OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

Effect of *Leptin* Gene Polymorphism on Reproductive Efficiency in Awassi Ewes

Laith Sofian Younis[1*], Hayder Abd Alkarem Al-Mutar[2], Ali Aziz Abid[1]

[1]College of Veterinary Medicine, Tikrit University, Iraq; [2]College of Veterinary Medicine, Baghdad University, Iraq.

Abstract | *LEP*tin (*LEP*) is a hormone that strongly associate with nutritional state, glucose homeostasis and reproduction. Study performed to identify the linkage between *LEP* polymorphism and reproductive efficiency such as *Seasonal*ity and litter size. Forty mature non-pregnant Awassi ewes were utilized between 1st July/2017 to 1st May/2018 in Salah Aldin province/Iraq. Twenty ewes were demonstrated estrus heat at August/2017which considered *Seasonal* group, and the others showed estrus signs at April/2018, which considered *Non-Seasonal* group.Genomic DNA was extracted from blood specimens and four primers were utilized to amplify *exon* II, *intron* II (fragment 1) and *exon* III (fragment 1) of *LEP* geneby polymerase chain reaction (PCR). Polymorphisms were revealed via sequencing and compared with the sequencing of the ovine *LEP* gene in NCBI. One single nucleotide polymorphism (SNP) A(99)R was detected in *intron* II and three SNPs G(425)R, T(541)K and G(587)R were in *exon* III. Two genotypes of each SNP were observed with higher significant differences (P<0.01) between frequencies 47.50 and 52.50 for AA and AG of A(99)R, 42.50 and 57.50 for GG and GA of G(425)R, 55.00 and 45.00 for TT and TC of T(541)K, lastly, 37.50, 62.50 for GG and GA of G(587)R. The finding demonstrated AG genotypic frequency of *Non-Seasonal* ewes (55.00) was significantly increased (P<0.05) than AA (45.00) for A(99)R SNPs. The mutant GA, TC and GA genotypic frequencies (80.00, 40.00 and 85.00) were recorded higher significant increased (P<0.01) than wild genotype GG, TT and GG (20.00, 60.00 and 15.00) for G(425)R, T(541)K and G(587)R SNPs in *Non-Seasonal* group. Higher significant increased (P<0.01) were observed in GG, TT and GG genotypic frequencies (65.00, 70.00 and 60.00) than GA, TC and GA (35.00, 30.00 and 40.00) for G(425)R, T(541)K and G(587)R SNPs respectively in *Seasonal* group, while non-significant differences were observed between AA and AG (50.00 for each) genotypic frequency for A(99) R SNPs of *Seasonal* group. Non-significant differences in litter size were recorded between GA (1.39) and GG (1.29) for G(425)R and between GG (1.4) and GA (1.32) for G(587)R, while significant differences (P<0.05) were observed between AG (1.26) and AA (1.42) and between TT (1.18) and TC (1.55) for A(99)R and T(541)K respectively. In Awassi breed, *exon* III polymorphisms of *LEP* gene have an expected effect on the *Seasonal*ity and the wild genotypes find majorly in *Seasonal* ewes. *Intron* II and *exon* III polymorphisms (2 SNPs) caused an increment in litter size.

Keywords | *LEPtin*, Litter Size, Polymorphism, Reproduction, *Seasonal*ity.

Editor | Kuldeep Dhama, Indian Veterinary Research Institute, Uttar Pradesh, India.
Received | August 07, 2018; **Accepted** | October 04, 2018; **Published** | November 02, 2018
***Correspondence** | Laith Sofian Younis, College of Veterinary Medicine, Tikrit University, Iraq; **Email**: laith.vet89@tu.edu.iq
Citation | Younis LS, Al-Mutar HAA, Abid AA (2019). Effect of *leptin* gene polymorphism on reproductive efficiency in awassi ewes. Adv. Anim. Vet. Sci. 7(1): 17-23.
DOI | http://dx.doi.org/10.17582/journal.aavs/2019/7.1.17.23
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

LEPtin (*LEP*) is a protein release mostly from adipose tissue (the major source of *LEP*) (Houseknecht et al., 1998). It consists of 146 amino acids with molecular weight 16000 Dalton (Zhang et al., 1994). Chromosome four is containing the ovine *LEP* gene (Moravcikova et al., 2012). It's included two *intron*s and three *exon*s, the last two *exon*s are involved in *LEP* protein synthesis (167 amino acids) which is undergo cleavage of 21 amino acid (signal peptide) (De La et al., 1996; Zhang et al., 1994).

LEPtin receptors are categorized as class one cytokine receptors because of bilaterally symmetrical with a structure

of IL-6 receptors (Houseknecht and Portocarrero, 1998). Because the *LEP* mRNA receptors are extensively express in arcuate nuclei, ventromedial hypothalamus and adenohypophysis of ewes, *LEP* act directly on brain and pituitary levels to promote and coordinate gonadotropin (FSH/*LH*) secretion (Dyer et al., 1997). The *LEP* infusion cause restore *Kiss1* mRNA expression of *kisspeptin* neurons in an Arcuate nucleus and Preoptic area for poorly nourished ewes, and *kisspeptin* regulate brain control of reproduction by a reduction in *proopiomelanocortin* (*POMC*) and raised *neuropeptide Y* (*NPY*) gene expression (Backholer et al., 2010).

In sheep; *LEP* blood circulating level is correlate with nutritional levels (Marie et al., 2001), feeding value (Blache et al., 2000) and condition of fat mass (Delavaud et al., 2000). The main role of *LEP* is regulating *GnRH* and prevents reduction of pulsatile *LH* throughout fasting (Nagatani et al., 1998) because *GnRH* neurons affect directly by *LEP* (Sullivan and Meonter, 2004). A severe feed restriction for 48 h caused an apparent decline in *LEP* secretion concurrent with *LH* drooping in cow (Amstalden et al., 2000) and ewes (Henry et al., 2001; Morrison et al., 2001). Continual feed deprivation for seven days gives rise to decline both of serum *LEP* and *LH* ovariectomized (OVX) young sow (Whisnant and Harrell, 2002).

In fasted cows; *LEP* treatments stimulate adenohypophyseal *LH* secretion mediated by basal *GnRH* (Amstalden et al., 2003). Furthermore, *LEP* blocks the pulsating reduction of *LH* and promote *GnRH* secretion in heifers (Maciel et al., 2004). Moreover, sexual immaturity is correlating with low *LEP* levels (El-Eshmawy and Aal, 2010).

In ewes, *LH* secretion stimulated by intra cerebro ventricular (ICV) *LEP* injection in feed restricted OVX ewe (Henry et al., 2001). In spite of the fact that chronic ICV administration of *LEP* failed to stimulate *LH* secretion in well-fed ewes (Henry et al., 1999), the *LEP* injection caused rise of *LH* blood concentration and non-significantly enhance blood FSH level in feed deprivation; these facts give an indication about role of *LEP* as a metabolic signal on *GnRH-LH/FSH axis* in feed-limited ewes (Towhidi et al., 2007).

Several genetic SNPs contributed with *Seasonal*ity in sheep; *aryl alkyl amine-N-acetyl-transferase* gene (AA-NAT) and melatonin receptor 1A gene polymorphism correlated with ewes that breeding out of season (Giantsis et al., 2016; Hatif and Younis, 2018). As well as, *GDF9* gene SNPs in Araucana creole sheep breed linked with litter size (Bravo et al., 2016). In sow, *LEP* receptor gene polymorphism record increment in litter size for Yorkshire and Duroc breed (Chen et al., 2004a).

Numerous studies pointed out about the role of *LEP* gene polymorphisms and growth parameters in different domestic animals; dairy cattle production quality (Nkrumah et al., 2004), sheep muscles growth and meat features (Boucher et al., 2006), body weight and growth traits (Shojaei et al., 2011).

Because the role of *LEP* on *GnRH*/*LH* and since the available information regarding the effect of polymorphism of *LEP* gene on sheep reproduction aspect and (*hypothalamic-pituitary-gonadal axis*) is insufficient, therefore, the existing study was carried out to identify polymorphisms in ovine *LEP* gene and their possible association with the time of estrus cycle and litter size in Iraqi Awassi ewes.

MATERIAL AND METHODS

Animals Management and Samples Collection

Three ml blood specimens were collected randomly during April from forty mature multiparous Awassi ewes with average age three years in Salah Aldin province. The animals were disconnected into two categories depending on breeding season; about 20 of ewes showed estrus at august/2017 (*Seasonal* ewes) (group 1) and the rest were demonstrated estrus cycle at April /2018 (*Non-Seasonal* ewes) (group 2). At all time; animals were housed in one flock with a breeding ram in the animal house of veterinary college/Tikrit university; that locate in latitude 34 and longitude 43. The blood samples were septically aspirated from vena puncture of jugular vein into vitamin K containing collection tube (APTACA/Italy) and stored at -20°C for DNA extraction, amplification and sequencing of *exon* II and III of *LEP* gene.

Genomic DNA was extracted by utilized G-spin Kit (*Intron*/ Korea) according to manufacturer's protocol. Four Primers were designed based on genomic sequences of sheep (Genbank, AF310264, and AY831682) and (GenBank, U84247, and AY911719) according to Boucher et al. (2006). The sequence of primer 1 forward: 5´-CGCAAGGTCCAGGATGACACC-3´; and primer 1 reverse: 5´-GTCTGGGAGGGAGGAGAGTGA-3´ that's amplified part of *exon* II and *intron* II (fragment 1), the sequence of primer 2 forward: 5'CTCTTGATGTCCCCTTCCTC-3' and primer 2 reverse: 5' TGGTCCTTCGAGATCCATTC-3' that's designed to amplified all *exon* III and part of the 3′ UTR of the gene (fragment 2) (Mahmoud et al., 2014). The total volume of reactions were 25 µl; that containing 2 ul of genomic DNA, 1 µl of each primer (10 pM), PCR Master Mix Kit (*Intron*/ Korea) 12.5 ul, and nuclease-free water 8.5 ul in 30 cycles for fragment 1 and 2 (initial denaturation: 95°C for 5 min, denaturation: 95°C for 30s, annealing: 60°C for 30s for fragment 1 and 54 °C for 30 sec for fragment 2, extension: 72°C for 30s and final extension 72°C for 7 min).

Table 1: The SNPs location, number, nucleotide and changes of amino acidsfor Awassi ewes

	Location Of SNP	SNP Number	Nucleotide Change	Amino Acid Change & Number	Predicted Effect	Type of Mutation
1	A(99)R (*Intron* II)	Rs408463464	TAG>TAG& TAA			
2	G(425)R (*Exon* III)	Rs409584889	CGG> CAG& CGG	Arginine > Glutamine & Arginine (142)	Transition	Missense
3	T(541)K (*Exon* III)	Rs420693815	TTG> GTG& TTG	Leucine>Valine & Leucine(181)	Trasversion	Missense
4	G(587)R (*Exon* III)	Rs428185456	CGG> CAG& CGG	Arginine > Glutamine & Arginine (196)	Transition	Missense

Table 2: The observed genotypic and allele frequencies of *LEP* gene for Awassi ewes

No	Locus	Genotypes	Observed Genotypes	Genotypic Frequency %	Allele Frequency		Chi Square
1	A(99)R	AA	19	47.50	A	73.75	11.72 **
		AG	21	52.50	G	26.25	
2	G(425)R	GG	17	42.50	G	71.25	11.55 **
		GA	23	57.50	A	28.75	
3	T(541)K	TT	22	55.00	T	77.5	12.42 **
		TC	18	45.00	C	22.5	
4	G(587)R	GG	15	37.50	G	68.75	10.09 **
		GA	25	62.50	A	31.25	

** (P<0.01).

The DNA bands were detected by placing ethidium bromide staining gel electrophoresis in Trans illuminator (*Vilberlourmat*/ France).

Amplicons of *LEP* gene were sequenced separately by Macrogen Corporation/ Korea. The *LEP* sequences were edited and aligned by utilizing (BioEdit software). The samples homology done by applied BLAST option in NCBI GenBank database and Bioedit program.

Statistical Analysis

Statistical Analysis System - SAS (2012) program was utilized to monitor the effect of different factors in study parameters. T-test and Chi-square test were usedto significant compared between means and between percentages respectively in this study.

RESULTS AND DISCUSSION

PCR Amplification

Two primers that amplified particular regions (part of *exon*II and part of *intron*II) of *LEP* gene of Awassi ewes. The PCR amplified size was 260 bp. In addition to that, another twoprimers were amplified of *exon*III and part of 3′ UTR and the uniform fragments with size 1090 bp appeared after electrophoresed in 1% agarose gel (Figure 1).

Sequencing and Genetic Variability

The sequencing result revealed that one SNP in *Intron* II A(99)R and three SNPs in *exon* III (G(425)R, T(541)K

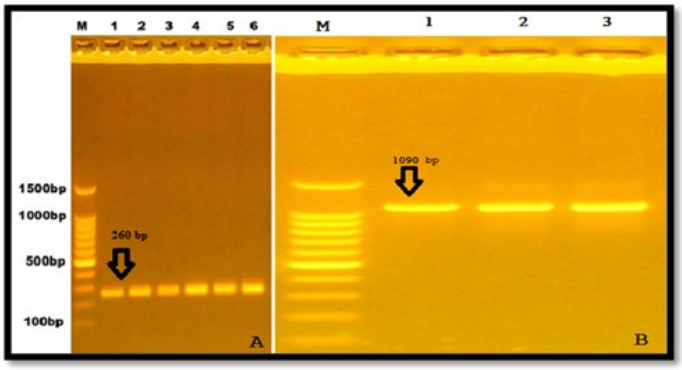

Figure 1: Results of the presence of *LEP* gene of samples were fractionated on 1% agarose gel electrophoresis stained with ethidium bromide Lane1:100bp DNA marker A. *Exon* II& *intron* II, B. *Exon* III.

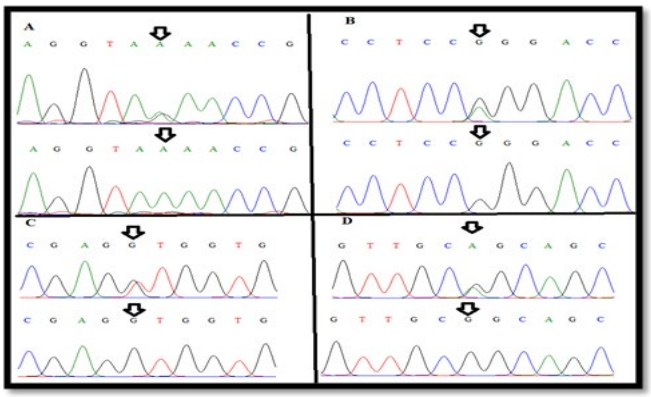

Figure 2: The wild-type and new variant (A) A(99)R , (B) G(425)R (C), (C) T(541)K and (D) G(587)R of *exon*II, *intron*II and *exon*III of *LEP* gene

Table 3: Chi- square of genotypic distribution of *LEP* gene in*Non-Seasonal* and *Seasonal* ewes.

No	Locus	Genotypes	Genotypic Frequency for Non-Seasonal Ewes	Chi- Square	Genotypic Frequency for Seasonal Ewes	Chi- Square
1	A(99)R	AA	9 (45%)	4.57 *	10 (50%)	0.00 NS
		AG	11 (55%)		10 (50%)	
2	G(425)R	GG	4 (20%)	13.86 **	13 (65%)	9.87 **
		GA	16 (80%)		7 (35%)	
3	T(541)K	TT	8 (40%)	7.25 **	14 (70%)	10.33 **
		TC	12 (60%)		6 (30%)	
4	G(587)R	GG	3 (15%)	13 .46 **	12 (60%)	7.25 **
		GA	17 (85%)		8 (40%)	

* (P<0.05), ** (P<0.01).

Table 4: T- test of the litter size for the *LEP* genotypes of Awassi ewes

	Locus	Genotypes	Ewe Number	Lambs Number	Litter Size	T- Test
1	A(99)R	AA	19	24	1.26	0.128 *
		AG	21	30	1.42	
2	G(425)R	GG	17	22	1.29	0.115 NS
		GA	23	32	1.39	
3	T(541)K	TT	22	26	1.18	0.196 *
		TC	18	28	1.55	
4	G(587)R	GG	15	21	1.4	0.107 NS
		GA	25	33	1.32	

* (P<0.05).

and G(587)R) of *LEP* gene in Awassi ewes (Figure 2). All these SNPs were recorded in NCBI and Ensembl gene browser (rs408463464, rs409584889, rs420693815 and rs428185456 respectively). The last three SNPs of coding region are missense mutation that changed amino acid to another; Arginine > Glutamine, Leucine> Valine and Arginine > Glutamine in position 142, 181 and 196 respectively (Table 1).

Significant differences were recorded between the genetic variability of *LEP* gene. Higher significant variation (P<0.001) were recorded between the (AA and AG), (GG and GA), (TT and TC) and (GG and GA) genotypes of A(99)R, G(425)R, T(541)K and G(587)R locus respectively, while GG, AA, CC and AA genotypes didn't noticed in these populations of the same locus (Table 2).These amino acids changes are impacted in *LEP* hormone function. These are in agreement with the Zhou et al. (2009) finding, which hypothesized that diversity of *LEP* gene might have an influence on *LEP* activity and function.

Correlation between Genotypes and Breeding Season in Awassi Sheep

The genotypic distributions of *LEP* gene in the two animal groups were recorded. In *Non-Seasonal* Awassi ewes group higher significantly increased (P<0.001) were showed in mutant genotypes;GA, TC and GA as compared with the wild genotypes; GG, TT and GG of G(425)R, T(541)K and G(587)R locus, also AG genotype of A(99)R locus recorded significantly increased (P<0.05) when compared with AA genotype . On the other hand, higher significantly increased (P<0.001) were recorded in wild genotypes; GG, TT and GG in comparison with other genotypes (GA, TC and GA) of G(425)R, T(541)K and G(587)R locus respectively, while non-significant differences were recorded between AG and AA genotypes for A(99)R locus for *Seasonal* Awassi ewes group (Table 3).

This finding leads to speculate that the mutations has affected on the final form of *LEP* protein and caused increasing its function in ewes that breed off season. Only a few studies have been performed to detect the relationship between polymorphism of *LEP* gene and ovine reproductive performance; Bravo et al. (2016) find a relationship between *LEP* gene polymorphism and litter size. When compared the sequence of *exon* III for the Najdi breed (ID: KF922846.1), the GG, TT and GG genotypic frequency in (G(425)R, T(541)K and G(587)R were present, while AG, TC and GA genotypes (mutant alleles) of the same locus were absent, the Najdi breed has shorter lambing interval compared with the Awassi breed (330 vs 286 days) (Abdelqader et al., 2012), that means the *Non-Seasonal* Awassi group has a longer breeding season than Najdi breed.

The *LEP* hormone is a metabolic factor that has an important role in up-regulation *GnRH* and *LH* output by an effect on *kisspeptin*, *POMC* and *NPY* expression in the arcuate nucleus and preoptic area of ewe's hypothalamus (*LEP* receptors are expressed in *kisspeptin* cells) (Backholer et al., 2010). Therefore, the SNPs in *LEP* gene were strongly contributed to reproduction by an effect on *kisspeptin* secretion because the missense mutation make structural changes in protein conformation and that may enhance *LEP* protein function. These observations come constantly with several studies that revealed the polymorphisms in *exon* III of *LEP* gene were contributed with body weight and growth rate in Kermani sheep (Shojaei et al., 2011) and in Baluchi sheep (Tahmoorespur et al., 2010). These facts find out that *LEP* gene polymorphism have a positive influence of reproduction through its effect on fat deposition because the adipose tissue is the central source of *LEP*.This hypothesis was accord with Buchanan et al. (2002) study; which showed that *LEP* gene polymorphism contributed to increased expression of mRNA *LEP* gene. These event lead to in plasma *LEP* elevation (Liefers et al., 2003), and when *LEP* level increased, the *LH* is also increased. These argue was agreement with Henry et al. (2001), who mentioned that the *LEP* cerebral injection elevated of *LH* secretion. Also, *GnRH* secretion is indirectly potentiated and regulated by *LEP* because itacts as a metabolic factor (Burcelin et al., 2003; Quennell et al., 2009).

Correlation between Genotypes and Litter Size in Awassi Sheep

The results of present study indicates that *LEP* gene contribute to the ovulation rate and subsequently litter size. The finding appeared that *LEP* AG and TC genotypes had significantly raised ($P<0.05$) in litter size when compared with AA and TT genotypes ofA(99)R and T(541)K respectively. Non-significant differences were noticed between each genotype of G(425)R and G(587)R (Table 4).

Litter size is correlated with ovulation rate, the finding of present study showed variance between SNPs and genotypes; non-significant differences in litter size were noticed between the genotypes of each (G(425)R and G(587)R, while the genotypes of in locus in A(99)R *intron* II and the genotypes of T(541)K locus in*exon* III were recorded significant differences that reflect the mediated effect of *LEP* gene polymorphism on litter size, these outcomes have not been described previously in sheep. In spite the fact that mRNA of *LEP* receptor not expressed in ovine granulosa and the ca cells (Spicer, 2001), but in this study the polymorphisms recorded increase in ovulation rate. These results accord with Chen et al. (2004b) outcomes, which find that the litter size was increased significantly in mutant allele of porcine *LEP* gene. As mentioned above; *LEP* increases *GnRH* and *LH* in ewes which reflected positively on ovulation rate (litter size). Additionally, according to Lagonigro et al. (2003), theincrease fat deposition and feed intake occurs because *LEP* gene polymorphism andthat explain the effect of polymorphism on enhancement the litter size. This speculation was corresponded with Kara et al. (2010), who mentioned that improving feed intake had a positive effect on reproduction and litter size Awassi ewes.

Taking into consideration the vast range of physiological *LEP* impact, it is expected that several phenotypic features have been detected with *LEP* gene SNPs. As a conclusion, heterozygote *LEP* gene polymorphisms of *exon* III were related with Awassi ewes that breeding out of season, while wild genotypic frequencies were higher in *Seasonal* Awassi ewes. Litter size was correlated with two SNPs, in *intron* II and *exon* III.

ACKNOWLEDGEMENTS

The authors thank the staff of laboratory of Biotechnology Research Center-Al-Nahrian University/Baghdad and laboratory of veterinary college/Tikrit University. Also special thanks to employers of animal house/veterinary college/Tikrit University.

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

Laith Sofian Younis supervised the project. Both Hayder Alkarem Al-Mutar and Laith Sofian Younis designed and performed the experiments of genotyping, while Ali Aziz Abd wrote the manuscript, inconsultation with Hayder Alkarem Al-Mutar and Laith Sofian Younis. Also Ali Aziz Abd analyzed the data.

REFERENCES

- Abdelqader A, Al Yacoub A, Gauly M (2012). Factors influencing productive longevity of Awassi and Najdi ewes in intensive production systems at arid regions. Small Rum. Res. 104(1): 37-44. https:/doi:org/10.1016/j.smallrumres.2011.11.007.
- Amstalden M, Garcia MR, Williams SW, Stanko RL, Nizielski SE, Morrison CD, Williams GL (2000). *LEP*tin gene expression, circulating *LEP*tin, and luteinizing hormone pulsatility are acutely responsive to short-term fasting in prepubertal heifers: relationships to circulating insulin and insulin-like growth factor I. Biol. Reprod. 63(1):127-133. https:/doi:org/10.1095/biolreprod63.1.127.
- Amstalden M, Zieba DA, Edwards JF, Harms PG, Welsh JT, Stanko RL, Williams GL (2003). *LEP*tin acts at the bovine adenohypophysis to enhance basal and gonadotropin-releasing hormone-mediated release of luteinizing hormone: differential effects are dependent upon

nutritional history. Biol. Reprod. 69(5): 1539-1544. https:/doi:org/10.1095/biolreprod.103.018119.
- Backholer K, Smith JT, Rao A, Pereira A, Iqbal J, Ogawa S, Clarke IJ (2010). *Kisspeptin* cells in the ewe brain respond to *LEP*tin and communicate with *neuropeptide Y* and *proopiomelanocortin* cells. Endocrinol. 151(5): 2233-2243. https:/doi:org/10.1210/en.2009-1190.
- Blache D, Tellam RL, Chagas LM, Blackberry MA, Vercoe PE, Martin GB (2000). Level of nutrition affects *LEP*tin concentrations in plasma and cerebrospinal fluid in sheep. J. Endocrinol. 165(3): 625-637. https:/doi:10.1677/joe.0.1650625.
- Boucher D, Palin MF, Castonguay F, Gariepy C, Pothier F (2006). Detection of polymorphisms in the ovine *LEP* (*LEP*) gene: Association of a single nucleotide polymorphism with muscle growth and meat quality traits. Can. J. Anim. Sci. 86(1): 31-35. https:/doi:org/10.4141/A05-052.
- Bravo S, Larama G, Paz E, Inostroza K, Montaldo H, Sepúlveda N (2016). Polymorphism of the *GDF9* gene associated with litter size in Araucana creole sheep. Anim. Genet. 47: 390-391.
- Buchanan FC, Fitzsimmons CJ, Van AG, Thue TD, Windkelman-Sim DC, Schmutz SM (2002). Association of a missense mutation in the bovine *LEP*tin gene with carcass fat content and *LEP*tin mRNA levels. Genet. Selec. Evol. 34: 105-116. https:/doi:10.1051/gse:2001006
- Burcelin R, Thorens B, Glauser M, Gaillard RC, Pralong FP (2003). Gonadotropin-releasing hormone secretion from hypothalamic neurons: stimulation by insulin and potentiation by *LEP*. Endocrinol. 144(10): 4484-4491. https:/doi:org/10.1210/en.2003-0457.
- Chen CC, Chang T, Su HY (2004a). Characterization of porcine *LEP* receptor polymorphisms and their association with reproduction and production traits. Anim. Biotechnol. 15(1): 89-102. https:/doi:org/10.1081/ABIO-120037903.
- Chen CC, Chang T, Su HY (2004b). Genetic polymorphisms in porcine *LEP* gene and their association with reproduction and production traits. Aust. J. Agri. Res. 55(6): 699-704 https:/doi:org/10.1071/AR03258.
- De La, Brousse FC, Shan B, Chen JL (1996). Identification of the promoter of the mouse obese gene. Proc. Natl. Acad. Sci. 93(9): 4096-4101. https:/doi:org/10.1073/pnas.93.9.4096
- Delavaud C, Bocquier F, Chilliard Y, Keisler DH, Gertler A, Kann G (2000). Plasma *LEP*tin determination in ruminants: effect of nutritional status and body fatness on plasma *LEP*tin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165(2): 519-526. https:/doi:10.1677/joe.0.1650519.
- Dyer CJ, Simmons JM, Matteri RL, Keisler DH (1997). *LEP*tin receptor mRNA is expressed in ewe anterior pituitary and adipose tissues and is differentially expressed in hypothalamic regions of well-fed and feed-restricted ewes. Domest. Anim. Endocrinol. 14(2): 119-128. https:/doi:org/10.1016/S0739-7240(96)00119-1.
- El-Eshmawy MM, Aal IA (2010). Association of ghrelin and *LEP*tin with reproductive hormones in constitutional delay of growth and puberty. Reprod. Biol. Endocrinol. 8(1): 153. https:/doi:org/10.1186/1477-7827-8-153.
- Giantsis IA, Laliotis GP, Stoupa O, Avdi M (2016). Polymorphism of the melatonin receptor 1A (MNTR1A) gene and association with *Seasonal*ity of reproductive activity in a local Greek sheep breed. J. Biol. Res. Thessaloniki 23(1): 9. https:/doi:org/10.1186/s40709-016-0050-y.
- Hatif SA, Younis LS (2018). Effect of *aryl alkyl amine-N-acetyl-transferase* gene polymorphism on melatonin in *Non-Seasonal* ewes. Onl. J. Vet. Res. 22(5): 356-361.
- Henry BA, Goding JW, Alexander WS, Tilbrook AJ, Canny BJ, Dunshea F, Clarke IJ (1999). Central administration of *LEP*tin to ovariectomized ewes inhibits food intake without affecting the secretion of hormones from the pituitary gland: evidence for a dissociation of effects on appetite and neuroendocrine function. Endocrinol. 140(3): 1175-1182. https:/doi:10.1210/endo.140.3.6604.
- Henry BA, Goding JW, Tilbrook AJ, Dunshea FR, Clarke IJ (2001). Intra cerebro ventricular infusion of *LEP*tin elevates the secretion of luteinising hormone without affecting food intake in long-term food-restricted sheep, but increases growth hormone irrespective of bodyweight. J. Endocrinol. 168(1): 67-77.
- Houseknecht KL, Portocarrero CP (1998). *LEP*tin and its receptors: regulators of whole-body energy homeostasis. Domest. Anim. Endocrinol. 15(6): 457-475. https:/doi:org/10.1016/S0739-7240(98)00035-6.
- Houseknecht KL, Baile CA, Matteri RL, Spurlock ME (1998). The biology of *LEP*tin: a review. J. Anim. Sci. 76(5): 1405-1420.
- Kara C, Orman A, Topal E, Carkungoz E (2010). Effects of supplementary nutrition in Awassi ewes on sexual behaviors and reproductive traits. J. Biol. Environ. Sci. 4(10): 15-21.
- Lagonigro R, Wiener P, Pilla F, Woolliams JA, Williams JL (2003). A new mutation in the coding region of the bovine *LEP* gene associated with feed intake. Anim. Genet. 34:371–374.https:/doi:org/10.1046/j.1365-2052.2003.01028.x.
- Liefers SC, Te Pas MF, Veerkamp RF, Chilliard Y, Delavaud C, Gerritsen R, van der Lende T (2003). Association of *LEP* gene polymorphisms with serum *LEP* concentration in dairy cows. Mammalian Genome. 14(9): 657-663. https:/doi:10.1186/1471-2156-11-73.
- Maciel MN, Zieba DA, Amstalden M, Keisler DH, Neves JP, Williams GL (2004). Chronic administration of recombinant ovine *LEP*tin in growing beef heifers: Effects on secretion of *LH*, metabolic hormones, and timing of puberty 1. J. Anim. Sci. 82(10): 2930-2936. https:/doi:10.2527/2004.82102930x.
- Mahmoud AH, Saleh AA, Almealamah NA, Abou-tarboush FM, Aljumaah RS, Shafey TM, Abouheif MA (2014). Molecular Characterization of *LEP Exon* 3 gene in sheep of Saudi Arabia. Res. J. Biotechnol. 9(7): 32-35.
- Marie M, Findlay PA, Thomas L, Adam CL (2001). Daily pattern of plasma *LEP*tin in sheep: effects of photoperiod and food intake. J. Endocrinol. 170(1): 277-286.
- Moravcikova N, Trakovicka A, Kasarda R (2012). Polymorphism within the *intron* region of the bovine *LEP* gene in Slovak Pinzgau cattle. Anim. Sci. Biotechnol. 45(1): 211-214. https:/doi:10.1134/S1022795408040169.
- Morrison CD, DanielJA, Holmberg BJ, Djiane J, Raver N, Gertler A, Keisler DH (2001). Central infusion of *LEP*tin into well-fed and undernourished ewe lambs: effects on feed intake and serum concentrations of growth hormone and luteinizing hormone. J. Endocrinol. 168(2): 317-324.
- Nagatani S, Guthikonda P, Thompson RC, Tsukamura H, Maeda KI, Foster DL (1998). Evidence for *GnRH* regulation by *LEP*tin: *LEP*tin administration prevents reduced pulsatile *LH* secretion during fasting. Neuroendocrinol. 67(6): 370-376.https:/doi:10.1159/000054335.
- Nkrumah JD, Li C, Basarab JB, Guercio S, Meng Y, Murdoch

B, Moore SS (2004). Association of a single nucleotide polymorphism in the bovine *LEP*tin gene with feed intake, feed efficiency, growth, feeding behaviour, carcass quality and body composition. Canadian J. Anim. Sci. 84(2): 211-219. https:/doi:org/10.4141/A03-033.

- Quennell JH, Mulligan AC, Tups A, Liu X, Phipps SJ, Kemp CJ, Anderson GM (2009). *LEP* indirectly regulates gonadotropin-releasing hormone neuronal function. Endocrinol. 150(6): 2805-2812. https:/doi:10.1210/en.2008-1693.
- SAS (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
- Shojaei M, Mohammad Abadi M, Asadi Fozi M, Dayani O, Khezri A, Akhondi M (2011). Association of growth trait and *LEP* gene polymorphism in Kermani sheep. J. Cell Mol. Res. 2(2): 67-73.
- Spicer LJ (2001). *LEP*: a possible metabolic signal affecting reproduction. Domest. Anim. Endocrinol. 2(2): 67-73.
- Sullivan SD, Moenter SM (2004). -Aminobutyric acid neurons integrate and rapidly transmit permissive and inhibitory metabolic cues to gonadotropin-releasing hormone neurons. Endocrinol. 145(3): 1194-1202. https:/doi:10.1210/en.2003-1374
- Tahmoorespur M, Taheri A, Valeh MV, Saghi DA, Ansary M (2010). Assessment relationship between *LEP* and ghrelin genes polymorphisms and estimated breeding values (EBVs) of growth traits in Baluchi sheep. J. Anim.Vet. Adv. 9(19): 2460-2465. https:/doi:10.3923/javaa.2010.2460.2465.
- Towhidi A, Khazali H, Zhandi M (2007). *LEP*tin is a metabolic signal for *GnRH-LH*/FSH axis in feed-restricted ewes. Asian-Aust. J. Anim. Sci. 20(7): 1039-1048. https:/dio:org/10.5713/ajas.2007.1039 .
- Whisnant CS, Harrell RJ (2002). Effect of short-term feed restriction and refeeding on serum concentrations of *LEP*tin, luteinizing hormone and insulin in ovariectomized gilts. Domest. Anim. Endocrinol. 22(2): 73-80. https:/doi:org/10.1016/S0739-7240(01)00126-6.
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994). Positional cloning of the mouse obese gene and its human homologue. Natr. 372(6505): 425. https:/doi:10.1038/372425a0.
- Zhou H, Hickford JG, Gong H (2009). Identification of allelic polymorphism in the ovine *LEP* gene. Mol. Biotechnol. 41(1): 22-25. https:/doi:10.1007/s12033-008-9090-3.