**ABSTRACT**

Inhibin A (INHA) a member of the transforming growth factor-β (TGF-β) family has been implicated in the negative feedback control mechanism of the pituitary follicle stimulating hormone (FSH). Inhibin has been reported to be associated with litter size, milk yield, fertility and reproductive traits in ruminants.

A total of ten amino acid sequences (four sheep and six goats) were downloaded from the National Centre for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/snp). The amino acid sequence alignment was carried out using ClustalW algorithm of Molecular Evolutionary Genetic Analysis software version 6.0. The functional effects of eighteen (18) amino acid substitutions of INHA gene in each of sheep and goats were predicted computationally using Polyphen-2, PROVEAN and SIFT algorithms while INHA gene functions and interactions with associated genes were investigated using GeneMANIA. Variants that were consensually predicted to be deleterious by the three algorithms utilised were referred to as ‘Cmutant’ and ‘Dmutant’ in sheep and goats, respectively. The MutPred was further used to determine the tolerance degree for each amino acid substitution of both the ‘Cmutant’ and ‘Dmutant’.
GeneMANIA revealed 20 genes that co-localised, co-expressed with, play functionally similar role or has physical or genetic interaction with INHA gene. Out of the studied eighteen (18) amino acid substitutions; there was a consensus by SIFT, PROVEAN and PolyPhen-2 algorithms in the prediction of variants C99R, C264D, L237T and F14C as being deleterious in sheep and variants D77F, L190K, R223D, L240N, C322T and P326C in goats. In goats, MutPred revealed that variants R223D and D77F were not harmful while, L190K, R223D, L240N and P326C mutations were observed to be highly harmful. In sheep, the ‘Dmutants’ (C99R, C264D, F14C and L237T) were predicted to be highly harmful. The obtained findings would be useful in planning research aiming at exploring the association between INHA gene variants and economically important traits of small ruminants.

Keywords: Inhibin; goat; sheep; SNP; amino acid variants.

1. INTRODUCTION

Single nucleotide polymorphism (SNP) is the variation in a genetic sequence that affects only one of the basic building blocks of a DNA molecule and that occurs in more than 1% of a population [1]. The SNPs could be grouped into coding SNPs, noncoding SNPs, or the intergenic [2,3]. Unlike other SNPs, which are quite natural in the animal/human genome, the non-synonymous coding SNPs (nsSNPs) often have main impact on phenotype by changing the protein sequence via amino acid alteration in the corresponding protein product. It can also exert deleterious effects on the structure, function and stability of proteins or by modifying DNA and transcriptional binding factors and impacting the phenotype by changing the protein sequence [4,5]. Single nucleotide polymorphism in inhibin, MTNR1, PAPPA2, DGAT1 etc have been postulated to contribute or be associated with both economic and adaptive traits of farm animals including but not limited to disease resistance, longevity, milk yield, wool production, fertility traits, reproductive traits, laying performance and heat tolerance. Inhibins are dimeric glyco-proteins that inhibits pituitary follicle-stimulating hormone secretion, follicular maturation and steroidogenesis by suppressing its receptor expression in granulosa cells, thus affecting the development of ovarian follicles [6,7]. Inhibins are made up of dimmer of alpha and beta-A which commonly encoded by INHA, INHbA, and INHbB [8]. The gene has been physically mapped to chromosome 2q41-42 of Capra hircus INHA gene; consisting of three exons [9]. The transcript of goat INHA contains 1123 bp (NM_001285606), including a 1083 bp open reading frame, a 15-bp 5’ UTR and a 25-bp 3’ UTR.

In goats, [10] reported that the INHA 651A/G polymorphism was a potential marker for the mean litter size of the second parity in Boer goats. Earlier studies have demonstrated that immunization against inhibin could improve the ovarian response to superovulation, resulting in increased ovulation rates and higher yields of transferable embryos in sheep [11], heifers or cows [12,13], and water buffalo [14]. In this study, in silico analysis of the functional and structural effects of the genetic variants of INHA gene of sheep and goats were investigated. The observed beneficial SNPs can be used as marker to improve economically important traits in these farm animals.

2. MATERIALS AND METHODS

2.1 Sequence Retrieval and Analysis

The amino acid sequence data on sheep and goats INHA gene were retrieved from the database of the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/snp). A total of six (6) goat sequences and four (4) sheep sequences were downloaded. Details of the downloaded sequences are presented in Table 1. The amino acid sequence alignment of the two species was carried out using ClustalW algorithm of Molecular Evolutionary Genetic Analysis software version 6.0 [15].

2.2 Functional Prediction of Non-synonymous Amino Acid Substitutions

The functional effects of the nsSNPs of INHA gene in sheep and goats were predicted computationally using Polyphen-2, PROVEAN and SIFT while INHA gene functions and interactions with associated genes were investigated using GeneMANIA.
2.2.1 Investigation of INHA gene’s interactions with functionally similar genes

GeneMANIA finds similar and functionally associated genes with the query gene using a wealth of genomics and proteomics data. The functionally related genes to INHA were obtained using the GeneMANIA (http://www.genemania.org). Genetic interactions, pathways, co-expression, co-localization and protein domain similarity of the INHA gene of sheep and goats were determined as previously described [16].

2.2.2 Prediction of structural impact of nsSNPs on protein by SIFT software

The Structural Impact of nsSNPs on the sheep and goats INHA gene sequences were predicted using SIFT (Separating Intolerant from Tolerant) software (http://sift.bii.a-star.edu.sg/). SIFT is a sequence homology-based tool that uses multiple alignment information to predict tolerated and deleterious substitutions for each position of the query sequence. It first searches for related/similar sequences, then chooses closely functionally related sequences to the query sequence and finally calculates normalized probabilities for all possible substitutions from the alignment. Positions with normalized probabilities less than 0.05 were predicted to be deleterious while those greater than or equal to 0.05 were predicted to be tolerated [17].

2.2.3 Function analysis of nsSNP using provean

Protein Variation Effect Analyser (PROVEAN) was used to predict the single amino acids substitutions and functional effect of protein sequence variations. Variants with a PROVEAN score above -2.5 are considered "NEUTRAL" while variants with a PROVEAN score equal to or below -2.5 are considered "DELETEROUS,” [18].

2.2.4 Prediction of deleterious nsSNPs by polyphen-2

Polymorphism Phenotyping version 2.0 software available: http://genetics.bwh.harvard.edu/pph2/ (Polyphen-2) was also used to predict the possible impact of amino acid substitutions on the stability and function of INHA proteins using structural and comparative evolutionary considerations. Polyphen performs functional annotation of SNPs, maps coding SNPs to gene transcripts, estimates the probability of the missense mutation being damaging among others.

2.2.5 Prediction of harmful mutations by mutpred

The Mutpred server (http://mutpred.mutdb.org/) was employed to classify an amino acid substitution as deleterious or neutral. Additionally, Mutpred predicts molecular cause of deleterious amino acid sequence. The output of Mutpred contains the probability that the amino acid substitution is deleterious/disease-associated and top 5 property scores (p), where p is the P-value that certain structural and functional properties are impacted [19].

Table 1. Amino acid sequences of sheep and goats

| Species | Accession number | Length (bp) |
|---------|------------------|-------------|
| Goats   |                  |             |
|         | ABR13681.1       | 80          |
|         | NP_001272535.1   | 360         |
|         | AB13682.1        | 263         |
|         | AEJ07666.1       | 360         |
|         | AEP40506.1       | 360         |
|         | AEP40507.1       | 360         |
| Sheep   |                  |             |
|         | AIW82618.1       | 360         |
|         | ABS82446.1       | 265         |
|         | AAA31553.1       | 265         |
|         | NP_001295508.1   | 360         |

3. RESULTS AND DISCUSSION

Fertility is one of the most economically important traits in farm animal production. Genetic improvement of fertility traits in indigenous domestic animals will enhance productivity and food security especially in developing countries [18]. The INHA gene (Fig. 1) play a crucial role in fertility and reproductive rate in farm animals by suppressing the FSH receptors in the granular cells thereby affecting ovarian development. The INHA gene has been postulated to be associated with superovulation in small ruminants [20]. A total of ten amino acid sequences (six goats and 4 sheep) were retrieved from NCBI database. The sequence lengths for goats ranged between 80 and 360 while those of the sheep ranged between 265 and 360 base pairs.

GeneMANIA revealed vital functions of INHA gene as well as the genes that co-localised, co-expressed with, play functionally similar role or has physical or genetic interaction with INHA (Fig. 1). These include Follicle stimulating
### Table 2. Gene description using GeneMania

| SN  | Genes   | Description                                                        |
|-----|---------|-------------------------------------------------------------------|
| 1   | INHA    | Inhibin alpha subunit                                              |
| 2   | FSHB    | Follicle stimulating hormone beta subunit                          |
| 3   | CGA     | Glycoprotein hormones, alpha polypeptide                           |
| 4   | ACVR2A  | Activin A receptor type 2A                                          |
| 5   | INHBB   | Inhibin beta B subunit                                             |
| 6   | PDIA3   | Protein disulphide isomerase family A member 3                      |
| 7   | FST     | Follistatin                                                        |
| 8   | INHBA   | Inhibin beta A subunit                                             |
| 9   | CALR    | Calreticulin                                                       |
| 10  | MAPK4   | Mitogen-activated protein kinase 4                                  |
| 11  | IKBK    | Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma |
| 12  | HMBOX1  | Homeobox containing 1                                               |
| 13  | CASP9   | Caspase 9                                                          |
| 14  | TRIM29  | Tripartite motif containing 29                                      |
| 15  | BMP4    | Bone morphogenetic protein 4                                       |
| 16  | ALX4    | Homeobox 4                                                         |
| 17  | ACVR1B  | Activin A receptor type 1B                                          |
| 18  | SERPINB3| Serpin family B member 3                                            |
| 19  | GRAMD1B | GRAM domain containing 1 B                                          |
| 20  | INHBC   | Inhibin beta C subunit                                             |
| 21  | KCNQ1   | Potassium voltage-gated channel subfamily Q member 1               |

Hormone beta subunit, Glycoprotein hormones, alpha polypeptide, Activin A receptor type 2A, Protein disulphide isomerase family A member 3, Follistatin and Mitogen-activated protein kinase 4 (Table 2). This association further lay credence to the important role this gene plays in follicular development and oocyte maturation. Out of the studied eighteen (18) amino acid substitutions in each of sheep and goats; there was a consensus by SIFT, PROVEAN and PolyPhen-2 algorithms in the prediction of variants C99R, C264D, L237T and F14C as being deleterious in sheep (Table 3) and variants D77F, L190K, R223D, L240N, C322T and P326C in goats (Table 4). These ten variants (four in sheep and six in goats) were therefore collectively referred to as ‘Cmutant’ and ‘Dmutant’, respectively for further confirmatory analysis. Variants D201W, L50W, A43K, D244H, Y251D, and C227R were predicted deleterious by only 2 out of the three algorithms, W138A, L136V, L219A and F214Q were predicted deleterious by only one out of the three algorithms while E127H, H19Q, A225F and S248N were predicted to be tolerated by SIFT, PROVEAN and PolyPhen-2 algorithms in sheep. Similarly, Q124A, L148K, S200Y and E94Y were observed to be deleterious by two out of the three algorithms, A201V, T152S and S112A were predicted deleterious by only one while SIFT, PROVEAN and PolyPhen-2 algorithms predicted V301A, H128T, V63T, A176G and A100E to be neutral in goats. The differences in prediction capabilities refer to the fact that every prediction algorithm uses different sets of sequences and alignments [21]. The MutPred was further used to determine the tolerance degree for each amino acid substitution of both the ‘Cmutant’ and ‘Dmutant’ as described by He et al. [22]. In goats, MutPred revealed that variants R223D and D77F were neutral while, L190K, R223D, L240N and P326C mutations were observed to be highly harmful. In sheep, the ‘Dmutants’ (C99R, C264D, F14C and L237T) were predicted to be highly harmful. This conforms with the earlier obtained results from SIFT, PROVEAN and PolyPhen-2 algorithms in this study. Details of the obtained results from MutPred are shown in Table 5. These results suggest that some nsSNPs such as the ‘Cmutants’ and ‘Dmutants’ may account for potential structural and functional changes in INHA protein. Similar observation was made by He et al. [22] in their study on in silico analysis of deleterious Single Nucleotide Polymorphisms (SNPs) in Human MutS Homolog6 (MSH6) gene. Earlier [14] have identified polymorphism in INHA gene with a significant association with litter size in three goat breeds. Tian et al. [23] observed significantly different genotype distributions in INHA gene between year-round
estrus goat breeds and seasonal estrous goat breeds. Similarly, A282G mutation in INHA promoter had significant effects on the average litter size of Small Tailed Han sheep (P < 0.05) [24] and mutation homozygous genotypes had 1.32 lambs more than those with wild type in Small Tail Han Sheep [25]. In a similar study, [26] reported significant correlation between MspI polymorphism in the bovine INHA gene and superovulation. However, INHβA C7639T mutation has no significant effect on superovulation traits in Chinese Holstein cows. In exon 2, 651A/G and 125 G/A have been associated with litter size of the second parity in Boer, Matou and Nubi goats [10] and Dazu Black and Nanjiang Yellow goats, respectively [27]. However, exon 1 of INHA was conserved with no polymorphisms in goats [10,28,29]. This suggests possibility of INHA variants as marker against seasonal breeding in goats.

Table 3. The effect of amino acid variant on the functions of INHA proteins of sheep using PROVEAN, SIFT and polyphen-2

| Amino Acid | SIFT prediction | SIFT score | PROVEAN Prediction | PROVEAN Score | PolyPhen-2 Prediction | PolyPhen-2 Score |
|------------|----------------|------------|-------------------|---------------|----------------------|-----------------|
| D201W      | Tolerated      | 0.68       | Deleterious       | -2.709        | POROBABLY DAMAGEING  | 0.976           |
| E127H      | Tolerated      | 0.68       | Neutral           | -1.619        | POSSIBLY DAMAGEING   | 0.865           |
| H19Q       | Tolerated      | 0.47       | Neutral           | -1.608        | POROBABLY DAMAGEING  | 0.999           |
| F214Q      | Tolerated      | 1.00       | Neutral           | -0.216        | BENIGN                | 0.001           |
| L219A      | Tolerated      | 1.00       | Neutral           | 0.108         | BENIGN                | 0.046           |
| Y251D      | Tolerated      | 1.00       | Deleterious       | -5.654        | POROBABLY DAMAGEING  | 1.000           |
| C99R       | Deleterious    | 0.47       | Deleterious       | -4.627        | POROBABLY DAMAGEING  | 1.000           |
| C264D      | Deleterious    | 1.00       | Deleterious       | -9.257        | POROBABLY DAMAGEING  | 1.000           |
| A225F      | Tolerated      | 1.00       | Neutral           | -2.376        | POSSIBLY DAMAGEING   | 0.553           |
| C227R      | Tolerated      | 1.00       | Deleterious       | -10.169       | PROBABLY DAMAGEING   | 1.000           |
| L237T      | Deleterious    | 1.00       | Deleterious       | -4.237        | POROBABLY DAMAGEING  | 1.000           |
| D244H      | Tolerated      | 0.95       | Deleterious       | -5.413        | PROBABLY DAMAGEING   | 1.000           |
| S248N      | Tolerated      | 1.00       | Neutral           | -0.254        | PROBABLY DAMAGEING   | 1.000           |
| F14C       | Deleterious    | 0.47       | Deleterious       | -3.068        | POROBABLY DAMAGEING  | 1.000           |
| L136V      | Tolerated      | 0.84       | Neutral           | 0.052         | BENIGN                | 0.000           |
| W138A      | Tolerated      | 0.68       | Neutral           | -0.883        | BENIGN                | 0.045           |
| A43K       | Deleterious    | 0.42       | Neutral           | -1.019        | POSSIBLY DAMAGEING   | 0.782           |
| L50W       | Deleterious    | 0.37       | Neutral           | -1.723        | PROBABLY DAMAGEING   | 0.997           |

PolyPhen-2 result: POROBABLY DAMAGING (more confident prediction) / POSSIBLY DAMAGING (less confident prediction). The amino acid substitution is predicted damaging if the score is ≤ 0.05, and tolerated if the score is > 0.05
Table 4. The effect of amino acid variant on the functions of INHA proteins of Goats using PROVEAN, SIFT and PolyPhen-2

| Amino Acid Change | PROVEAN Prediction | PROVEAN Score | PolyPhen-2 Prediction | PolyPhen-2 Score | SIFT Prediction | SIFT Score |
|-------------------|---------------------|---------------|-----------------------|------------------|-----------------|------------|
| A100E             | Neutral             | -0.342        | Benign                | 0.002            | Tolerated       | 0.15       |
| E94Y              | Neutral             | -0.951        | POSSIBLY DAMAGING     | 0.903            | Deleterious     | 0.15       |
| D77F              | Deleterious         | -2.587        | PROBABLY DAMAGING     | 1.000            | Deleterious     | 0.15       |
| S112A             | Neutral             | -1.620        | PROBABLY DAMAGING     | 1.000            | Tolerated       | 0.15       |
| T152S             | Neutral             | 0.958         | BENIGN                | 0.000            | Deleterious     | 0.15       |
| A176G             | Neutral             | -1.145        | BENIGN                | 0.023            | Tolerated       | 0.15       |
| S200Y             | Neutral             | -0.827        | PROBABLY DAMAGING     | 0.984            | Deleterious     | 0.15       |
| L190K             | Deleterious         | -3.134        | PROBABLY DAMAGING     | 0.000            | Deleterious     | 0.15       |
| L148K             | Neutral             | -0.469        | PROBABLY DAMAGING     | 1.000            | Deleterious     | 0.15       |
| Q124A             | Neutral             | -2.427        | POSSIBLY DAMAGING     | 0.862            | Deleterious     | 0.15       |
| V63T              | Neutral             | 0.158         | BENIGN                | 0.002            | Tolerated       | 0.15       |
| H128T             | Deleterious         | -2.811        | POSSIBLY DAMAGING     | 0.622            | Deleterious     | 0.15       |
| A201V             | Neutral             | -1.318        | BENIGN                | 0.003            | Deleterious     | 0.15       |
| R223D             | Deleterious         | -4.297        | PROBABLY DAMAGING     | 1.000            | Deleterious     | 0.23       |
| L240N             | Deleterious         | -2.578        | PROBABLY DAMAGING     | 1.000            | Deleterious     | 0.26       |
| V301A             | Neutral             | 0.131         | BENIGN                | 0.001            | Tolerated       | 0.83       |
| C322T             | Deleterious         | -8.808        | PROBABLY DAMAGING     | 1.000            | Deleterious     | 0.85       |
| P326C             | Deleterious         | -8.066        | PROBABLY DAMAGING     | 0.999            | Deleterious     | 0.85       |
| A100E             | Neutral             | -0.342        | BENIGN                | 0.002            | Tolerated       | 0.15       |
| E94Y              | Neutral             | -0.951        | POSSIBLY DAMAGING     | 0.903            | Deleterious     | 0.15       |
| D77F              | Deleterious         | -2.587        | PROBABLY DAMAGING     | 1.000            | Deleterious     | 0.15       |

*PolyPhen-2 result: PROBABLY DAMAGING (more confident prediction) / POSSIBLY DAMAGING (less confident prediction). The amino acid substitution is predicted damaging if the score is ≤ 0.05, and tolerated if the score is > 0.05*
Table 5. Prediction of the functional impact of nsSNPS on INHA protein of sheep and goats by MutPred

| Species | Amino acid change | Probability of deleterious mutation | Top 5 features | Affected PROSITE and ELM Motifs |
|---------|-------------------|-------------------------------------|----------------|---------------------------------|
| Goats   |                   |                                     |                |                                 |
|         | R223D             | 0.428 (Not harmful)                 | None           | None                            |
|         | L190K             | 0.842 (Highly harmful)              | Altered Stability (P = 0.00017) | ELME000045, ELME000148         |
|         |                   |                                     | Gain of Strand (P = 0.0026)      |                                 |
|         |                   |                                     | Loss of Loop (P = 0.0097)        |                                 |
|         | L240N             | 0.551 (Harmful)                     | Altered Disordered interface (P = 0.04) Gain of Loop (P = 0.02) | None |
|         |                   |                                     | Loss of Proteolytic cleavage at R241 (P = 0.02) |                                 |
|         | D77F              | 0.245 (Not harmful)                 | None           | None                            |
|         | P326C             | 0.728 (Highly harmful)              | Altered Metal binding (P = 0.01) | ELME000336                     |
|         |                   |                                     | Gain of Disulfide linkage at C321 (P = 0.00031) |                                 |
|         |                   |                                     | Gain of Helix (P = 0.04)          |                                 |
|         |                   |                                     | Altered Transmembrane protein (P = 0.01) |                                 |
|         |                   |                                     | Gain of Catalytic site at C322 (P = 0.04) |                                 |
| Sheep   | C99R              | 0.715 (Highly harmful)              | Gain of Intrinsic disorder (P = 0.04) | ELME000012, ELME000102, ELME000162 |
|         |                   |                                     | Loss of Strand (P = 0.05)         |                                 |
|         | C264D             | 0.922 (Highly harmful)              | Gain of Intrinsic disorder (P = 0.000073) | ELME000108, ELME000162         |
|         |                   |                                     | Altered Metal binding (P = 0.000046) |                                 |
|         |                   |                                     | Altered Transmembrane protein (P = 0.00) |                                 |
|         |                   |                                     | Altered Ordered interface (P = 0.0017) |                                 |
|         |                   |                                     | Altered Disordered interface (P = 0.02) |                                 |
|         |                   |                                     | Gain of Relative solvent accessibility (P = 0.0001) |                                 |
|         |                   |                                     | Gain of Disulfide linkage at C264 (P = 0.0046) |                                 |
|         |                   |                                     | Gain of Catalytic site at C262 (P = 0.03) |                                 |
|         | F14C              | 0.864 (Highly harmful)              | None           |                                 |
|         |                   |                                     | Altered Transmembrane protein (P = 0.03) | ELME000155, ELME000163, ELME000182, ELME000202, ELME000328 |
|         | L237T             | 0.845 (Highly harmful)              | Altered Transmembrane protein (P = 0.00015) | ELME000012, ELME000053, ELME000062, ELME000106 |
|         |                   |                                     | Gain of ADP-ribosylation at R235 (P = 0.03) |                                 |
|         |                   |                                     | Altered Stability (P = 0.04)       |                                 |
4. CONCLUSION

This study provides information on the potential deleterious effects of single nucleotide polymorphism on functions and structures of INHA gene of sheep and goats. All the four nsSNPs that were consensually predicted to be the deleterious by SIFT, PROVEAN and PolyPhen-2 were also observed to be deleterious by Mutpred in sheep, while R223D and D77F were observed to be neutral in goats after been predicted to be deleterious by the three software.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ng PC, Henikoff S. Predicting the effects of amino acid substitutions on protein function. Annual Review of Genomics and Human Genetics. 2006;7:61–80.
2. Carninci P, Kasukawa T, Katayama S et al. The transcriptional and cpe of the mammalian genome. Science. 2005; 309:1559–1563.

3. Liu J, Gough J, Rost B. Distinguishing protein-coding from non-coding RNAs through support vector machines. PLOS Genetics. 2006;2:4-29.

4. Smith EP, Boyd J, Frank GR et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. The New England Journal of Medicine. 1994;331:1056–1061.

5. Lande E. The new genomics: Global views of biology. Science. 1996;274:536–539.

6. Thomas RR, McConnell J, Whittacker P, Kirkpatrick, Bradley J. Identification of mutations in the repeated part of the autosomal dominant polycystic kidney disease type 1 gene, PKD1, by long-range PCR. The American Journal of Human Genetics. 1999;65:39–49.

7. deKretser DM, Hedger MP, Loveland KL, Phillips DJ. Inhibins, activins and follistatin in reproduction. Hum Reprod Update. 2002;8(6):529-541.

8. Goldammer T, Brunner RM, Hendleder S, Schwerin M. Comparative mapping of sheep inhibin subunits alpha (INHA) and beta B(INHBB) to chromosome 2 in goat by FISH. Mamm Genome. 1995;6(9):685-686.

9. Wu, WS, Hua GH, Yang LG, Wen QY, Zhang CY, Zheier KM. Association analysis of the INHA gene with litter size in Boer goats. Small Ruminant Res. 2009;82(2):139-143.

10. D’Alessandro A, Martemucci G, Iaffaldano N. Active immunization with a synthetic fragment of pig inhibin alpha-subunit increases ovulation rate and embryo production in superovulated ewes but season affects its efficiency. J. Reprod. Fertil. 1999;115:185–191.

11. Li C, Zhu YL, Xue JH, Zhang SL. Immunization against inhibin enhances both embryo quantity and quality in Holstein heifers after superovulation and insemination with sex-sorted semen. Theriogenology. 2009a;71:1011-1017.

12. Mei C, Li MY, Zhong SQ, Lei Y. Enhancing embryo yield in superovulated Holstein heifers by immunization against inhibin. Reprod. Domest. Anim. 2009;44:735-739.

13. Li DR, Qin GS, Wei YM, Lu FH, et al. Immunisation against inhibin enhances follicular development, oocyte maturation and superovulatory response in water buffaloes. Reprod. Fertil. Dev. 2011;23: 788-797.

14. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution. 2013;30: 2725-2729. Available:http://dx.doi.org/10.1093/molbev/msw197

15. Fatai RB, Akinyemi MO, Osaiyuwu OH. Computational Analysis of the Sequences of LIPE Gene of Selected Ruminants and Non-ruminants. Journal of Advances in Biology & Biotechnology. 2019;1-10.

16. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 2016;33(7):1870-4. DOI: 10.1093/molbev/msw054 Epub 2016 Mar 22

17. Akinyemi MO, Osaiyuwu HO, Ismail AA. Computational molecular analysis of the sequences of PAPPA2 gene of selected ruminants and non-ruminants. Biotechnology Journal International. 2017; 19(1):1-8.

18. Li B, Krishnan VG, Mort ME, Xin F, Kamati KK, Cooper DN. Automated inference of molecular mechanisms of disease from amino acid substitutions. Bioinformatics. 2009b;25:2744–2750.

19. Yang WC, Li SJ, Chen L, Yang LG. Polymorphism of the inhibin βA gene and its relationship with superovulation traits in Chinese Holstein cows. Genetics and Molecular Research. 2014;13(1):269-275.

20. Yakubu A. Computational molecular analysis of deleterious mutations in serum amyloid A3 gene in goats and cattle. Nigerian Journal of Biotechnology. 2018;35(2):80-90.

21. Abdeirahim NE, Osman MM, Elgamaab OM, Alia AA, Ismail MM, Osman SA. Computational Analysis of Deleterious Single Nucleotide Polymorphisms (SNPs) in Human MutS Homolog6 (MSH6) Gene. American Journal of Bioinformatics Research. 2016;6(2):56-97.

22. He YQ, Ma XK, Liu XY, Zhang CX, Li J. Candidate genes polymorphism and its association to prolificacy in Chinese goats. Journal of Agricultural Science. 2010; 2(1):88-92
23. Tian XE, Sun HX, Wang YJ. Genetic polymorphism of INHA gene and its effect on litter size in three sheep breeds. Journal of Northwest A & F University. 2010; 38(1):23-29.

24. Zhou WR, Chu MX, Sun SH, Fang L, Ye SC. A candidate gene INHA for prolificacy of small Tail Han sheep. J Agric Biotechnol. 2007;15(1):32-36.

25. Tang KQ, Yang WC, Li SJ, Yang LG. Polymorphisms of the bovine growth differentiation factor 9 gene associated with superovulation performance in Chinese Holstein cows. Genet. Mol. Res. 2013;12:390-399.

26. Zhao ZQ, Li ZQ, Zhang JH. Association analysis of polymorphism in INHα gene with goat litter size. Chinese Journal of Animal Science. 2012;(5):11-13:42.

27. Hou JX, An XP, Li G, Wang Y, Song YX, Cao BY. Exploring polymorphisms and their effects on reproductive traits of the INHA and INHβA genes in three goat breeds. Anim Science Journal. 2012; 83(4):273-278.

28. Wang RF, Zhu TG, Pang XS, Wang ZY, Ding XL, Wang F. Single nucleotide polymorphism detection of inhibin-A 5' region from three breeds of goat. Jiangsu Journal of Agricultural Science. 2008; 24(5):687-691.

29. Wang YN, Yan QM, Han D, Li L, Zhu GQ, Song YX. Polymorphism of the promoter of INHA gene and its relationship with litter size and milk production in xinongshaaneng dairy goat. Chinese Journal of Veterinary Science. 2010;30: 552-555.

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