

Molecular and Physiological Role of BMP 15 Gene Associated with Ovulation Rate and Fertility of Iraq Goat

Hayder Abdul-Kareem Hasan AL-Mutar

Department of Surgery and Obstetrics, College of Veterinary Medicine, Baghdad University,
Baghdad, Iraq

Abstract: In the present study, the investigation ovulation rate and fertility of Iraq goat by BMP-15 gene after estrus synchronization by using 20 mg impregnated sponges with MAP for 12 days with 400 IU PMSG on day before sponges withdrawal. All 25 Iraqi goats showed (100%) estrus after sponge withdrawal. Twinning percentage 57.9% while single percentage 42.1%. The polymorphisms of BMP-15 gene were analyzed as a genetic marker in Iraqi goats have shown that natural mutations in prolific goat breeds in BMP-15 gene mutations are crucial for increasing litter size as well as ovulation rate. FecXR, FecXB and FecXH mutations increased prolificacy in goats, PCR primers were used to polymorphisms in female goats by using PCR-RFLP method. Although, the sequencing data showed four novels polymorphic sites in goat, mutations in base number. 102 G>A, 156 T>G, 207 G>A and 218 G>T were identified, all these mutations were reported and registered in NCBI with Accession No. of JX860305.1. Amino acid change is Arginine>Lysine, Methionine>Arginine, Arginine>Glutamine and Valine>Leucine with Accession No. of AFX69269.1. The conclusion of BMP15 gene may be a major gene which affects the prolificacy in goat and could provide basic molecular data on the reproductive characteristics of Iraq breeds and a scientific basis for the conservation and utilization of goat.

Key words: BMP 15 gene, ovulation rate, fertility, goat, mutations, impregnated

INTRODUCTION

Fertility is the ability of an individual to produce live offspring and also the birth rate of a population refers to fertility (Frank, 2012). In particular, the proteins secreted from oocytes play a vital role in ovulation rate with the follicular growth regulation (Juengel and McNatty, 2005; Kelly Moore *et al.*, 2003; Otsuka *et al.*, 2011). Estrus synchronization is a key element of all protocols and has a major influence to increase the overall efficiencies of these programmes (Baldassarre and Karatzas, 2004). Estrus synchronization plays a major role in fixed time breeding, the value of estrus synchronization is vital in goats as the duration of both estrous cycle and estrus is variable and estrus detection cannot be accomplished safely without a buck (Jainudeen *et al.*, 2000). One limitation of ECG is its long-acting biological activity causing it to continually recruit antral follicles which results in a large number of unovulated follicles particularly when given at dose levels to induce superovulation (Baruselli *et al.*, 2004). BMP15 gene is X-linked expresses in oocytes involved in regulation of granulosa cell proliferation and differentiation by promoting granulosa cell mitosis, suppressing follicle stimulating hormone receptor expression and engaged in

the stimulation of kit ligand expression. The function of gene Bone Morphogenetic protein 15 (BMP-15) is not absolutely appreciated or understood even if gene collaborate to regulate granulosa cells function (McNatty *et al.*, 2005). BMP15 gene has a vital role and necessary for folliculogenesis in sheep. If the same gene carrying two copies of naturally occurring inactivating BMP15 mutations are infertile and the follicular development will be blocked at the primary stage. In sheep it is furthermore clear that if heterozygotes mean carrying inactivating mutation in only one copy of BMP15 gene, whereby the other copy of the gene produces active protein, this situation likely increased ovulation rate (Galloway *et al.*, 2000).

MATERIALS AND METHODS

The present study was conducted on goat breeds. A total of 25 healthy goat range in age from 3-4 years and fertile bucks about 2-4 years with history of single and twinning birth. Estrus detection before treatment and Insemination by natural mating. Treated goats had received intra vaginal sponge which were impregnated with 20 mg of (MAP) and coated with an antiseptic cream. Sponge was left on for 12 days on day 11 all goat were

Table 1: Primer sequences and PCR amplification parameters

Gene BMP-15	Forward and reverse Primers (5'-3')	Amplicon size (bp)	Annealing temperature (°C)	Enzyme	References
FecXR	(forward) 5- CTCTGAGACCAACCGGGTA -3 (reverse) 5- CATGCCACCAGA ACTCAAGA -3	312	55		Hanrahan <i>et al.</i> (2004)
FecXB	(forward) 5- GCCTTCCTGTGTCCCTTATAAGTA TGTTCCCTTA-3 (reverse) 5- TTCTTGGGAAACCTGAGCTAGC-3	154	60	DdeI	Hanrahan <i>et al.</i> (2004)
FecXH	(forward) 5- TATTTCAATGACACTCAGAG-3 (reverse) 5- GAGCAATGATCCAGTGATCCCA-3	250	50	SpeI	Galloway <i>et al.</i> (2002)

injected I.M with 400 I.U PMSG and natural insemination (Baruselli *et al.*, 2004). Five mL blood sample was collected aseptically from jugular vein of selected goat into 50 mL falcon tubes containing 200 µL anticoagulants (0.5 M EDTA). DNA was extracted by using the standard protocol by intron kit procedure. After extraction of genomic DNA, gel electrophoresis was used to detect to the presence and integrity of the extracted DNA and presence of PCR product, three used of primers BMP 15 (Table 1).

Sequencing of BMP15 gene was performed by National Instrumentation Center for Environmental Management (NICEM) online at (http://nicem.snu.ac.kr/main/?en_skin=index.html), Biotechnology Lab, machine DNA sequencer 3730XL, Applied Biosystem), Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (<http://www.ncbi.nlm.nih.gov>) and BioEdit program. PCR-FRFLP method was also used to investigate the FecXB (primer pair B2F/B2R) and FecXH (primer pair B4F/B4R) mutations which includes digestion with restriction enzymes DdeI and SpeI, respectively.

RESULTS AND DISCUSSION

All goats used in this experiment showed no signs of estrous during progesterone treatment till 12 days, post-treatment while 100% of experimental animals were showed estrous signs within 24-66 h. After ECG injected and included plenty of clear viscous mucous vaginal secretion, hyperemia of vaginal mucosa, mild edema of vulva, tail shaking, restlessness, homosexual behavior (riding a goat another in common), does in estrous always seek male finally they accept the ride by the male. The present results agreed with suggestion with other study that, the expressions of estrous behavior was associated with ovulation (Bearden and Fuquay, 2000; Menchaca and Rubianes, 2001; Dogan *et al.* 2008). As well as the manifestation of estrous behavior may vary either the duration or intensity, depending on the breed, age, health status, season and by the presence of males. Fertility result in this study was evaluated by kidding rate which

Table 2: Distribution of study according to pregnant and non-pregnant in does

Animal	Number	Percentage
Pregnant	19	76
Non-pregnant	6	24
Total	25	100

Table 3: Distribution of parturition of pregnant animal according to type of birth in does

Type of birth	Number	Percentage
Twin	11	57.9
Single	8	42.1
Total	19	100

proved that 19 does out of 25 (76 %) gave birth. However, the percentage of does returning to estrus was 6 (24%) (Table 2) which might be attributed to fertilization failure or early embryonic mortality, this finding is in agreement with (Kadhim, 2014) but it disagrees with the finding of (Moaeeen-ud-Din *et al.*, 2008).

The distribution of parturition in pregnant animals according to type of birth showed 11 out of 19 (57.9%) pregnant does had a twin birth while 8 out of 19 (42.1%) had single birth (Table 3).

Thus the conception could increase the percentage of twinning. Similar suggestion was recorded by Dias *et al.* (2001). The present protocol was recommended outside of breeding response by Hashemi *et al.* (2006) to improve ovulation rate (Greyling and Van Niekerk, 1990).

The analysis of BMP-15 gene polymorphism was carried out using PCR method. Genomic DNA of goat was successfully amplified by pair of primer that covers entire coding sequence of BMP-15 gene. Genomic DNA of white blood cells was also used for amplification of BMP-15 gene using PCR specific primers. The amplified fragment which is yielded of single band of the desired product of FecXR, FecXB and FecXH with a molecular weight of 312, 154 and 250 base pair appeared sharp in agarose gel through Gel electrophoreses technique and loaded with (100-1000 bp) DNA ladder (Fig. 1-3). Agarose gel electrophoresis of FecXR did not reveal any size variation within this amplicons in the 204 analysed subjects we always obtained a 312 bp long fragment, instead of 295 bp. Another important genotype of BMP15 gene is FecXB which corresponds to a G/T transversion at nt 1100 of the BMP15 cDNA. No subject carrying this mutation was found in the population analysed.

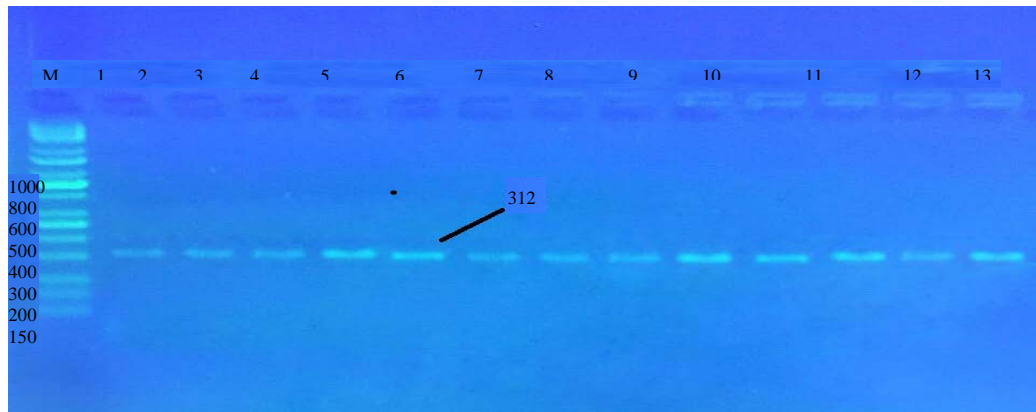


Fig. 1: The product was electrophoresis on 2% agarose gel at 5 Volt/cm², 1×TBE buffer for 2 h. M:DNA ladder (100-10000 bp), Lane 1-13 product for BMP 15 (FecXR) gene of goat, PCR product of band size 312 bp. visualized under U.V light after staining with red stain safe

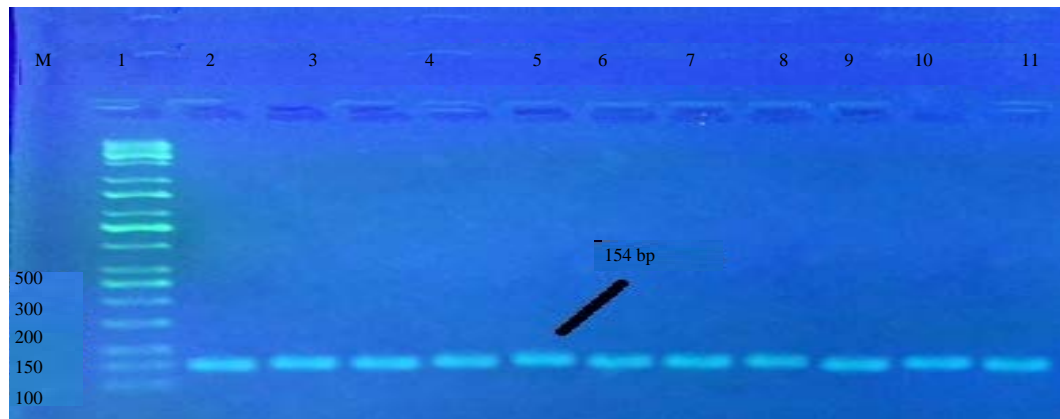


Fig. 2: The product was electrophoresis on 2% agarose gel at 5 Volt/cm², 1×TBE buffer for 2 h. M:DNA ladder (100-10000 bp), Lane 1-11 product for BMP 15 (FecXB) gene of goat, PCR product of band size 154 bp. visualized under U.V light after staining with red stain safe

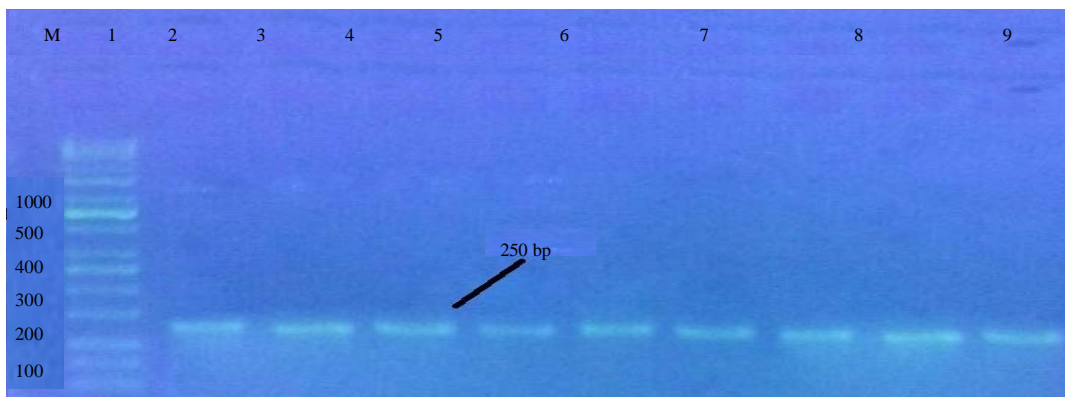


Fig. 3: The product was electrophoresis on 2% agarose gel at 5 Volt/cm², 1×TBE buffer for 2 h. M:DNA ladder (100-10000 bp), Lane 1-9 product for BMP 15 (FecXH) gene of goat, PCR product of band size 250 bp. visualized under U.V light after staining with red stain safe

Table 4: Type of polymorphism and amino acid change in sense of BMP15 gene in goats

Location of gene bank	Nucleotide change	Amino acid change	Predicted effect	Type of mutation
G102A	AGA>AAA	Arginine>Lysine	Missense	Transition
T156G	ATG>AGG	Methionine>Arginine	Missense	Transversion
G207A	CGA>CAA	Arginine>Glutamine	Missense	Transition
G218T	GTA>TTA	Valine>Leucine	Missense	Transversion

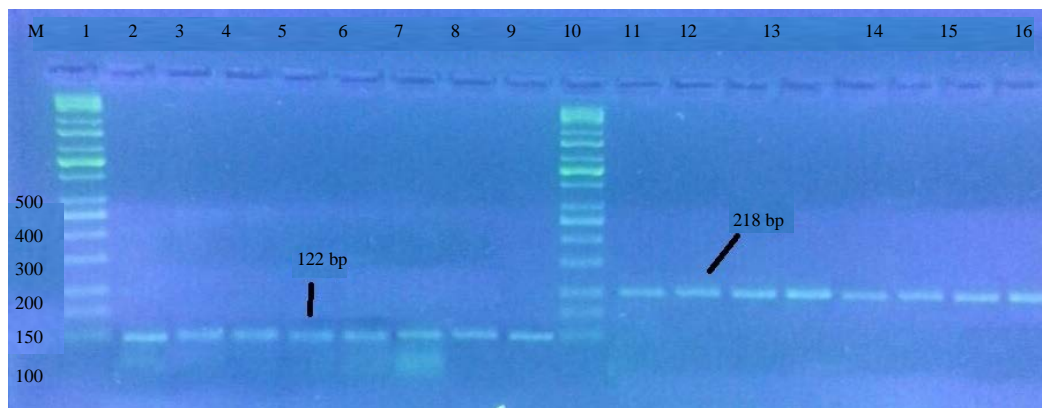


Fig. 4: An agarose gel electrophoresis pattern of PCR restriction enzyme for Lane's 1-8 FecXB (DdeI) and Lane's 9-16 FecXH (SpeI) product digested, showing genotypes in goats

PCR-RFLP analysis of the exon 10 of goat BMP-15 gene, allelic polymorphism was found with restriction endonuclease FecXB (DdeI) and FecXH (SpeI) which cuts the amplicon to fragments. There are one cleavage sites (122 and 218 bp) allelic polymorphism showed the (Table 4). All individuals were homozygous for the whole tested mutations as a result, none of the samples carried above mutations in BMP15 gene. The goat code region of BMP15 has similarity (Hua *et al.*, 2008). The mutations in the BMP15 gene increase ovulation rate in heterozygous individuals. On the other hand, most of the mutations in this gene block follicular development in homozygous individuals (Montgomery *et al.*, 2001). The PCR-RFLP methods similarity (Davis *et al.*, 2002). BMP15 mutants had higher ovulation rates in the heterozygote but homozygous mutant's primary ovarian failure resulting in complete sterility (Monteagudo *et al.*, 2009). Regarding the substantial roles of BMP15 played in the folliculogenesis and ovogenesis (McNatty *et al.* 2005). PCR-RFLP used for detection of FecXH and FecXB mutations showed several of polymorphism in goats (Fig. 4). The genetic factors affecting fecundity should be investigated further by goat due to its higher twinning rates.

The sequencing of amplified product of BMP-15 gene from goat, out of them appeared 100 and 98%

compatibility with standard *Capra hircus* breed bone Morphogenetic Protein 15 (BMP15) gene from 28 to 249 number of nucleotide from gene of gene Bank results as shown in Fig. 5a, b, Sequence ID: gb|JX860305.1| and have number score (261) bits and Fig. 6a, b the Sequence protein ID AFX69269.1 and have number score (391) bits. This result is similar with (Ahlawat *et al.*, 2013). The lack of similarity of conformation of protein as in Fig. 7a, b, the difference in the order of the amino acids and the emergence of site side of acids as in shape and the difference in body protein leads to variation in the function of BMP15 in seq ID: 193378 and 193379 (Kallbery *et al.*, 2012) in Fig. 8, This mutation which we label B5 (following nomenclature of Hanrahan *et al.*, 2004).

The number of live lambs born per breeding goat is an important trait in commercial goat breeding, since, the litter size largely determines the amount of meat produced per goat. In this study we have investigated the phenotypic effect of a mutation in the goat BMP-15 that recently has been reported to segregate in the sheep (Vage *et al.*, 2013).

The study of genes associated with fertility is important and has many applications in the animal health sector, thus, sheep can provide a genetic model for the study of fecundity genes and ovulation rate (Montgomery *et al.*, 2001). BMPs are essential for female fertility and knowledge of its function allows direct

(a) A= *Capra hircus* bone morphogenetic protein 15 (BMP15) gene, partial cds
Sequence ID: [JX860305.1](#) Length: 762 Number of Matches: 1
Related Information
Map Viewer aligned genomic context
Range 1: 28 to 249 [GenBank Graphics](#) Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
411 bits(222)	4e-111	222/222(100%)	0/222(0%)	Plus/Plus
Query 1	TCAT TCCACCTCTCTGCCATGTGGAGCCCTGGGGCAGAAAAGCCAAACCAATCACTT	60		
Sbjct 28	TCAT TCCACCTCTCTCTGCCATGTGGAGCCCTGGGGCAGAAAAGCCAAACCAATCACTT	87		
Query 61	TCCTCTTTCAGGAAGAGGCTCCCAAAGCCTTCCCTGTTGCCCAAACCTTGACAGAGAT			
Sbjct 88	TCCTCTTTCAGGAAGAGGCTCCCAAAGCCTTCCCTGTTGCCCAAACCTTGACAGAGAT			
Query 121	GGATATCATGGAACATGTTGGGCAAAAGCTCTGGAATCACAAGGGCGCAGGCTTCTACG			
Sbjct 148	GGATATCATGGAACATGTTGGGCAAAAGCTCTGGAATCACAAGGGCGCAGGCTTCTACG			
Query 181	ACTTCGCTTCGTATGTCAGCAGCCAAAGCTAGTGAGGTCT	222		

(b) B= *Capra hircus* bone morphogenetic protein 15 (BMP15) gene, partial cds
Sequence ID: [JX860305.1](#) Length: 762 Number of Matches: 1
Related Information
Map Viewer aligned genomic context
Range 1: 28 to 249 [GenBank Graphics](#) Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
388 bits(210)	2e-104	218/222(98%)	0/222(0%)	Plus/Plus
Query 1	TCAT TCCACCTCTCTCTGCCATGTGGAGCCCTGGGGCAGAAAAGCCAAACCAATCACTT	60		
Sbjct 28	TCAT TCCACCTCTCTCTGCCATGTGGAGCCCTGGGGCAGAAAAGCCAAACCAATCACTT	87		
Query 61	TCCTCTTTCAGGAAGAGGCTCCCAAAGCCTTCCCTGTTGCCCAAACCTTGACAGAGAT			
Sbjct 88	TCCTCTTTCAGGAAGAGGCTCCCAAAGCCTTCCCTGTTGCCCAAACCTTGACAGAGAT			
Query 121	GGATATCAGGAAACATGTTGGGCAAAAGCTCTGGAATCACAAGGGCGCAGGCTTCTACA			
Sbjct 148	GGATATCATGGAACATGTTGGGCAAAAGCTCTGGAATCACAAGGGCGCAGGCTTCTACG			
Query 181	ACTTCGCTTCGTATGTCAGCAGCCAAAGCTAGTGAGGTCT	222		
Sbjct 208	ACTTCGCTTCGTATGTCAGCAGCCAAAGCTAGTGAGGTCT	249		

Fig. 5a, b: Sequencing of sense flanking the BMP-15 gene for goat, obtained from gene bank Query represents of sample Subject represent of database of National Center Biotechnology Information (NCBI)

(a) A= bone morphogenetic protein 15, partial [*Capra hircus*]
Sequence ID: [AFX69269.1](#) Length: 253 Number of Matches: 1
Related Information
Range 1: 10 to 82 [GenPept Graphics](#) Next Match Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
155 bits(391)	8e-46	Compositional matrix adjust.	73/73(100%)	73/73(100%)	0/73(0%)	+2
Query 2	HSLSCHVEFWQKSPNHPFSSGRGSPKPSLLPKTWTEMDIMEHVQKQLWNHKGRRVLR					
Sbjct 10	HSLSCHVEFWQKSPNHPFSSGRGSPKPSLLPKTWTEMDIMEHVQKQLWNHKGRRVLR					69
Query 182	LRFVCQPPRGSEV	220				
Sbjct 70	LRFVCQPPRGSEV	82				

(b) B= bone morphogenetic protein 15, partial [*Capra hircus*]
Sequence ID: [AFX69269.1](#) Length: 253 Number of Matches: 1
Related Information
Range 1: 10 to 82 [GenPept Graphics](#) Next Match Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
115 bits(289)	2e-30	Compositional matrix adjust.	69/73(95%)	72/73(98%)	0/73(0%)	+2
Query 2	HSLSCHVEFWQKSPNHPFSSGRGSPKPSLLPKTWTEMDIMEHVQKQLWNHKGRRVLR					
Sbjct 10	HSLSCHVEFWQKSPNHPFSSGRGSPKPSLLPKTWTEMDIMEHVQKQLWNHKGRRVLR					69
Query 182	LRFLCQPPRGSEV	220				
Sbjct 70	LRFVCQPPRGSEV	82				

Fig. 6a, b: Amino acid sequence of the translated exon 2 in BMP15 represent all type of parturited does (single and twine) to a protein sequence, Query represents of sample Subject represent of database of National Center Biotechnology Information (NCBI)

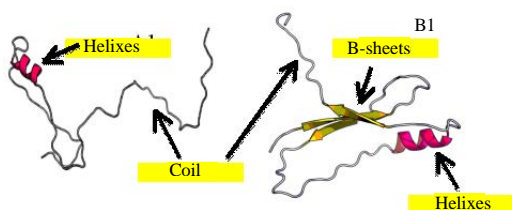


Fig. 7: (A1+B1): Conformation of protein BMP15 gene in goats used Raptorx software for drawing structure protein (Red color helixes, brown color B-sheets and black coils) of amino acid (symbol represent A: sample single goat and B: sample twin goat)

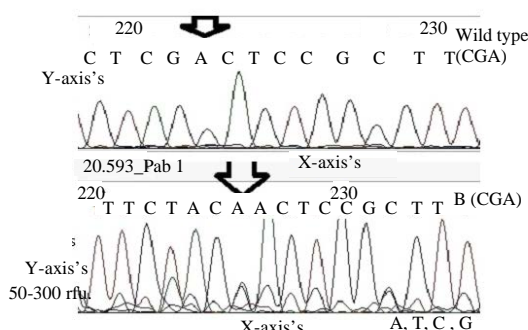


Fig. 8: Nucleotide substitution of the BMP 15 mutation compared with wild-type goat sequence. X axis represent nucleotide (A, T, C, G): Y axis represent relative fluorescence unite Single to Noise ratio (S/N) should optimally be between 50-300 rfu

manipulation of ovulation rate and litter size in farm animals and can also provide useful information in treating infertile individuals. The study of fecundity genes is also important to gain knowledge about genetic disorders associated with reproduction (Pramod *et al.*, 2013).

CONCLUSION

There are fecundity genes with major effect on ovulation rate and litter size in different sheep breeds. The genes that are involved in ovulation rate and litter size and the effects they have provides useful information for breeding and selection on those traits.

REFERENCES

Ahlawat, S., R. Sharma and A. Maitra, 2013. Screening of indigenous goats for prolificacy associated DNA markers of sheep. *Gene*, 517: 128-131.
Baldassarre, H. and C.N. Karatzas, 2004. Advanced Assisted Reproduction Technologies (ART) in goats. *Anim. Reprod. Sci.*, 82-83: 255-266.

Baruselli, P.S., E.L. Reis, M.O. Marques, L.F. Nasser and G.A. Bo, 2004. The use of hormonal treatments to improve reproductive performance of anestrus beef cattle in tropical climates. *Anim. Reprod. Sci.*, 82: 479-486.
Bearden, H.J. and J.W. Fuquay, 2000. *Applied Animal Reproduction*. 5th Edn., Prentice Hall, London, UK., ISBN:9780130819765, Pages: 382.
Davis, G.H., S.M. Galloway, I.K. Ross, S.M. Grogan and J. Ward *et al.*, 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. *Biol. Reprod.*, 66: 1869-1874.
Dias, E.T.F., E.S. Lopesjunior, A.B.S. Villarroel, D. Rondine and J.B. Limaverde *et al.*, 2001. Synchronization of estrus, ovulation induction and fertility in ewes after hormonal treatment woolless with equine chorionic gonadotropin. *Br. J. Vet. Med.*, 53: 618-623.
Dogan, I., A. Konyali, C. Tolu and S. Yurdabak, 2008. Different estrus induction protocols during the transition period in lactating Turkish Saanen does following AI. *Acta Veterinaria*, 58: 259-266.
Frank, O., 2012. *Reproductive health: The demography of fertility and infertility*. Geneva Foundation for Medical Education and Research, Geneva, Switzerland. http://www.gfmer.ch/Books/Reproductive_health/The_demography_of_fertility_and_infertility.html
Galloway, S.M., K.P.E. McNatty, L.M. Cambridge, M.P. Laitinen and J.L. Juengel *et al.*, 2000. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nat. Genet.*, 25: 279-283.
Galloway, S.M., S.M. Grogan, T. Wilson, K.P. McNatty and J.L. Juengel *et al.*, 2002. BMP15 mutations and ovarian function. *Mol. Cell. Endocrinol.*, 191: 15-18.
Greyling, J.P.C. and C.H. van Niekerk, 1990. Ovulation in the Boer goat doe. *Small Rumin. Res.*, 3: 457-464.
Hanrahan, J.P., S.M. Grogan, P. Mulsant, M. Mullen and G.H. Davis *et al.*, 2004. Mutations in the genes for Oocyte-Derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biol. Reprod.*, 70: 900-909.
Hashemi, M., M. Sadarian and M. Kafi, 2006. Estrous response to synchronization of estrus using different progesterone treatments outside the natural breeding season in ewes. *Small Rumin. Res.*, 65: 279-283.
Hua, G.H., S.L. Chen, J.T. Ai and L.G. Yang, 2008. None of polymorphism of ovine fecundity major genes fecb and fecx was tested in goat. *Anim. Reprod. Sci.*, 108: 279-286.

- Jainudeen, M.R., H. Wahid and E.S.E. Hafez, 2000. Sheep and Goats. In: Reproduction in Farm Animals, Hafez, B. and E.S.E. Hafez (Eds.). Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, USA., ISBN:9780683305777, pp: 172-181.
- Juengel, J.L. and K.P. McNatty, 2005. The role of proteins of the transforming growth factor- β superfamily in the intraovarian regulation of follicular development. *Hum. Reprod. Update*, 11: 144-161.
- Kadhim, Q.J., 2014. Clinical study and biochemical values during pregnancy and parturition in goat. MSc Thesis, University of Baghdad-College of Veterinary Medicine, Baghdad, Iraq.
- Kallberg, M., H. Wang, S. Wang, J. Peng, Z. Wang, H. Lu and J. Xu, 2012. Template-based protein structure modeling using the RaptorX web server. *Nat. Protocols*, 7: 1511-1522.
- Kelly Moore, R., F. Otsuka and S. Shimasaki, 2003. Molecular basis of bone morphogenetic protein-15 signaling in granulosa cells. *J. Biol. Chem.*, 278: 304-310.
- McNatty K.P., P. Smith, L.G. Moore, K. Reader and S. Lun *et al.*, 2005. Oocyte-expressed genes affecting ovulation rate. *Mol. Cell. Endocrinol.*, 234: 57-66.
- Menchaca, A. and E. Rubianes, 2001. Effect of high progesterone concentrations during the early luteal phase on the length of the ovulatory cycle of goats. *Anim. Reprod. Sci.*, 68: 69-76.
- Moaeeen-ud-Din, M., L.G. Yang, S.L. Chen, Z.R. Zhang and J.Z. Xiao *et al.*, 2008. Reproductive performance of Matou goat under sub-tropical monsoonal climate of Central China. *Trop. Anim. Health Prod.*, 40: 17-23.
- Monteagudo, L.V., R. Ponz, M.T. Tejedor, A. Lavina and I. Sierra, 2009. A 17 bp deletion in the Bone Morphogenetic Protein 15 (BMP15) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed. *Anim. Reprod. Sci.*, 110: 139-146.
- Montgomery, G.W., S.M. Galloway, G.H. Davis and K.P. McNatty, 2001. Genes controlling ovulation rate in sheep. *Reprod.*, 121: 843-852.
- Otsuka, F., K.J. McTavish and S. Shimasaki, 2011. Integral role of GDF-9 and BMP-15 in ovarian function. *Mol. Reprod. Dev.*, 78: 9-21.
- Pramod, R.K., S.K. Sharma, R. Kumar and A. Rajan, 2013. Genetics of ovulation rate in farm animals. *Vet. World*, 6: 833-838.
- Vage, D.I., M. Husdal, M.P. Kent, G. Klemetsdal and I.A. Boman, 2013. A missense mutation in Growth Differentiation Factor 9 (GDF9) is strongly associated with litter size in sheep. *BMC. Genet.*, 14: 1-8.