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Research Article

TEMPLATE BASED STRUCTURE MODELLING AND INTERACTOMIC STUDY OF BOVINE FERTILIN-β: A SPERM SURFACE PROTEIN INVOLVED IN HANDSHAKING WITH EGG DURING FERTILIZATION

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ABSTRACT

Fertilin-β is a member of "A Disintegrin and Metalloprotease" domain protein family (ADAMs) localized on the surface of sperm. The protein is involved in binding of sperm with the zona pellucida and plasma membrane of oocytes and subsequent membrane fusion in mouse. How this important sperm surface protein executes its handshaking mechanism and its interacting partners on oocyte-side are still obscure. Our study describes physicochemical properties of bovine Fertilin- β, predicts its secondary 3-dimensional structure and interacting partners of the protein by *in silico* analysis. It is a negatively charged, hydrophilic, relatively unstable, metalloendo peptidase involved in sperm-oocyte adhesion process. β-1,4-galactosyl transferase 1, Zona pellucida sperm binding protein 4 (ZP4), Calmegin (CLGN), Integrin alpha-9 precursor (ITGA9) are the few binding partners on the oocyte side. The structure-function relationship analysis and interactomic study of the protein helps to dissect the mechanism of fertilization in better way.

Keywords: Bovine, Fertilin β, Sperm, Fertilization, 3-D

Artificial insemination (AI) with frozen semen from superior bulls is the mainstay of cattle breeding program in Indian dairy industry as good quality germplasm can be disseminated in a short duration of time over a large geographical area. One of the major obstacles to increase coverage of AI in breedable Indian cattle population is the availability of bulls with sound fertility potential. Male fertility is defined as the ability of sperm to fertilize the egg and sustain embryo development (Parisi *et al.*, 2014). Fertilization involves multitude of processes leading to attachment of sperm and oocyte membranes, their cytoplasmic unity and fusion of genomes, starting the development of a new individual. Several molecular players and their timely function in the process of sperm-oocyte membrane contribute to handshaking of sperm and oocyte and successful fertilization event in mammals.

Fertilin, a heterodimer complex composed of two integral membrane glycoproteins named Fertilin-α (ADAM-1) and Fertilin-β (ADAM-2) (also previously named PH-30 alpha and PH-30 beta in guinea pig), is a crucial sperm surface protein involved in sperm-oocyte recognition and in membrane fusion (Blobel, 2000; Cho *et al.*, 2000; Edwards *et al.*, 2009). Both proteins are members of the "A Disintegrin And Metalloprotease" domain protein family (ADAMs) (Edwards *et al.*, 2009). Prediction of the three-dimensional structure of proteins leads to paradigm shift in the understanding of cellular process conferred by the protein. Thorough understanding of the structure of this protein will help to delineate the sperm and oocyte handshaking mechanism during fertilization. Therefore, present study is focused to predict the 3-dimensional structure of Fertilin-β, their interacting partners on oocytes.

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MATERIALS AND METHODS

Protein sequence retrieval and phylogenetic analysis

Amino acid sequences of Fertilin-β from different mammalian species were retrieved from NCBI. The sequences were aligned, curated and phylogenetic tree was constructed using Phylogeny. fr (Dereeper *et al.*, 2008, 2010).

Physicochemical properties

Physicochemical properties of bovine Fertilin-β was analyzed using ProtParam tool (Gasteiger *et al.*, 2005). Various physicochemical parameters vis-à-vis molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index, and grand average of hydropathy (GRAVY) have been tabulated in Table 1.

Prediction of secondary structure

Secondary structure of Fertilin-β containing α-helix, β-sheet, coil and turn was predicted using Psipred and RaptorX (Kallberg *et al.*, 2012).

Prediction of 3D structure and post translational modification sites on Fertilin- β

3-D structure of Fertilin-β was predicted using RaptorX (Kallberg *et al.*, 2012).The amino acid sequence of Fertilin-β was provided as inputs in RaptorX. Upon a PSI-BLAST search against the Protein Data Bank (PDB), 2dw0A was identified as the best template (p-value 2.66e-

Table1

Physicochemical properties of Bovine Fertilin- β predicted by ProtParam tool

Physicochemical properties	Bovine Fertilin-β	Remarks
Molecular weight	83150.4 Da	-
Isoelectric point	5.26	Negatively charged protein
Instability index	41.38	Probably unstable in test tube
Aliphatic index	76.11	-
Grand average of hydropathicity (GRAVY)	-0.250	-

18)for modelling. The glycosylation sites were predicted by using NetOGlyc, NetNGlyc and Yin-O-Yang tools provided by Centre for Biological Sequence Analysis, Technical University of Denmark (CBS DTU) (Gupta and Brunak, 2002).

Prediction of functional protein association network

Search Tool for the Retrieval of Interacting Genes/ Proteins (STRING) database was used to map the interaction network of Fertilin-β. The data was predicted by neighborhood, gene fusion, co-occurrence, co-expression experiments, databases and text-mining with confidence value of 0.400 (Jensen *et al.*, 2009).

RESULTS AND DISCUSSION

Present study retrieved the amino acid sequences of Fertilin-β of different mammalian species and a phylogenetic tree was created (Fig. 1). Different physiochemical properties were analyzed by ProtParam from the amino acid sequences. Bovine Fertilin-β is a 83150.4 Dalton negatively charged protein with isoelectric point 5.26. The negative GRAVY index of -0.025 is indicative of a hydrophilic and soluble protein. Different physicochemical properties of the protein molecule has been mentioned in Table 1.

Conserved domain structure and secondary structure prediction

NCBI-CDD was used to find out the conserved domains within the protein (Fig. 2). Amino acid sequence 34 to 140 represents Reprolysin family propeptide (Pep_M12B_propep), a propeptide for members of peptidase family M12B. The propeptide contains a sequence motif similar to the "cysteine switch" of the matrixins. This motif is found at the C terminus of the alignment. Amino acid sequence 178 to 373 represents Zinc-dependent metalloprotease; adamalysin_II_like subfamily. Adamalysin II is a snake venom zinc endopeptidase. This subfamily contains other snake venom metalloproteinases, as well as membrane-anchored metalloproteases belonging to the ADAM family. ADAMs are glycoproteins, which play roles in cell signaling, cell fusion, and cell-cell interactions. Amino acid sequence 178 to 375 is Reprolysin (M12B) family zinc metalloprotease; the members of this family are enzymes that cleave peptides. These proteases require zinc for catalysis.

8-class secondary structure was predicted by Raptor X (Fig. 3). It shows α-helix, β-helix, π-helix, extended strand in β-ladder, isolated β-bridge, hydrogen bonded turn, bend and coil. According to structure predicted by the software, 18% of amino acid residues conform α-helix, 21% conforms a β-sheet and 60% conforms coil.

3-D Structure Prediction of bovine Fertilin- β

The 3D model of a protein is essential to understand the structure-function relationship. Numerous online servers and tools are available for structure prediction of protein by homology modeling. RaptorX is a statistical method for template-based protein modeling. RaptorX consists of three major components: single-template threading, alignment quality prediction and multiple-template threading. Raptor X utilizes structural information in a single or multiple templates and thus improves alignment accuracy (Peng and Xu, 2011). The query sequences and template structure were then matched to generate the 3D model of bovine Fertilin-β protein. (Fig. 4). RaptorX modelled 617 amino acids (82%) divided the whole protein in two major domains: 1 to 171 amino acids as domain 2 and 172 to 617 amino acids as domain 1. 44 positions (5%) were considered as disordered by the software. Fig. 5 shows the structures of two domains of Fertilin- β.

Network prediction of N-Glycosylation sites and O-linked glycosylation

NetNGlyc predicted 4 potential glycosylation sites at 122, 220, 353 and 458 positions (Fig. 6a). N-glycosylation is known to occur on Asparagines which occur in the Asn-Xaa-Ser/Thr stretch (where Xaa is any amino acid except Proline). NetNGlyc attempts to distinguish glycosylated sequons from non-glycosylated ones. By default, predictions are only shown on Asn-Xaa-Ser/Thrsequons. Yin-O-Yang predicted potential O-glycosylation sites at 28, 148, 149, 240, 299, 345, 346, 535 and 663 position of the protein (Fig. 6b). Simultaneous running of NetPhos software predicted 3 phosphorylation sites among these O-glycosylation sites vis-à-vis 148, 149 and 663.

Prediction of interacting partners of Fertilin-β

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database found out several

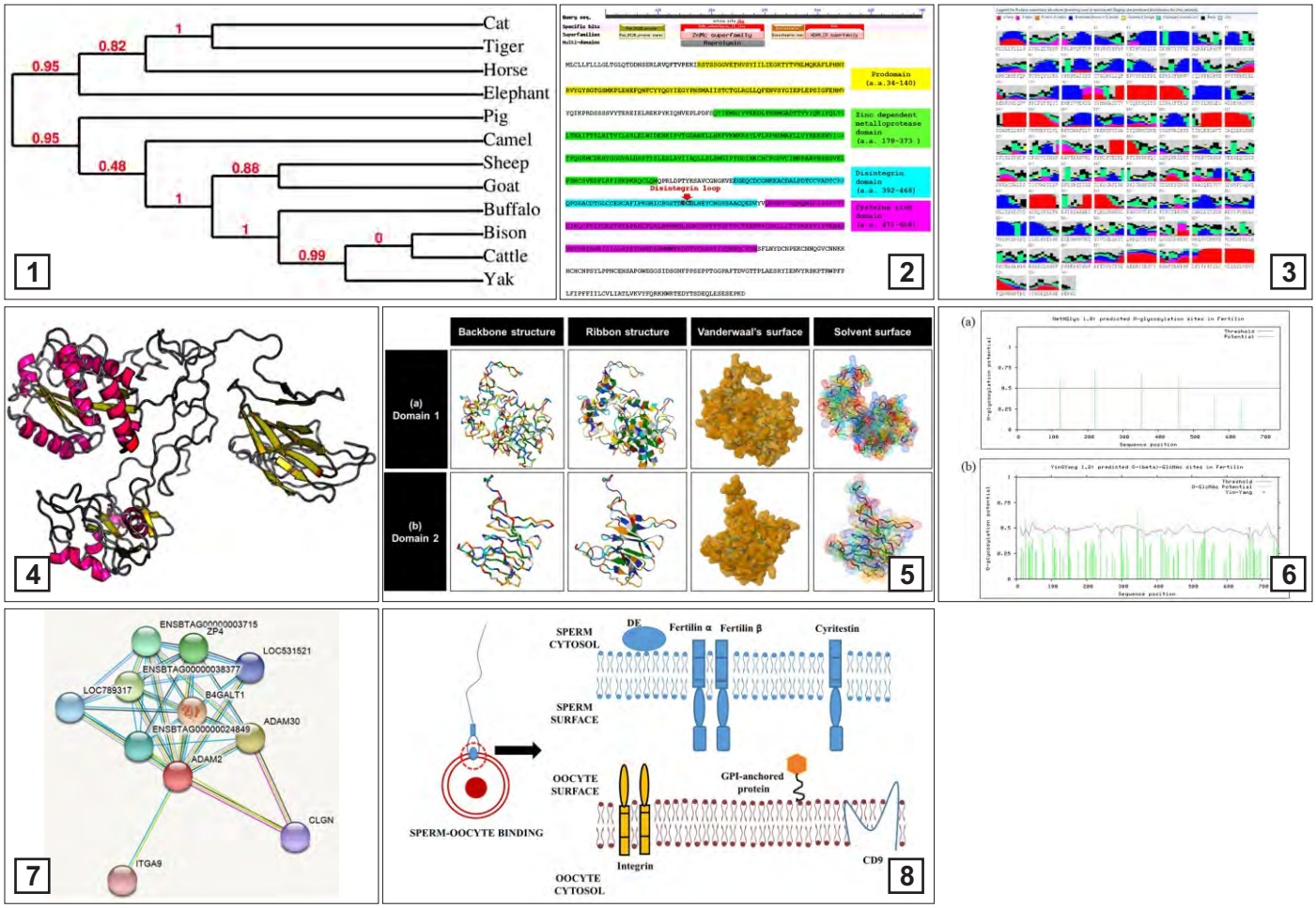

Fig. 1. Neighbour-joining phylogenetic analysis of mammalian Fertilin- β protein. Evolutionary distance was determined by number of amino acid substitution per site. **Fig. 2.** Conserved domain structure of Fertilin-β in cattle determined by NCBI-CDD. Amino acid number 34-140, 178-373, 392-468, 471-608 represent prodomain, metallo-protease domain, disintegrin domain and cysteine-rich domain respectively. A glutamic acid-cysteine-aspartic acid (ECD) motif within disintegrin domain participates in sperm-oocyte interaction. **Fig. 3.** RaptorX predicts 8-class secondary structure of Fertlin-β in cattle. According to structure predicted by the software 18% of amino acid residues conform α-helix, 21% conforms a β-sheet and 60% conforms coil. **Fig. 4.** Homology modelling and structure prediction of cattle Fertlin-β using Raptor X. The amino acid sequence of Fertilin-β was provided as inputs in RaptorX. Upon a PSI-BLAST search against the Protein Data Bank (PDB), Vascular apoptosis-inducing proteins (VAPs) from hemorrhagic snake venom (Crotalusatrox) (2dw0A) was identified as the best template (p-value 2.66e-18) .617 amino acids (82%) was modelled by RaptorX. **Fig. 5.** Domain structure prediction of cattle Fertlin-β using Raptor X. RaptorX divided 617 amino acids in two domain -1 to 171 amino acids as domain 2 and 172 to 617 amino acids as domain 1.44 positions (5%) were considered as disordered by the software. Panel (a) & (b) shows different structures of domain1 and domain 2, respectively. **Fig. 6.** Glycosylation site prediction of cattle Fertilin-β. (a) NetNGlyc version 1.0 predicts 4 potential glycosylation sites at 122, 220, 353 and 458 positions. (b) YinOYang version 1.2 predicts potential O-glycosylation sites at 28, 148, 149, 240, 299, 345, 346, 535 and 663 position of the protein. **Fig. 7.** Prediction of interacting partners of cattle Fertlin-β (ADAM2). Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database found out several interacting partners of Fertlin-β. With a confidence cut-off value of 0.4, 10 different interacting protein partners were retrieved for the sperm surface protein Fertlin-β. **Fig. 8.** ADAM proteins participate in sperm-oocyte binding. ADAM proteins i.e. Fertilin α, Fertilin β, Cyritestin along with their oocyte counterpart CD9, Integrin, GPI anchored protein contribute to sperm oocyte attachment leading to successful fertilization.

interacting partners of Fertilin-β (ADAM 2) (Fig. 7). β -1,4-galactosyl transferase 1, Zona pellucida sperm binding protein 4 (ZP4), Calmegin (CLGN), Integrin alpha-9 precursor (ITGA9) are few of these proteins found on oocyte surface. Interaction between Fertilin-β and these proteins strengthen sperm-oocyte adhesion.

Molecular Function prediction of Fertilin-β

Consensus results from ProKnow and Kihara Protein Function Prediction (PFP) servers show that Fertilin-β has metalloendopeptidase activity, metallopeptidase activity and Zn ion binding activity. Biochemical studies show that Fertilin-α and Fertilin-β pair together and form a heterodimeric complex on sperm membrane (Ikawa *et al.*, 2010). Various experimental data confirm that heterodimeric complex along with another cysteine-rich protein cyritestin contribute to sperm-egg adhesion (Fig. 8). The exact domain of ADAM proteins that mediate interaction with the egg membrane protein has also been determined by experimental approaches. Site specific deletion or mutation studies of these proteins have revealed that a specific sequence glutamic acid-cysteine-

aspartic acid (ECD) in disintegrin domain, known as disintegrin loop is responsible for this interaction. Here in our study we have identified different domain and marked the disintegrin loop in Fertilin-β sequence (Fig. 2).

CONCLUSION

Here we have computationally predicted the structural features and various interacting partners of bovine Fertilin-β, a key player in the process of sperm-oocyte handshaking during fertilization process. Also conserved domain structure and functional role of the protein in the process of fertilization has been revealed. Overall, this study will provide better understanding of the role of the protein in bovine fertilization process.

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