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Analysis and Importance of FSHβ Gene in Pig Reproductive Performance

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ABSTRACT

FSHβ plays an important role in the reproductive performance of pigs, but there are no studies on its protein structure and function. In this study, bioinformatics tools were used to predict the physicochemical properties, secondary and tertiary structure, hydrophobicity/hydrophilicity, transmembrane domain, and sites of phosphorylation and glycosylation of signal peptide FSHβ protein of sows. The results showed that the number of amino acids of FSHβ was 185, that is, the theoretical isoelectric amino acid point was 53.8, the instability index was 46.23, and the average hydrophilicity coefficient was 0.732. FSHβ protein was found to be a hydrophobic protein without a transmembrane domain, with 33 phosphorylation sites. None of the signal peptides was found to be distributed in the inner complete sequence. The secondary structure was mainly composed of α-helix, extended strand, β-turn, and random coil, with values of 29.97, 27.53, 10.10 and 32.40%, respectively. In summary, this study suggests that the amino acid sequence (988~1146aa) of FSHβ can be used to express antigens. It provides a reliable basis for further study of FSHβ protein function, purification of FSHβ protein, preparation of FSHβ antibody, and drug screening to improve reproductive performance of pigs.

Key words: FSHβ; pig; Reproductive performance.

INTRODUCTION

Reproductive traits play a key role in the productive performance of the pigs. The breeding performance of sows is an important biological index to measure the benefits of pig farms, and it is a key indicator of factor that restrict the improvement of breeding performance and production efficiency of sows at large-scale sow farms (Wang et al. 2018; Hickmann et al. 2021). In pigs, reproductive activity is controlled and regulated by the brain-pituitary-gonadal (BPG)-endocrine axis (Koketsu and Iida 2017). The classical reproductive hormones of the BPG-axis are gonadotropin-releasing hormone (GnRH), pituitary gonadotropins (GtHs), and gonadal steroids, as along-with a host of other regulatory molecules that act on the BPG-axis in a paracrine/autocrine manner (Khan and Chaudhary 2021; Uenoyama and Tsukamura 2023). The pars distalis of the anterior pituitary gland secretes the GtHs, which are glycoproteins in nature (Weiss et al. 2019). The both pituitary gonadotropins, luteinizing hormone (LH) and the follicle-stimulating hormone (FSH), are complex heterodimer glycoproteins, composed of a common alpha (α) subunit and a species-specific beta (β) subunit (Sand et al. 2013; Gagnon et al. 2018). These subunits bind non-covalently to form a biologically active dimeric peptide hormone (Boime and Ben-Menahem 1999; Gilbert et al. 2018).

However, the FSHβ gene is linked to many other genes controlling pig litter size and is the main gene controlling this trait in pigs (Prabhudesai et al. 2021). FSHβ plays a key role in follicular development and estrogen production in female animals, and the level of estrogen in the peripheral blood of sows and other animals during estrus is a decisive factor for their reproductive performance (Koketsu and Iida 2017; Gagnon et al. 2018; Simon et al. 2019). At the same time, a recent study has shown that FSH can be used as a useful marker for the identification of ovarian cytoma (Matsuoka et al. 2022). Moreover, LHR expression has been detected in the blood of sows at the estrus stage, indicating that the expression of LHR in relevant tissues is related to the changes occurring during estrus cycle in the animal (Guan et al. 2021; Kopycińska et al. 2022). In the current study, bioinformatics analysis was performed to have a reference for the effective prokaryotic extraction/expression and protein purification of the FSHβ gene and to provide a strong basis for the association between the FSHβ gene and the reproductive performance of sows.

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MATERIALS AND METHODS

Amino Acid Sequences

The complete amino acid sequences of *FSHβ* protein (Species, *Sus scrofa* (pig), NC_010444, Gene id: 396895) were obtained from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/).

Prediction of Physicochemical Parameters

The online *FSHβ* STAT3 proteins was used for the prediction of physicochemical parameters including the amino acid composition, molecular weight, total atomic number, theoretical isoelectric point, instability index, extinction coefficient, parent level mean, and other physicochemical parameters of *FSHβ* protein.

Prediction of Hydrophilicity, Hydrophobicity, and Transmembrane Domains

The hydrophilicity and hydrophobicity of the FSHβ protein was analyzed by the Kyte and Doolittle algorithm of the ExPASy server ProtScale module (https://web.expasy.org/protscale/). The transmembrane domains of the *FSHβ* proteins were further analyzed by the TMHMM Server (http://www.cbs.dtu.dk/services/TMHMM-2.0/).

Analysis of Phosphorylation and Glycosylation Sites

The potential N-linked phosphorylation and glycosylation sites of STAT3 were predicted using NetPhos 3.1 (http://www.cbs.dtu.dk/services/NetPhos/) and NetNGlyc 1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc/), respectively.

Subcellular Localization and Signal Peptide Identification

The subcellular localization of *FSHβ* protein was predicted from the amino acid sequence software "Predictprotein" available online (https://www.predictprotein.org/). The presence and location of signal peptide cleavage sites in the amino acid sequences were predicted by the SignalP 4.0 (http://www.cbs.dtu.dk/services/SignalP-4.0/) with a D-cutoff score of 0.5.

Secondary and Tertiary Structure Prediction

The secondary and tertiary structures of the FSHβ protein were predicted by the SOPMA (https://npsa-prabi.ibcp.fr/NPSA/npsa_sopma.html) and SWISSMODEL (https://swissmodel.expasy.org/) software, respectively. In addition, the conserved domain of FSHβ protein was analyzed in the Conserved Domain Database (CDD) of NCBI. (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

RESULTS

Physical and Chemical Properties

The online prediction and analysis of the physical and chemical properties of FSHβ3 protein revealed the presence of 149 amino acids, with the molecular weight of 12398.89 and total number of atoms as 1574. The molecular formula was $C_{449}H_{751}N_{149}O_{190}S_{35}$, with the theoretical isoelectric point was 5.38. The instability index was computed to be 46.23, which classified the protein as unstable. The aliphatic index was 24.16 and the grand average of hydropathicity (GRAVY) was 0.732, which indicated that FSHβ may be a hydrophobic protein.

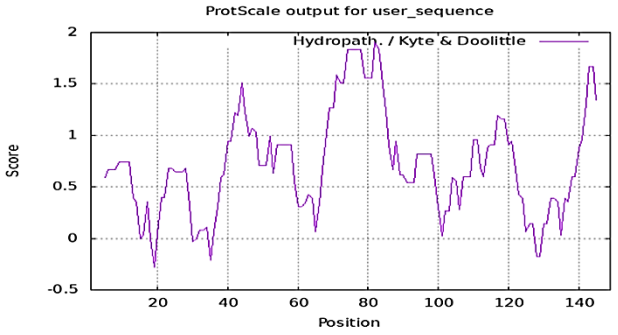

Fig. 1: Hydrophilicity prediction: Positive scores specify hydrophobicity, while negative scores signify hydrophilicity. Higher the absolute value, higher hydrophilicity degree is.

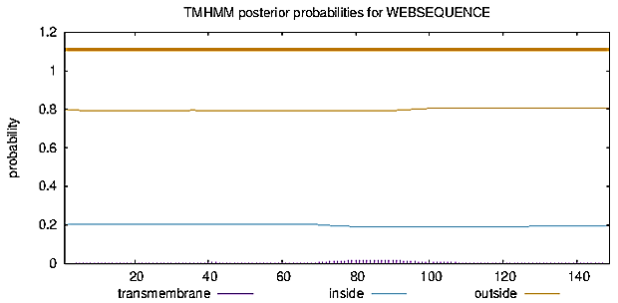

Fig. 2: Transmembrane domain of *FSHβ* protein. The dark yellow line represents the *FSHβ* protein, the yellow line below represents the outer cell membrane, and the blue line represents the inner cell membrane.

Hydrophilicity and Hydrophobicity and Transmembrane Domains

The evenly distributed hydrophobic amino acids were found to form most of the FSHβ polypeptide. The software analysis showed that the highest hydrophobicity score of FSHβ protein was -0.278 and the highest hydrophilicity value was 1.911. The tide chain and the whole polypeptide chain showed hydrophobic amino acids (Fig. 1). This was also stable with the average hydrophobicity index already projected by the ExPASy ProtParam online analysis system. No evidence of a hydrophobic region was found in FSHβ, so it was speculated that there is no transmembrane region in it. TMHMM analysis showed that there was no obvious transmembrane region in the amino acids sequence of FSHβ protein.

Sites of Phosphorylation and Glycosylation

A gene expression regulatory protein, directly linked to the functions, is an important characteristic of phosphorylation. The results of predictive analysis of the phosphorylation site of FSHβ protein using the bioinformatics software NetPhos 3.1 showed that in the FSHβ peptide chain when the threshold of potential phosphorylation sites was 0.5, there were multiple phosphorylation sites of FSHβ protein (Fig. 3), of which serine (Ser), threonine (Thr) and tyrosine (Tyr) had 33, 16 and 6 sites, respectively. As is evident in Fig. 4, there were

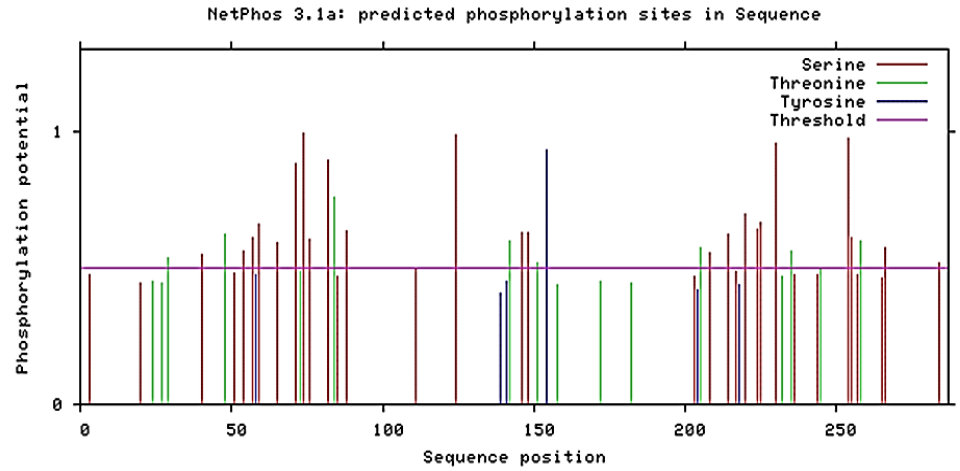

Fig. 3: Phosphorylation site of FSHβ protein. Serine phosphorylation sites are shown by red lines at 33 sites (3, 20, 40, 51, 54, 57, 59, 65, 71, 74, 76, 82, 85, 88, 111, 124, 146, 148, 203, 208, 214, 217, 220, 224, 225, 230, 236, 244, 254, 255, 257, 266, 283), while green and blue lines indicate threonine phosphorylation (16 positions: 24, 27, 29, 48, 73, 84, 142, 151, 158, 172, 182, 205, 232, 235, 245, 258) and tyrosine phosphorylation sites (6 sites: 58, 139, 141, 154, 204, 218), respectively. The threshold is indicated by purple lines.

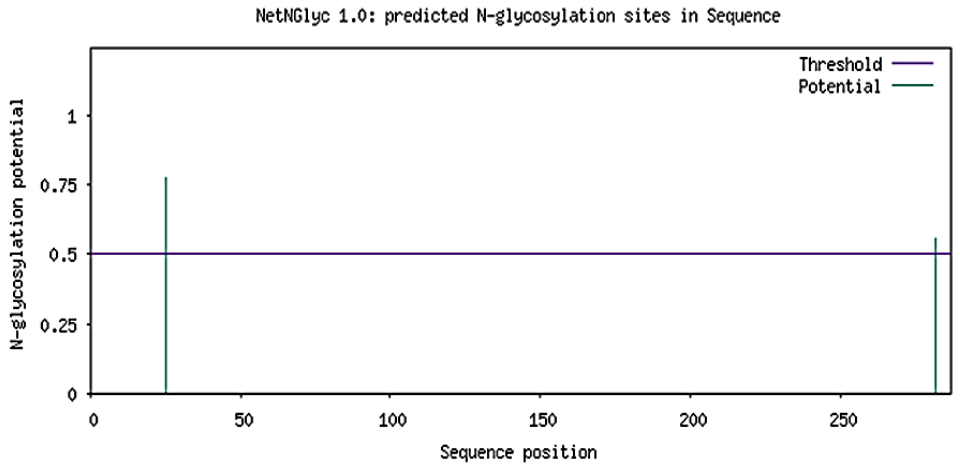

Fig. 4: Prediction of glycosylation sites. The potential glycosylation sites are shown by green lines, while threshold is indicated by purple lines.

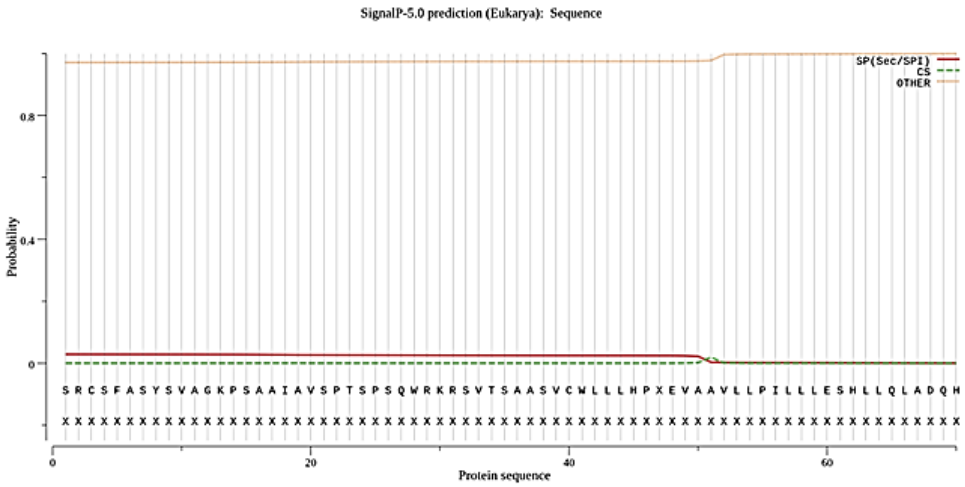

Fig. 5: Signal peptide of FSHβ protein. The red, green and blue lines represent C, S and Y scores.

two n-glycosylation reformed sites at 25(0.7746) NITI and 285(0.5569) NCSD, indicating that these sites may be encompassed at more than one peptide.

Subcellular Localization and Signal Peptide Identification

Results obtained from the neural network algorithm (Fig. 4) indicated that there was a 0.0282 probability of having sec signal peptide, while no signal peptide probability was 0.9718. These results indicate that this could not be a secretory protein, which was coherent with the results of glycosylation.

Secondary and Tertiary Structure

The prediction of the secondary structure of FSHβ protein by SOPMA software showed that there were 86 α-helixes, 79 extended strands, 29 β-turns and 93 random coils in the FSHβ amino acid sequence, accounting for 29.97, 27.53, 10.10 and 32.40% of the secondary structure, respectively (Fig. 6). According to the prediction of SWISS-MODEL, there were many α-helixes in the tertiary structure of FSHβ protein; these were mainly α-helix and random coil (Fig. 7), which was basically coherent with the estimation of secondary form.

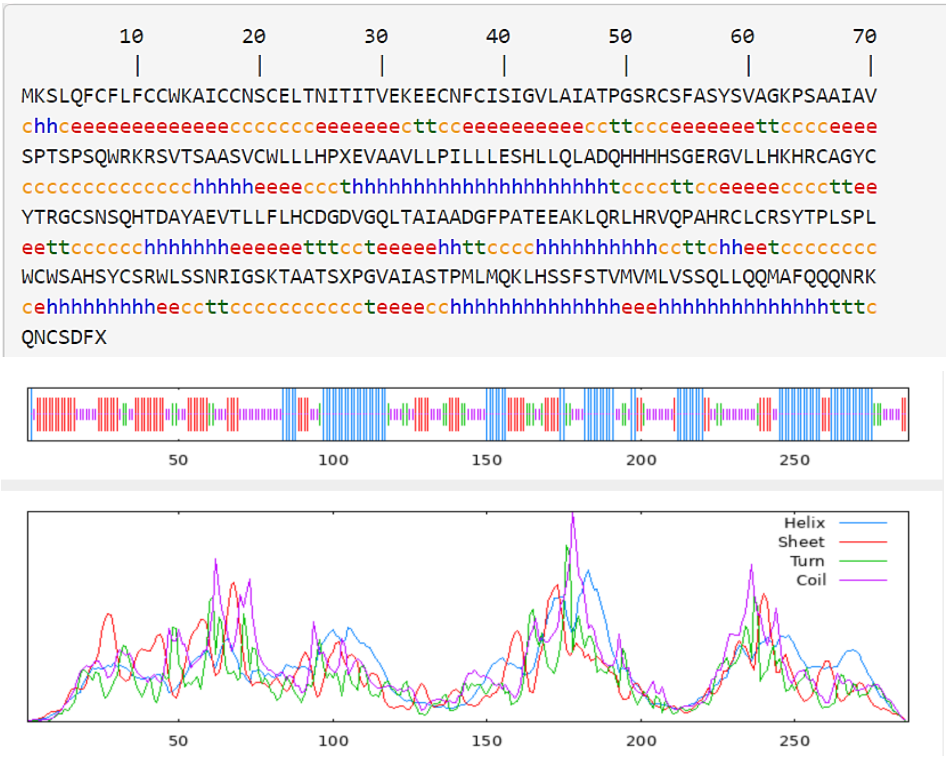

Fig. 6: FSHβ secondary structure. H = α-helix; C = Disordered crimped state. Blue = α-helix; purple = irregular crimping; red = extension chain and green = β-rotation.

Fig. 7: Tertiary structure of FSHβ protein. Red colour indicates α-helix, yellow indicates β-turning angle, green indicates Random coil, and blue indicates transmembrane region.

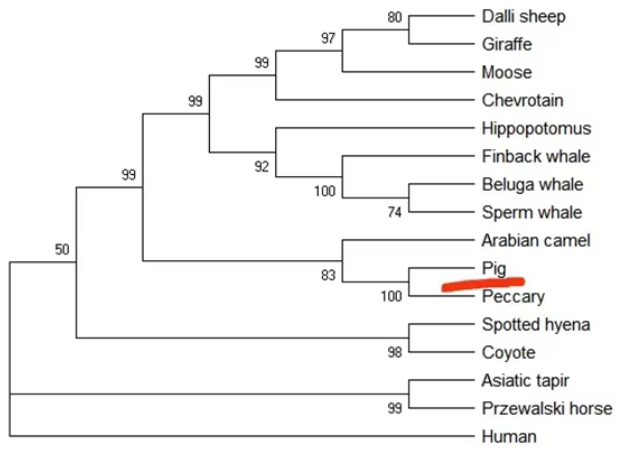

Fig. 8: Construction of FSHβ gene phylogenetic tree. The red triangle marks the species in this study.

Overall Analysis and Phylogenetic Analysis

Sequencing results and the NCBI (https://www.ncbi.nlm.nih.gov/) published homologous gene sequences, and built the system evolutionary tree, as shown in Fig. 8. The results showed that the homology between the porcine FSHβ gene and the porcine nucleotide sequence was 100%.

DISCUSSION

Reproductive traits are known to play an important role in the productive performance of pigs (Li et al. 2017b; Bovula et al. 2021; Ma et al. 2022). Published literature indicates that FSHβ is directly linked to reproductive performance (Zhao et al. 1998; Li et al. 2017a; Ye et al. 2018; Petrovas et al. 2020; Morton et al. 2023). In the present study, the bioinformatics analysis of FSHβ gene and its encoded protein was carried out, and the prediction of the property and structure of FSHβ protein provided the basis for the research and development and scientific basis of reproductive performance traits for the pig industry (Chen et al. 2018; Niu et al. 2019; Stamatiades et al. 2019; Lizneva et al. 2019; Zhu et al. 2020).

Bioinformatics has been widely and wisely used in the prediction of protein functions, gene recognition, determination of the physiological range of proteins, and prediction of advanced structure of protein to ensure that predicted results are accurate and efficient (Trevisan et al. 2019; Song et al. 2021; Brandes et al. 2022). In the present study, the basic information of FSHβ protein was obtained by bioinformatics analysis for further studies (Bernard and Tran 2013; Prabhudesai et al. 2021). Combined physicochemical properties of FSHβ protein showed that the protein was hydrophobic and did not have a transmembrane domain and also without the presence of signal peptide, which indicates that FSHβ protein may belong to wabbly hydrophilic non-secretory proteins (Kanasaki et al. 2013; Wang et al. 2021).

The FSHβ protein can freely diffuse into prokaryotic cells after its expression. In the prediction of subcellular

localization, our results indicated that FSHβ protein was also expressed in the nucleus (Liu et al. 2020; Tian et al. 2023). In addition, further prediction using TMHMM confirmed that there was no obvious transmembrane domain and signal peptide distribution in the FSHβ protein (Palmerini et al. 2016; Niu et al. 2019). Therefore, FSHβ protein could not be a protein present in the membrane; it was also coherent with the prediction that it is not a secreted protein, indicating that FSHβ protein can receive foreign signals and participate in cell regulation. It has a specific role in maintaining a steady state.

Protein glycosylation and phosphorylation play a significant role in cell signal transduction, immunity, gene expression adaptation, and protein degradation (Ulintz et al. 2019; Li et al. 2023). The role of glycosylation in the processing, stability, and function of secondary cellular proteins cannot be overlooked. In this study, the FSHβ protein was predicted to have more phosphorylation and glycosylation sites. It also indicates the complexity of FSHβ protein function. The prediction of the secondary structure of FSHβ protein by SOPMA showed that irregular curling and β-turning accounted for a large proportion of the secondary structure (Prabhudesai et al. 2020). Different secondary structures and super-secondary structures combine to form independent stable structural regions. These domains are functionally important, immutable, and highly stable (Lan et al. 2011). The domain rich and stable FSHβ sequence should be selected to express FSHβ protein, which is beneficial to the successful expression and purification of amino acids in the protein.

Conclusion

The FSHβ gene is highly conserved, has multiple phosphorylation sites with one glycosylation site, and is a hydrophilic protein. Because it is concentrated in the nucleus, the FSHβ gene does not have a transmembrane structure and is not a secreted protein. It can participate in transcriptional regulation during ontogeny and may have good immunogenicity.

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Authors' Contribution

QHG conceived the idea and designed the experiment. XL, SPC and SFC executed the experiments on bacterial sample collection, bacterial DNA extraction, PCR amplification, and gene sequence. XL analyzed the data and wrote the manuscript. All authors interpreted the data, critically reviewed the manuscript for important intellectual contents, and approved the final version.

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