Indian J Anim Health (2022), 61(2): 300- 306
DOI: https://doi.org/10.36062/ijah.2022.14022

Research Article

In-silico ab initio 3D structure prediction of goat kisspeptin precursor protein (135 amino acid residues)

I. Mukherjee[1*] and I. Roy[2]

[1]Animal Physiology Division, ICAR- National Dairy Research Institute, Karnal- 132 001, Haryana, India; [2]Animal Genetics & Breeding Division, ICAR- National Dairy Research Institute, Eastern Regional Station, Kalyani, Nadia– 741 235, West Bengal, India

Abstract

Goat farming is one of the profitable ventures nowadays. The profitability of goat rearing entirely depends on its reproductive superiority. In tropics, goats often suffer from anestrus and acyclicity. Lack of Gonadotropin-releasing hormone (GnRH) surge is one of the prime reasons behind this. External application of GnRH secretagogue can solve this problem. Kisspeptin is one of such secretagogue. But the 3D structure of goat kisspeptin still needs to be discovered. That's why an attempt was made to elucidate the 3D structure of the kisspeptin-135 by ab initio method for the first time in domestic animals. Three machine learning-based tools, AlphaFold, RoseTTAFold and RaptorX, were used. Further, the result was validated by analyzing the Ramachandran plot and ProTSAV score. Final score indicated that the RaptorX predicted model is superior to the other two. Physicochemical characteristics of the kisspeptin-135 protein were also predicted. We also constructed the protein-protein interaction network of kisspeptin-135, which indicates its relationship with GnRH1 and neurokinin B. But the analysis revealed absence of co-expression between kisspeptin and neurokinin B, which is absolutely contrary to experimental evidence in human and mice. This suggested either species specificity or lack of experiment in this aspect. Overall, this 3D predicted model can be used for molecular docking in further analysis.

Key words: AlpaFold, GnRH secretagogue, Kisspetin-135, RoseTTAFold, RaptorX, Protein-protein interaction network

Highlights

- Kisspeptin 3D structure have been predicted using 3 machine learning based tools AlphaFold, RoseTTAFold and RaptorX. Structures were further refined using GalaxyRefine2 online tool. Ramachandran plot and ProTSAV score were used to predict the best model.
- ProTSAV score indicated RaptorX predicted model was best among the others. Protein-protein interaction network analysis by STRING indicated several associations of kisspeptin with other important proteins. But absence of co-expression of kisspeptin and neurokinin B indicated probable species difference or absence of experiments in particular direction.

INTRODUCTION

Goat farming nowadays is a profitable enterprise. Goats are often considered as "poor man's cow" as those are reared by the poorest of the society (Hasan *et al*., 2015). The Goat population in the country is 148.88 million heads showing an increase of 10.1% over the previous census (20th Livestock Census). The entire profitability of goat farming depends on its higher prolificacy rate. This kind of reproductive soundness of goat farming makes it superior to any other farming practices. But in tropical countries, goat often shows anoestrus due to the fact that goats are originally short-day breeders (Fatet *et al*., 2011). The predominant cause behind this reproductive incompetence is the lack of secretion of GnRH. And here comes the role of novel neuropeptides that could facilitate the surge of GnRH. Kisspeptin is one of such novel secretagogue of GnRH.

*Corresponding Author, E mail: indranilaloke@gmail.com

Kisspeptin is a neuropeptide, which initiates puberty and regulates ovulation in sexually mature females (Hu *et al*., 2018). The kisspeptin secreting neurones are located mainly in two areas- caudal portion of the Arcuate nucleus of the hypothalamus, extending to around the pre-mammillary nucleus, and medial preoptic area / antero- ventral periventricular region (Scott *et al*., 2019). Kisspeptin is the product of KiSS1 gene (Mondal *et al*., 2015). The first translated product of the KiSS1 gene is a 135 amino acid peptide in goat (Zheng *et al*., 2018). This peptide or its post-transcriptionally cleaved products act on GnRH secreting neurones via a G-Protein coupled receptor- GPR-54 with equal potency (Kotani *et al.*, 2001).

Previous work revealed the role of kisspeptins in augmenting GnRH secretion, but major hindrance in kisspeptin research lies in its unknown 3D structure, and the absence of proper 3D structure also hinders the prediction of its exact mode of action. Novel ligand prediction by multiple docking could further extend our understanding about the potential role of kisspeptin in several aspects of reproduction. Computational prediction of the 3D structure may be a possible and cost-effective solution to this problem (Zhang, 2009). So, in this present study, we attempted to predict the goat kisspeptin precursor protein (135 aa) structure. No experimental or theoretically resolved structure of homologues protein (template) is available in the database. Hence, it was planned to construct the structure of kisspeptin by ab-initio method of structure prediction.

MATERIALS AND METHODS

Retrieval of the sequence: Protein sequence was retrieved from NCBI protein database. The sequence accession number is ALA27389.1. The FASTA sequence is- MNVLLSWQLMLFL CATAFRETLEKVAPMENPRTTGSQLGPA TLRAPWEQSPRCAAGKPTAAGPRPR GAALCPSESSAGPRQPGPCASRSRLIP APRGAVLVQREKDVSAYN WNSFGLRY GRRQAALP GGRGAARG

Analysis of primary protein sequence: Protein homologues from other species are precursor proteins which later cleaved to generate smaller peptides; thus, instability of the query protein is possible. That's why we estimated different physicochemical properties like isoelectric point (pI), molecular weight, extinction coefficient, predicted half-life, total number of positively and negatively charged residues, and instability and aliphatic indices of the query protein with the help of ProtParam tool (Gasteiger *et al*., 2007; ProtParam tool).

Subcellular localisation and Gene ontology (GO) prediction: Protein location in subcellular organelle and several GO parameters like molecular functions of the query protein, biological process in which the query protein is or thought to be involved and cellular component where the protein is located was analysed by CELLO2GO web server (Yu *et al.*, 2014; CELLO2GO).

Three-dimensional structure prediction: Three-dimensional structure was predicted using AlphaFold, RoseTTAFold and RaptorX as suitable template for designing of similar protein was not found. Similarity between other homologs was less than 50 percent. So, we couldn't use homologues and orthologues to predict the protein structure. AlphaFold is a machine learning based algorithm that can predict protein structure based on physical and biological information even when the homologs are absent (Jumper *et al*., 2021). RoseTTAFold was designed by DeepMind and generates an accurate 3D structure by transformation and integration of three-track network i.e. information at the 1D sequence level, 2D distance map level and 3D coordinate level (Baek *et al*., 2021; RoseTTAFold). RaptorX creates protein 3D structure with the help of its three components- single-template threading, alignment quality prediction and multiple-template threading (Peng and Xu, 2011; RaptorX).

Validation of model: The best predicted models using these softwares were then refined using GalaxyRefine2 server. GalaxyRefine2 uses an iterative optimization approach to refine both local

and global structures (Lee *et al*., 2019; GalaxyRefine2). Ramachandran plot was used to predict the stereochemical stability of the devised models. This plot was analysed by PROCHECK module (Laskowski *et al.*, 2005; PROCHECK) and indicated proportion of residues in preferred and allowed zones, amount of glycine and proline residues, dihedral angle orientation including phi (ϕ) and psi (ψ), and backbone conformation. The ProTSAV score (http://www.scfbio-iitd.res.in/software/proteomics/ProTSAV.jsp) was calculated as a combined value of the quality evaluation at several interfaces. Using Discovery Studio, several areas of the protein structure were identified in terms of aromatic receptor surface, hydrogen bonds and hydrophobic regions (Biovia).

Analysis of PPIN (Protein-protein interaction network): To determine the network of interactions with other proteins, the STRING (Version 11.5) (Search Tool for the Retrieval of Interacting Proteins) analysis was performed with a minimum needed interaction score of 0.700 (Szklarczyk *et al*., 2021; STRING Version 11.5).

RESULTS

Analysis of primary protein sequence: Caprine kisspeptin-135 is a 135 amino acid residue long protein with a molecular weight of 14.38 kDa and primarily displayed extremely alkaline features with a calculated pI value of 11.23. (Table 1). The CELLO2GO server verified that the protein is mostly found in the extracellular space and the nucleus, and it is predominantly involved in the signal transduction process (Fig. 1).

Prediction of 3D structure and model validation: The 3D structure was predicted by AlphaFold with the help of AlphaFoldColab. RoseTTAFold and RaptorX also predicted 3D structure without any prior template information. All these servers used ab-initio methods of protein prediction. All these predicted models were further refined by Galaxyrefine2 server. Ramachandran plot analysis revealed that AlphaFold and RoseTTAFold predicted structures had higher accuracy than RaptorX predicted model since more residues were present in acceptable region. Comparison between Ramachandran plots were enlisted in Table 2 and shown in Fig. 2.

ProTSAVscore was also compared to evaluate the protein models. ProTSAV score indicates different rmsd values for different models. ProTSAV scores were 0.5416, 0.6477 & 0.787 for RaptorX, RoseTTAFold & AlphaFold, respectively. For RaptorX model (which is the best predicted model according to rmsd score), the model resided at faint yellow region i.e., the rmsd value is in between 2-5 Å, whereas for all other predicted model rmsd

Table 1. Physicochemical parameters of caprine kisspeptin-135 proteins

Physical and chemical parameters	Kisspeptin (135)
Number of amino acids	135
Theoretical pI	11.23
Molecular weight	14383.53
Instability index	41.81 (Unstable)
Aliphatic index	66
Total number of negatively charged residues (Asp + Glu)	7
Total number of positively charged residues (Arg + Lys)	19
Grand average of hydropathicity (GRAVY)	-0.41
Half Life	30 hrs (Mammalian reticulocyte, *In Vitro*) >20 hrs (Yeast, *In Vivo*) >10 hrs (E. Coli, *In Vivo*)

Table 2. Comparison between amino acid residues of different protein models in several regions of Ramachandran plot

Amino acid residue in Ramachandran plot	Model prediction Software	Percentage
Residues in most favoured region	AlphaFold	95.20%
	RoseTTAFold	88.60%
	RaptorX	80.00%
Residues in additional allowed region	AlphaFold	4.80%
	RoseTTAFold	10.50%
	RaptorX	16.20%
Residues in generously allowed region	AlphaFold	0.00%
	RoseTTAFold	1.00%
	RaptorX	1.00%
Residues in disallowed region	AlphaFold	0.00%
	RoseTTAFold	0.00%
	RaptorX	2.90%

score is more than 5 Å. This indicates the unstable nature of the protein. RaptorX model ProTSAV graph is provided in Fig 3.

Protein-protein interaction network: Protein-protein interaction network (PPIN) predicted by STRING (Version 11.5) revealed 10 probable interacting partners with higher confidence (0.700) viz. Kiss1R (G-Protein-Receptor F12 domain-containing protein), GNRH1 (Gonadoliberin), TAC3 (Neurokinin B), TAC1 (Tacykinin precursor 1), TMEM252 (Transmembrane protein 252), HCRT (Orexin), NPSR1 (Neuropeptide S receptor 1), TACR3 (Tachykinin receptor 3), TSHB (Cys-knot domain-containing protein), MLNR (Motilin receptor).

DISCUSSION

Analysis of primary sequence revealed that the kisspeptin-135 is an unstable protein as kisspeptin-135 is a pre-pro hormone which is cleaved inside the cell and ultimately forms kisspeptin10 which is physiologically active isoform (Tena-Sempere, 2006). Kisspeptin protein is basic in nature. GRAVY value indicates as negative i.e., the protein is hydrophilic in nature (Chang and Yang, 2013). Ramachandran plot analysis of the 3D structures proved AlphaFold predicted model was superior to other models, but later analysis by ProtSAV ranked RaptorX predicted model to be superior. ProtSAV takes into account various validation softwares which were listed in Fig. 3, and the basis of their validation were also enlisted in the same. We have taken ProtSAV score as the final basis of selection of superior model as it used various validation techniques including Ramachandran plot analysis. Localization analysis revealed extracellular location, and major biological process related to kisspeptin, is signal transduction. This is because of the fact that the kisspeptin act as neurohormone which is secreted near the vicinity of GnRH secretory nerves by the Anteroventral periventricular Neurones (AVPV) and Arcuate nucleus (ARC) neurones and serves as a potent secretagogue of GnRH (Dungan *et al*., 2006). Major molecular function is protein binding as kisspeptin binds with its receptor Kiss1R/ GPR54 (G protein couple receptor 54) in GnRH neurone to exert its action (Franssen and Tena-Sempere, 2018).

Intra-protein characterization is an essential aspect of protein modelling as it indicates various molecular functions. Aromatic-aromatic interaction is one of the bases of prediction of stability of protein conformation. Individually, these interactions give modest structural stability; nevertheless, when combined, they greatly contribute to the protein structure's conformational stability (Anjana *et al*., 2012). Fig. 6a represents a 3-dimensional surface

3D structure prediction of goat kisspeptin precursor protein

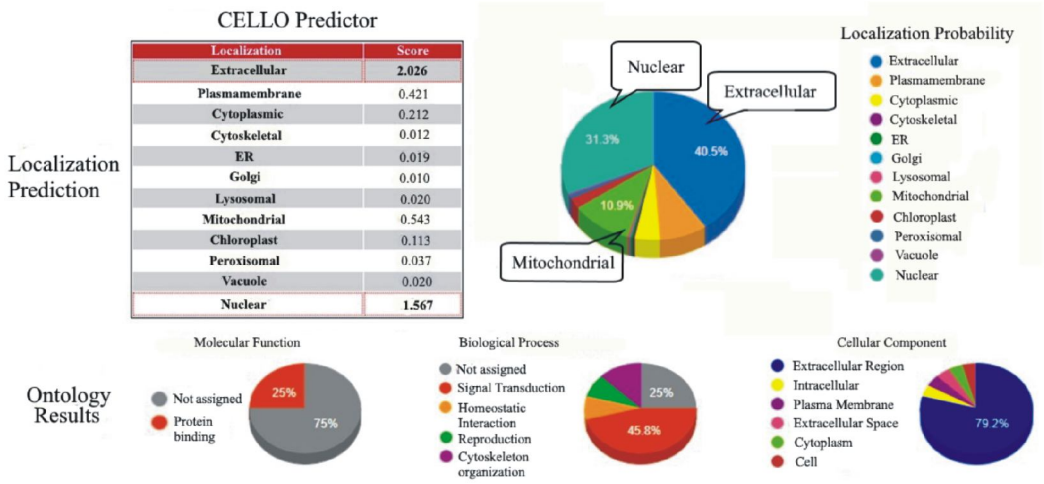


Fig. 1. Functional annotation in terms of ontology predicted by CELLO2GO server

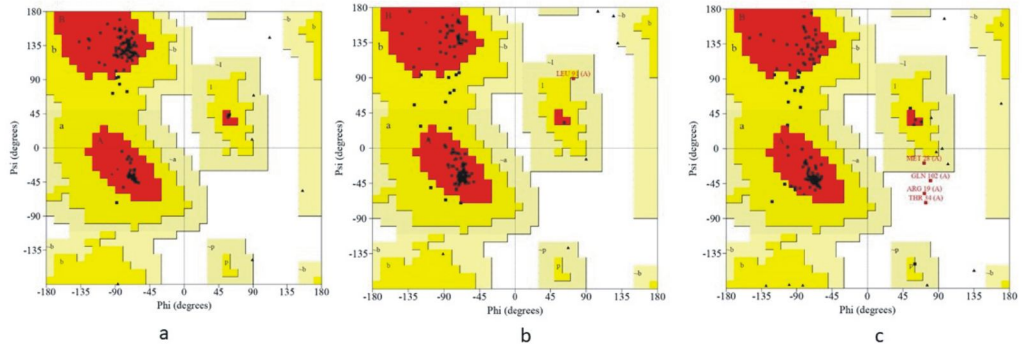
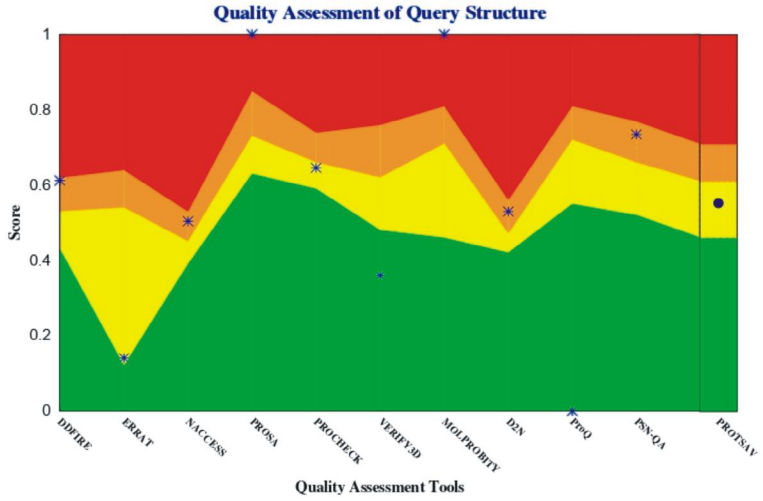


Fig. 2. Ramachandran plot of protein models predicted by different software- a. AlphaFold, b. RoseTTAFold, c. RaptorX



[ProTSAV uses different online tertiary structure validation tools like DDFIRE, ERRAT, NACCESS, PROSA, PROCHECK, VERIFY3D, MOLPROBITY, D2N, ProQ, PSN-QA&PROTSAV. It checks various feature of protein like Non-covalent interactions, Residue based contact potential, Burial preference of residue contacts, Accessible surface area, Residue packing preference, Contact order, Globularity, Secondary structure Information, ϕ and ψ distribution in Ramachandran plot, Energy based scorings&Side chain packing (Singh *et al*., 2016).]

Fig. 3. ProTSAV score of RaptorX predicted kisspeptin protein model

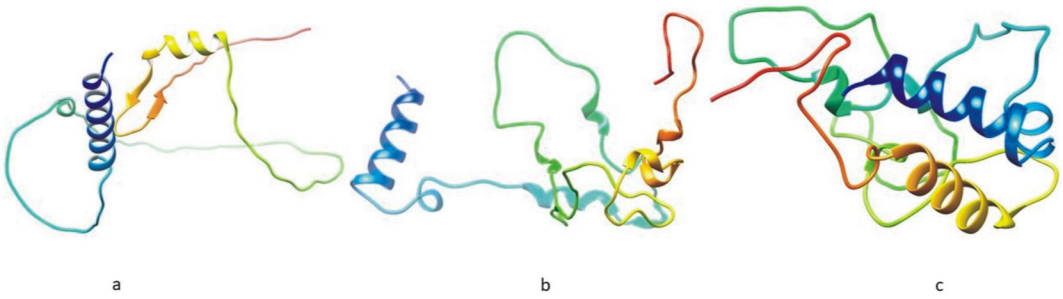


Fig. 4. Predicted tertiary structure of caprine kisspeptin- 135 protein by a. AlphaFold b. RoseTTAFold and c. RaptorX

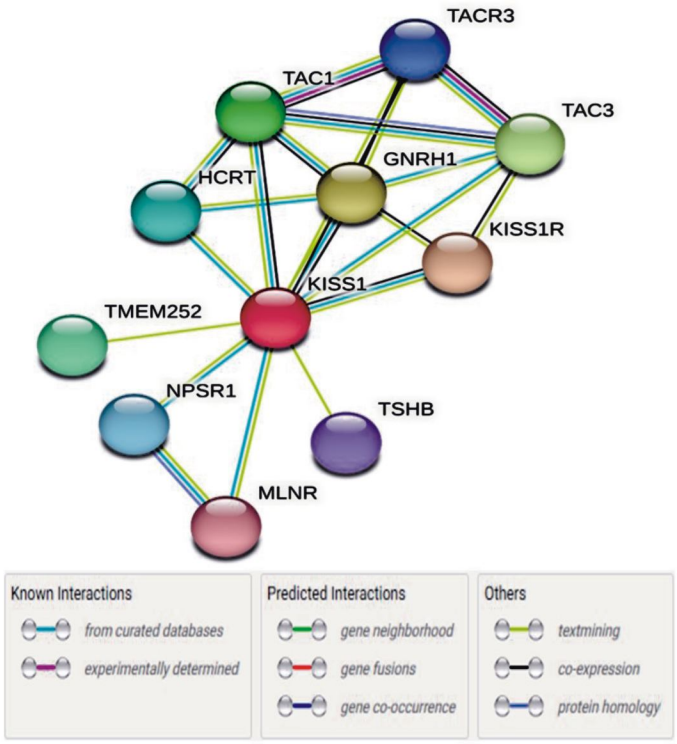


Fig. 5. Protein-protein interaction network of caprine kisspeptin protein (KISS1) revealed both experimentally derived and predicted interactions of physically and functionally related proteins with KISS1

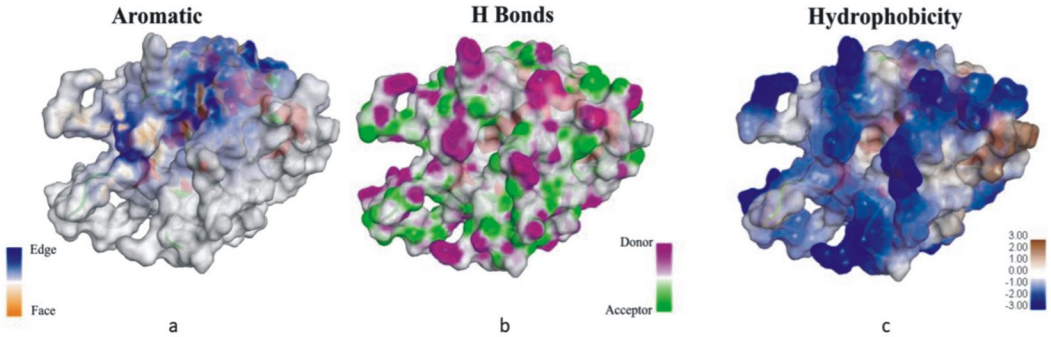


Fig. 6. Different regions of RaptorX predicted caprine Kisspeptin-135 protein according to a. aromatic receptor surface, b. hydrogen bond & c. Hydrophobic interaction

structure of edge and face given different colour to depict aromatic residues. Non-covalent hydrogen bonds ("hbonds") form, when a donor atom gives its covalently linked hydrogen atom to an electronegative acceptor atom. The oxygens in -OH (e.g., the sidechains of Ser, Thr, Tyr), HOH, and nitrogen in $-NH_3^+$ (as in the sidechains of Lys, Arg) or -NH- are typical donor atoms (as in the main chain peptide bond, and the sidechains of Trp, His, Arg, and nucleotide bases). These same donors' lone electron pairs (when they lack hydrogen atom) can act as hbond acceptor sites. Typical examples are- carbonyl oxygens =O (as in the main chain of proteins) or nitrogens with three covalent bonds =N- (as in the sidechains of His, Trp, or in nucleotide bases) (https://proteopedia.org/wiki/index.php/Hydrogen_bonds). Fig. 6b depicts H bonds in 3D structure revealing all donor and acceptor sites. Hydrophobic interactions are the key elements to be considered regarding protein folding (Camilloni *et al*., 2016). More positive value in the area of the protein structure indicates more hydrophobic region. Our structure of Fig. 6c indicates both more hydrophobic and less hydrophobic regions of the protein.

Protein-protein interaction network analysis by STRING provide top 10 physically and functionally related interacting partners with high confidence (0.700). Most potent interaction is with Kiss1R which is the receptor of the kisspeptin. This fact is quite obvious. Another interesting fact is its interaction with Gonadoliberin-1 which is a member of GnRH peptide family and responsible for the secretion of LH & FSH (NCBI-Gene Database- https://www.ncbi.nlm.nih.gov/gene/2796). TAC1 & TAC3 are tachykinin genes which encode some pre-pro-tachykinins which ultimately undergo post-translational proteolytic cleavage (Steinhoff *et al*., 2014). Post modification products of TAC1 are neurokinin A (NKA), neuropeptide γ, neuropeptide K and substance P (SP) whereas for TAC3, the product is neurokinin B (De Biasi *et al*., 2016). These products are important novel neuro-peptide and related to GnRH secretion. Kisspeptin and Neurokinin B neurones are part of kisspeptin, neurokinin B and dynorphin (KND_y) system of Arcuate nucleus (ARC) which can potentially upregulate or downregulate GnRH secretion according to the needs (Moore *et al*., 2019).

TACR3 is one of the most common receptors of tachykinins. Interestingly, our observation from PPIN analysis indicates co-expression of kisspeptin with Kiss1R, GnRH1, TAC1 and TACR3, but we couldn't identify any such co-expression between kisspeptin and TAC3 (neurokinin B) in goat which is absolutely contrary from the findings in human and rodent experimental studies. This indicates either species variance or lack of experiment in the particular direction in caprine species. Hypocretin neuropeptide precursor (HCRT) encodes a hypothalamic neuropeptide precursor protein which later cleaves to form Orexin A and Orexin B protein (NCBI Gene Database- https://www.ncbi.nlm.nih.gov/gene/3060). Orexin, a neuropeptide, mainly controls arousal, wakefulness and appetite (Davis *et al*., 2011). Orexin can modulate GnRH secretion via its effect on KNDy system (Hosseini and Khazali, 2018).

Overall, we have predicted kisspeptin-135 protein structure by ab initio structure prediction strategy, which is probably for the 1st time in any domestic animal species. This structure can be used for further analysis of molecular docking studies with its receptor and other orphan G protein couple receptor.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: IM: Planned and executed the whole research work, involved in manuscript preparation; IR: Helped to execute the work, checked the manuscript for error and also corrected the reference section.

ACKNOWLEDGEMENT

The authors are extremely grateful to the authorities of NDRI for providing facilities to carry out the work & also acknowledged the National Centre for Biotechnology Information (NCBI) and all other authorities of the online tools used in this work for providing free access to their databases and software.

REFERENCES

20th Livestock Census: https://vikaspedia.in/agriculture/agri-directory/reports-and-policy-briefs/20th-livestock-census [Accessed on 29/09/2022]

Anjana R, Vaishnavi MK, Sherlin D, Kumar SP, Naveen K *et al*., 2012. Aromatic-aromatic interactions in structures of proteins and protein-DNA complexes: A study based on orientation and distance. Bioinformation, 8(24): 1220-1224, doi: 10.6026/97320630081220

Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S *et al*. 2021 Accurate prediction of protein structures and interactions using a three-track neural network. Science, 373(6557): 871-876. doi: 10.1126/science.abj8754

Camilloni C, Bonetti D, Morrone A, Giri R, Dobson CM *et al*., 2016. Towards a structural biology of the hydrophobic effect in protein folding. Sci Rep, 6: 28285, doi: 10.1038/srep28285

CELLO2GO tool available in: http://cello.life.nctu.edu.tw/cello2go/alignment.php

Chang KY and Yang JR, 2013. Analysis and prediction of highly effective antiviral peptides based on random forests. PLoS One, 8(8): e70166, doi: 10.1371/journal.pone.0070166

Davis JF, Choi DL and Benoit SC, 2011. "24. Orexigenic Hypothalamic Peptides Behavior and Feeding - 24.5 Orexin". In Preedy VR, Watson RR, Martin CR (eds.). Handbook of Behavior, Food and Nutrition. Springer. pp 361-362. ISBN 9780387922713

De Biasi M, McLaughlin I and Klima ML, 2016. Nicotine and neurokinin Signaling. In: Neuropathology of drug addictions and substance misuse. Elsevier, Amsterdam, pp 189-200

Dungan HM, Clifton DK and Steiner RA, 2006. Minireview: kisspeptin neurons as central processors in the regulation of gonadotropin-releasing hormone secretion. Endocrinology, 147(3): 1154-1158, doi: 10.1210/en.2005-1282

Fatet A, Pellicer-Rubio MT and Leboeuf B, 2011. Reproductive cycle of goats. Anim Reprod Sci, 124(3-4): 211-219, doi: 10.1016/j.anireprosci.2010.08.029

Franssen D and Tena-Sempere M, 2018. The kisspeptin receptor: A key G-protein-coupled receptor in the control of the reproductive axis. Best Pract Res Clin Endocrinol Metab, 32(2): 107-123, doi: 10.1016/j.beem.2018.01.005

GalaxyRefine2 tool available in: (http://galaxy.seoklab.org/refine2)

Gasteiger E, Hoogland C, Gattike A, Duvaud S, Wilkins MR *et al.*, 2007. A protein identification and analysis tools on the ExPASy server, Humana, Louisville, pp 571-607

Hasan MJ, Ahmed JU, Alam MM, Mojumder MLO and Ali MS, 2015. Reproductive performance of Black Bengal goat under semi-intensive and extensive condition in Rajshahi district of Bangladesh. Asian J Med Biol Res, (1): 22-30

Hosseini A and Khazali H, 2018. Central Orexin A affects reproductive axis by modulation of hypothalamic kisspeptin/neurokinin B/dynorphin secreting neurons in the male Wistar rats. Neuromolecular Med, 20(4): 525-536, doi: 10.1007/s12017-018-8506-x

Hu KL, Zhao H, Chang HM, Yu Y and Qiao J, 2018. Kisspeptin/ kisspeptin receptor system in the ovary. Front Endocrinol, 8: 365, doi: 10.3389/fendo.2017.00365

Jumper J, Evans R, Pritzel A, Green T, Figurnov M *et al*., 2021. Highly accurate protein structure prediction with AlphaFold. Nature, 596: 583-589 doi:10.1038/s41586-021-03819-2

Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM *et al*., 2001. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. J Biol Chem, 276(37): 34631-34636, doi: 10.1074/jbc.M104847200

Laskowski RA, Chistyakov VV and Thornton JM, 2005. PDBsum more: new summaries and analyses of the known 3D structures of proteins and nucleic acids. Nucleic Acids Res, 33(sppl-1): D266-D268, doi: 10.1093/nar/gki001

Lee GR, Won J, Heo L and Seok C, 2019. GalaxyRefine2: simultaneous refinement of inaccurate local regions and overall protein structure. Nucleic Acids Res, 47(W1):W451–W455, doi: 10.1093/nar/gkz288

Mondal M, Baruah KK and Prakash BS, 2015. Determination of plasma kisspeptin concentrations during reproductive cycle and different phases of pregnancy in crossbred cows using bovine specific enzyme immunoassay. Gen Com Endocrinol, 224: 168-175, doi: 10.1016/j.ygcen.2015.08.014

Moore AM, Coolen LM and Lehman MN, 2019. Kisspeptin/neurokinin B/bynorphin (KNDy) cells as integrators of diverse internal and external cues: evidence from viral-based monosynaptic tract-tracing in mice. Sci Rep, 9: 14768, doi: 10.1038/

s41598-019-51201-0

Peng J and Xu J, 2011. Raptor X: exploiting structure information for protein alignment by statistical inference. Proteins, 79(Suppl 10): 161-71, doi: 10.1002/prot.23175

PROCHECK tool available in: https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/ [Accessed on 22.09.2022]

Proteopedia available in: https://proteopedia.org/wiki/index.php/Hydrogen_bonds [Accessed on 22/09/2022]

Protparam tool available in: http://web.expasy.org/protparam/ [Accessed on 22/09/2022]

RaptorX tool available in: http://raptorx.uchicago.edu/ [Accessed on 22/09/2022]

RoseTTAFold tool available in: https://robetta.bakerlab.org/submit.php [Accessed on 22/09/2022]

Scott CJ, Rose JL, Gunn AJ and McGrath BM, 2019. Kisspeptin and the regulation of the reproductive axis in domestic animals. J Endocrinol, 240: R1-R16, doi: 10.1530/JOE-18-0485

Singh A, Kaushik R, Mishra A, Shanker A and Jayaram B, 2016. ProTSAV: A protein tertiary structure analysis and validation server. Biochim Biophys Acta, 1864(1): 11-19, doi: 10.1016/j.bbapap.2015.10.004

Steinhoff MS, von Mentzer B, Geppetti P, Pothoulakis C, and Bunnett NW, 2014. Tachykinins and their receptors: contributions to physiological control and the mechanisms of disease. Physiol Rev, 94(1): 265-301, doi: 10.1152/physrev.00031.2013

STRING Version 11.5 tool available in: https://string-db.org/ [Accessed on 22/09/2022]

Szklarczyk D, Gable AL, Nastou KC, Lyon D, Krisch R *et al*., 2021. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res, 49(D1): D605-D612, doi: 10.1093/nar/gkaa1074

Tena-Sempere M, 2006. GPR54 and kisspeptin in reproduction, Hum Reprod Update, 12(5): 631-639, doi: 10.1093/humupd/dml023

Yu CS, Cheng CW, Su WC, Chang KC, Huang SW *et al*., 2014. CELLO2GO: A Web Server for Protein subCELlularLOcalization prediction with functional gene ontology annotation. PLoS One, 9(6): e99368, doi: 10.1371/journal.pone.0099368

Zhang Y, 2009. Protein structure prediction: is it useful? Curr Opin Struct Biol, 19(2): 145-155, doi: 10.1016/j.sbi.2009.02.005

Zheng J, Raza SHA, Zi XD and Lu JY, 2018. Comparing KISS1 and GPR54 genes between the two breeds of goats with low and high prolificacy. Gen Mol Res, 17(4): 1 -11, doi: 10.4238/GMR16039922

Received- 19.10.2022, Accepted- 22.11.2022, Published- 28.11.2022(Online), 01.12.2022 (Print)
Section Editor: Prof S. K. Nandi, Associate Editor