoresis The reaction was run of PCR program at 94°C for 5 min, 35 cycles of 95°C for 1 min, 56°C for 1 min, 72°C for 1 min and a final extension at 72°C for 5 min.The RFLP-PCR technique bydigestedof PCR product by using restriction enzyme; *Xmn*I(cat no# R0194s/ NEB/USA).

RESULTS AND DISCUSSION

KISS1plays decisive permissive role in controlling the onset from puberty by regulating the release of gonadotrophin-releasing hormone (GnRH) of hypothalamic neurons (Tassigny et al., 2007; Smith et al., 2007).In a perspective of the significance of KISS1 as a regulator of puberty onset, there is a hypothesis that the polymorphisms of KISS1 have a few relationships in goat (Cao et al., 2010). Some studies from the KISS1 gene as a candidate gene for reproductive traits in animals, which directory that the KISS1 gene plays an important part in animal reproduction (Tomikawa et al., 2010).We pointed in this study to identify RFLP and SNP polymorphisms of KISS1 gene in Iraq goat. The primers used in this study flanked a 377 bp fragment from intron 1 of KISS1 gene in goat and goat. The amplified fragments sections acquired from all tested sheep and goat animals were at 377bp (Fig1). These PCR amplified fragments (377bp) were digested with *Xmn*I endonuclease. Relyon the presence or absence of the restriction site (GAANN^NNTTC) (N = A or T or C or G) at position 121^122, we can easily differentiate between 3 different genotypes: CC with undigested fragment at 377bp, TT with digested fragments at 256 and 121bpand TC with digested fragments at 377, 256 and 121bp. The results showed the presence of three genotypes; CC, TC and TT genotype in 25 animals for this gene (Fig 2). The mean value ± SE of FSH hormone concentration according the genotyping as shown respectively in (Table 1). The Diagram of peak nucleotide KISS1gene appeared location of single nucleotide polymorphism, CC and TT homozygote, and C/T heterozygote as seen in (Fig4) which were detected in this study declared the presence of one SNP substitution (T→C) at position98 in the amplified fragments of goat and goat *KISS1* gene (Fig 3) which is responsible for the elimination of the restriction site GAACT^TCTTC and consequently the appearance of two different alleles T and C. An et al., (2013a, b) detected polymorphisms of the goatKISS1 gene in three Chinese goat breeds utilizing PCR-RFLP and DNA sequencing techniques. SNPs were distinguished in the intron 1 of the KISS1 gene. The 2270 C>T SNPs were significantly higher associated with litter size where the combined alleles of T in both loci with greater litter size than the concerted alleles of C. On the other hand, Cao *et al*. (14)utilized three pairs of primers to clone the goat KISS1 and scan polymorphisms and four pairs to detect polymorphisms in sexual precocious and sexual late maturing goat breeds. The genotype distribution did not show demonstrate difference between sexual precocious and sexual late-maturing goat breeds and no consistency inside the sexual late-maturing breeds. This study preliminarily indicated an association between allele C in KISS1 gene and high litter size in goats. Chu *et al*. (2012) analyzed SNPs in exon 1 of *KISS1* gene in high fecundity sheep , Polymorphisms inexon 1 of *KISS1* gene were detected in prolific sheep (AA, AB and BB genotypes) ,no polymorphism was found in low fruitfulness sheep breeds (just AA genotype). These outcomes preliminarily demonstrated that the KISS1 gene may have a few relationship with productivity in sheep. Our results matches with the past outcomes got by (11 and 16), where they contemplated the hereditary polymorphism of KISS1 quality in three goats breeds and recorded the association relationship of T→A substitution with the litter size. They reported in prepubertal and pubertal of Iraqi goat. These frequencies very close to the frequencies of T and C alleles in our animals.The mean value ± SE ofhormone Estrogenin prepubertal and pubertal as seen respectively in (Table 2). While Standard curveof FSHand estrogen as shown in (Fig 5).It is the time when estrus isfor first time followed by characteristic ovarian movement and ovulation in female shown (Snyman, 2010; Haliu*et al*., 2006; Greyling, 2000). Puberty is generally considered to berelated more to growth and body weight rather thanage in tropical goats (Bushara and Abu-Nikhaila, 2012, Delgadillo*et al*., 2007, Zeshmarani*et al*., 2007; Sodiq*et al.,* 2002). Generally breeding may bedelayed until the animal has attained 60 to 70% of itsadult body weight (Devendra, 2007; Grayling, 2010).

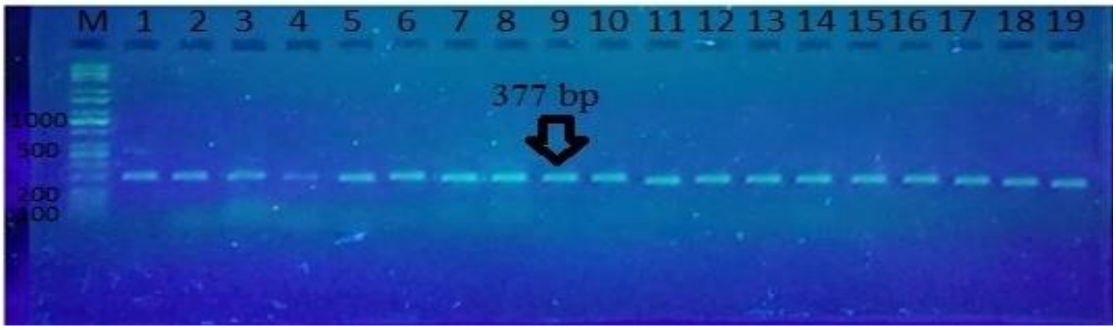

Figure 1: *KISS1*gene in Iraqi goat by PCR technique which revealed size of band 377 bp with red stain safe, resolved by (2%) agarose gel electrophoresis (1.5 hr/70v), Lane M, DNA molecular weight marker.

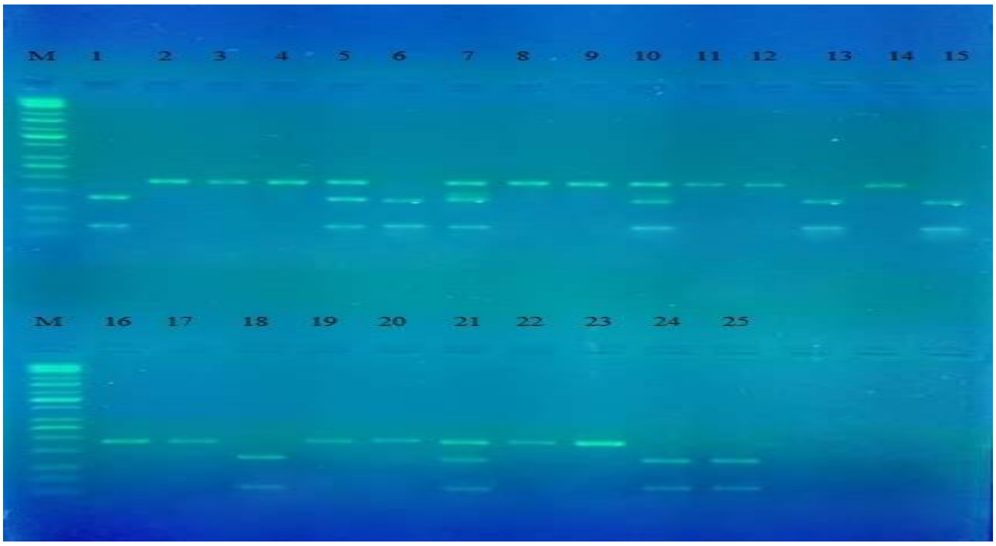

Figure 2: PCR-RFLP technique observed *the PCR products of the KISS1 gene after Xmn*I *enzyme digestion and electrophoresis at 4% agarose gel (2.5 hr/70v).Show lane :(1,613,15,18,24,25) homozygous (TT-256bp,121pb) and lane (2,3,4,8,9,11,12,14,16,17,19,20,22,23) homozygouse (CC-377bp), and lane (5,7,10,21) heterozygous (CT-377bp,256bp,121bp)* in Iraqi Goat.Capra hircuskisspeptin (KISS1) gene, KISS1-T allele, intron 1

Sequence ID: [KP835799.1]

Score	Expect	Identities	Gaps	Strand
392 bits(212)	2e-105	212/212(100%)	0/212(0%)	Plus/Plus

```
Query  61   ACTTCTTCTCTCCTGGGATCGGGTGCTCTTTCTGGGTAAGGGAGGATCCCCCGGAGAATA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  119  ACTTCTTCTCTCCTGGGATCGGGTGCTCTTTCTGGGTAAGGGAGGATCCCCCGGAGAATA  178
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Figure 3: Alignment of nucleotide sequencingCaprahircuskisspeptin (KISS1) genewithgene bank of NCBI, and appeared compatibility of gene under Sequence ID: KP835799.1

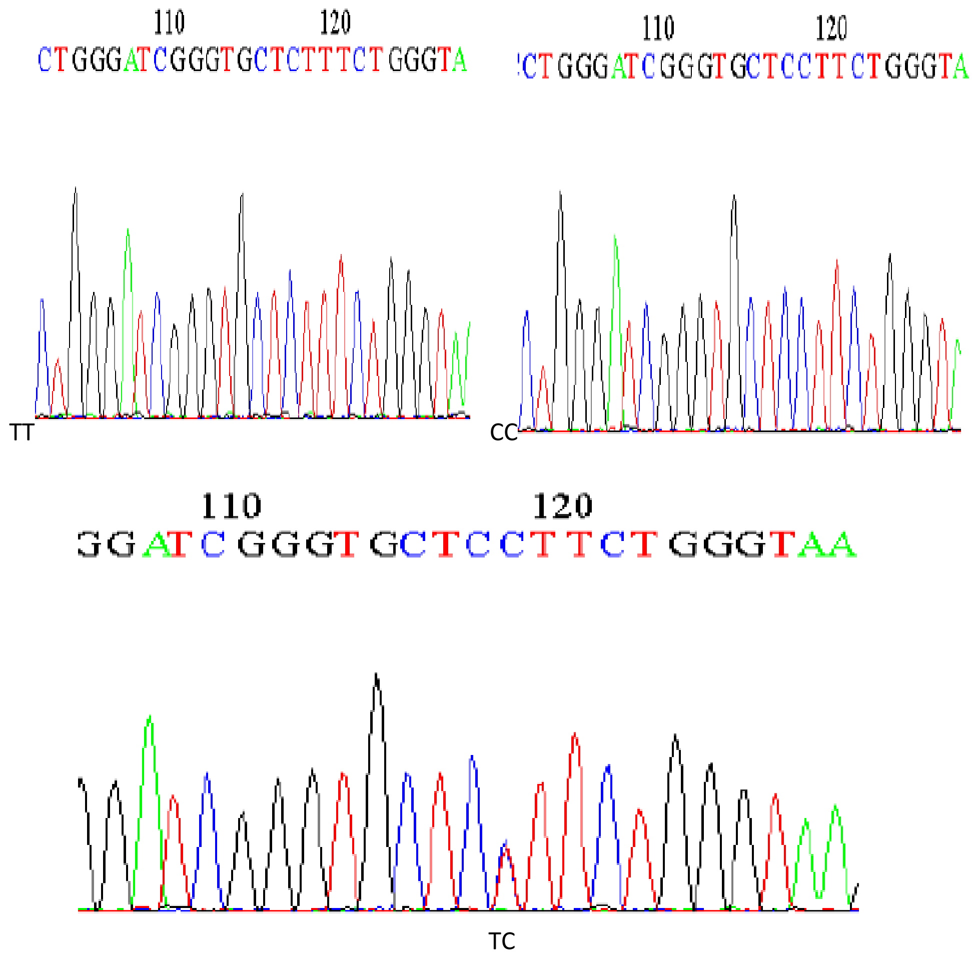

Figure (4): Diagram of peak nucleotide KISS1gene appeared location of single nucleotide polymorphism, chromatogram representing two Genotype, the App arrow is a C/C and TT homozygote, and the down arrow is a C/T heterozygote.

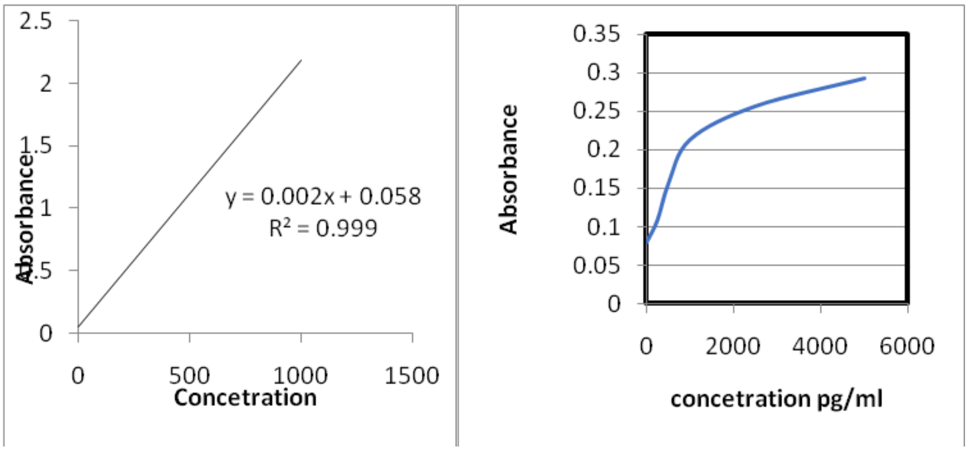

Figure (5): Standard curveof FSH(left graphic) and estrogen (Right graphic).

The mean value ± SE of FSH hormone as shown in table (1). The results show a significant changes in 3 months when comparison between allele frequency TT and allele frequency TC , as that show a significant changes when comparison between allele frequency TT and allele frequency CC , on the other hand allele frequency TC shows a non- significant with allele frequency CC. While the results show a significant changes in 9 months when comparison between allele frequency TT and allele frequency TC , as that show a significant changes when comparison between allele frequency TC and allele frequency CC , on the other hand allele frequency TT shows a non- significant with allele frequency CC. the result show high significant in FSH hormone when comparedprepubertal(3 months)Iraqi Goat with pubertal(9 months) ($P<0.0001$)

Table (1): the distribution of FSH hormone concentration according the genotyping

3 months (mean ± SE)				9 months (mean ± SE)				*P*
TT	TC	CC	Total	TT	TC	CC	Total	
10.2 ± 4.3^{B}	23.0 ± 4.9^{A}	18.8 ± 3.4^{A}	19.1 ± 2.6	58.0 ± 10.5^{A}	36.2 ± 8.6^{B}	50.5 ± 5.1^{A}	46.8 ± 4.3	0.0001

P: Two tailed probability

The mean value ± SE of Estrogen hormone as shown in table (2). The results show a significant changes in 3 months when comparison between allele frequency TT and allele frequency TC , as that show a significant changes when comparison between allele frequency TT and allele frequency CC , on the other hand allele frequency TC shows a significant with allele frequency CC. While the results show a significant changes in 9 months when comparison between allele frequency TT and allele frequency TC , as that show a significant changes when comparison between allele frequency TC and allele frequency CC , on the other hand allele frequency TT shows a significant with allele frequency CC. the result show high significant in Estrogen hormone when compared prepubertal(3 months) Iraqi Goat with pubertal(9 months) ($P<0.0001$)

Table (2): the distribution of Estrogen hormone concentration according the genotyping

3 months (mean ± SE)				9 months (mean ± SE)				*P*
TT	TC	CC	Total	TT	TC	CC	Total	
1.8 ± 0.7^{C}	7.6 ± 1.0^{A}	5.0 ± 0.7^{B}	5.5 ± 0.6	12.8 ± 1.8^{B}	13.6 ± 0.6^{A}	12.3 ± 0.9^{AB}	12.4 ± 0.6	0.0001

P: Two tailed probability

CONCLUSION

The KISS1 quality polymorphism has been appeared to impact the beginning of conceptive movement and litter size in the goat breed. What's more, this polymorphism affect the reproductive response. The analysis from the other exons of the *KISS1* gene not only may provide additional information to clarify their role on the trigger of puberty and on seasonal reproduction in goat, but it can also stimulate further research.

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