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Characterization and Structural Properties of *Glycam1* Gene of Some Domestic Animals

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ABSTRACT

Glycosylation-dependent cell adhesion molecule 1 (*Glycam1*) gene is sulfated glycoprotein that appears to mediate the adhesion of leukocyte-endothelial cells. In this study *Glycam1* was characterized in *Bos taurus*, *Bubalus bubalis*, *Ovis aries*, *Equus caballus* and *Sus scrofa*. *Glycam*1 gene was amplified from DNA and mRNA samples revealed that different lengths. The coding region of *Glycam*1 gene is divided into four exons and three introns. Translation the coding region reported the presence of 154 amino acids for both of *O. aries* and *S. scrofa*, 153 amino acids in *B. bubalis*, *B. taurus* and *E. caballus*. Glycam1 peptide was unstable, heat stable and the GRAV was negative, so the peptide was hydrophilic. The domain position was 19-154 in *O. aries* and *S. scrofa*, 19-153 in *B. bubalis* and *E. caballus* and 19-152 in *B. taurus*. The peptide contained many O-linked glycosylation sits with rich in Ser sites. The helices constitute 69.9-82.4% of the secondary structure of the five animals, where strand units were 15.5-33.3%. The Glycam1 peptide are folded in 2-5 helical unites and 5-7 loops in their mucin domain. The fourth helical peptide in *B. bubalis*, *B. taurus* and *E. caballus,* the third in *S. scrofa* and the second in *O. aries* were the longest (115-143, 115-143, 117-137, 116-144 and 116-144, respectively). The *Glycam*1 gene is characterized by four exons and three introns with very short exon II.

Keywords: *Glycam1*; glycosatation; secondary structure; domestic animals

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INTRODUCTION

The mucosal tissue in the mammary gland is one of the frequently bacterial attack sites (1). The mammary gland infection causes significant loss in the dairy sector economic (2). The mammary gland has different defensive mechanisms to protect itself, similar to other lymphoid tissues associated with the mucosa (3), This also has an important immune role, such as shielding infants or pups from infection with milk immune reagents, lymphocytes and immunoglobulins (4). Immunoglobulins in milk are essential for the neonatal immune function development (5). The milk contains several lymphocytes that can also protect newborn infants against infection (6). Many investigators have observed lymphocyte migration to the mammary gland (7), a few molecules have been identified that mediate lymphocyte migration (8). Adhesion molecules belong to one of these families which mediate lymphocyte and chemokine trafficking (8). Some of these molecules, such as mucosal address in cell adhesion molecule-1 (*Madcam1*) and *Glycam1* are expressed in mammalian gland (4,9,10).

Glycam1 gene is identified as sulfated glycoprotein that appears to mediate the adhesion of leukocyte-endothelial cells by presenting carbohydrate ligands in the L-selectin lectin domain (11). *Glycam1* gene seems to be a mucin like glycoprotein, as carbohydrates share in 70% of its native molecular weight which found in two O-linked serine/threonine rich domains (11). This mucin's tissue-specific expression on the luminal surface of the high endothelial venules (HEV) of mesenteric lymph nodes and peripheral lymph nodes (PLN) is consistent with the role that *Glycam1* plays in the regional lymphocyte traffic to these lymph organs. In addition to *Glycam1*'s expression in these lymphoid sites, this glycoprotein's mRNA has been proved in the lung although there has been no clarification of *Glycam1*'s anatomical position. The interaction between *Glycam1* and L selectin depends on the side chains of O-linked carbohydrate which are introduced to the leukocyte selectin by the mucin. These carbohydrates have been proved to contain a sialic acid component that is essential for the recognition of these carbohydrate ligands in the L-selectin lectin domain (10). Furthermore, the data showed that the sulfate modification of the carbohydrates attached to *Glycam1* is also necessary for L-selectin to recognize this glycoprotein adhesively (12). The expression of *Glycam1* protein and mRNA appears to be modulated by afferent lymphatic flow, as peripheral lymph nodes differentiation results in expression loss of these components and a significant reduction in lymphocyte traffic to these treated sites (13).

Several studies also show *Glycam1*'s role in radiation-mediated protection. The results show that the entry of immune cells from the vasculature to neural tissues is regulated by *Glycam1* levels. As *Glycam1* deficiency influences cell entry more deeply than neurodegeneration (14). *Glycam1* expressed in mammary gland and the lung. The gene expressed by epithelial cells in the bovine and rat mammary gland and secreted into the milk (15). *Glycam1's* function in these organs has yet to be identified.

In order to determine the possible role of *Glycam1* in different tissues, we have determined the complete sequence of the mRNA and gene encoding *Glycam1* of *Bos taurus* (cattle), *Bubalus bubalis* (buffalo), *Ovis aries* (sheep), *Equus caballus* (horse) and *Sus scrofa* (pig) compared the genomic organization with the bovine, *Capra* and with other *Glycam1* gene in different species reported in the database. Also, we can predict the chromosomal locus of *Glycam1* gene in the five species *in silico* by using comparative mapping tool. Genetic information present in this study may be important for research, educational and informational purposes. Genetics research not only furthers understanding which animals are at risk for specific diseases but can also lead

to the development of diagnostic tests and potential gene therapies, leading to earlier disease intervention and improved health outcomes.

MATERIALS AND METHODS

Collection of samples

This study was executed in the Cell Biology Department, National Research Centre, Giza, Egypt. Tissue samples from mammary gland were obtained from healthy Egyptian buffalo (*Bubalus bubalis*), cattle (*Bos taurus*) and sheep (*Ovis aries*) at the slaughterhouse of Giza. Blood samples were collected from healthy horse *(Equus caballus*) which kindly provided from EL-Zahraa horse stud farm, EAO, Cairo, Egypt and pig (*Sus scrofa*) from Meit Okba pig farms in Giza, beside the aforementioned animals (*B. bubalis*, *B. taurus* and *O. aries*) in 0.5 ml of 0.5 M EDTA and processed as soon as possible whereas the tissue fragments were minced and frozen in liquid nitrogen.

DNA extraction

Genomic DNA was extracted from blood samples according to the procedure described by (16) with minor modifications. The Nanodrop 1000 spectrophotometer (Thermo Scientific Co.) was used to measure DNA concentration. The absorbance 260/280 ratio was between 1.7 and 1.9. The DNA solution was diluted to the final concentration of a 50 ng/µl.

RNA extraction

In 2000 *xg* at 4°C for 10 min., 1.5 ml of blood sample was centrifuged and them plasma was removed. Add 3 volumes of the RBC lysis buffer, then vortexed the tubes for 15 sec and incubated for 15 min at room temperature. The tubes were centrifuged in 2000 *xg* for 10 min (4°C). The supernatant was discarded. RNA isolation from WBC pellet and tissue sample were performed using one ml TRIzol® reagent. After homogenization, the tissue samples were kept for 5 min at room temperature. Samples (tissue or blood) were shaken vigorously by hand after addition of 0.2 ml of chloroform for 15 sec and then they were kept for 3-10 min at room temperature. The samples were centrifuged at 12000 *xg* for 10 min at 4°C. The aqueous phase was transferred to a fresh tube and the RNA was precipitated using 0.5 ml of isopropanol. The samples were kept on ice for 5-15 min and centrifuged for 10 min at 4°C at 12000 *xg*. The RNA pellet was precipitated at the bottom. The supernatant was carefully eliminated, and the RNA was washed twice with 75% ethanol and subsequent centrifugation at 7500 *xg* (4°C) for 8 min. The RNA was resuspended in RNAase-free DEPC treated water.

cDNA synthesis

cDNA was synthesized using Ready-To-Go You-Prime First-Strand Beads. Three µl of the total RNA sample (5 µg) and 25 µl DEPC treated water were added in RNAase-free microcentrifuge tube, and heated at 65°C for 10 min, and then chilled on ice for 2 min. Five µl (5 µg) oligo (dT) primer was added to the RNA solution and was kept at room temperature for approximately 1 min. The contents of the tube were mixed by gentle vortex, or by repeatedly pipetting of the mixture and then incubated at 37°C for 60 min in water bath.

PCR amplification

DNA and RNA fragment which are represent *Glycam1* gene was amplified using forward and reverse primers which designed by the web-based software Primer3Plus (17) from the published sequence of *Glycam1* gene deposited in the gene bank. The primers sequences and the product size are shown in Table 1. The polymerase chain reaction (PCR) was conducted at a 25 µl reaction volume comprising 0.2 mM dNTPs and 1.25 U *Taq* DNA polymerase. The Master Mix, with 100 ng of buffalo, cattle, sheep, horse and pig DNA or cDNA, was aliquot in PCR tubes. The reaction was cycled under the following conditions: the initial denaturation at 94°C for 5 minutes followed by 35 denaturation cycles at 94°C, annealing at (58-62°C) and extension at 72°C, each step for 1 min and final extension at 72°C for 5 min. GeneJET Gel Elution Kit (Thermo Scientific, Germany), was used to purify the PCR products of selected samples. The purified PCR products were sequenced (Macrogen, South Korea).

Sequence analysis

Sequences were analyzed using many web-based programs such as NCBI BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for sequence homology and databases comparison searches (18), sequence alignments and comparisons to reveal nucleotide or amino acid substitutions were carried out using Clustal Omega version (1.2.4) (https://www.ebi.ac.uk/Tools/msa/clustalo) as described by (19), the six-frame translation protein (http://molbiol.ru/eng/scripts/0113.html) and/or Open Reading Frame Finder (ORF) (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), translations of amino acid sequences from *B. bubalis*, *B. taurus*, *O. aries*, *E. caballus* and *S. scrofa* were conducted using Clustal programs, these sequences which are the most similar frames were matched with the amino acid sequences of the corresponding sequences in the database. The phylogenetic relationships among the *Glycam1* nucleotide or amino acids sequences of *B. bubalis*, *B. taurus*, *O. aries*, *E. caballus* and *S. scrofa* with the other species carried out by using unweighted pair group method with arithmetic mean (UPGMA). The Clustal Omega Program (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used to evaluate the differences and identity of the five animals DNA, mRNA, and protein sequences and also to the *Glycam1* gene in other organisms accessible in the GenBank database.

Chromosomal assignment

In this study, to identify the locus of Glycam1 gene of buffalo, cattle, sheep, horse and pig in their genomes, *B. bubalis* genome assembly version (UOA WB 1), *B. taurus* genome assembly version (ARS UCD1.2), *O. aries* genome assembly version (Oar rambouillet v1.0), *E. caballus* genome assembly version (EquCab 2.0) and *S. scrofa* genome assembly version (Sscrofa11.1) were used, which are available via the NCBI database (http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi).

The obtained DNA and mRNA sequences in current study had been deposited in GenBank. Therefore, the unknown sample is highly likely to match a sequence of DNA or mRNA from a reference sample deposited in the database. These results will therefore contribute to the development of the GenBank database and may be used for further phylogenetic and forensic studies on other animal species.

Physicochemical properties analysis

The bioinformatics website ExPASy (http:/www.expasy.ch/tools/) and the structural prediction software ProtParam were used to predict the physicochemical properties of peptides, including molecular weight, isoelectric point (PI), half-life in mammalian reticulocytes (*in vitro*), yeast (*in vivo*) and *E.*

coli (*in vivo*), instability index, Aliphatic index, and GRAVY.

The detection of glycosylation sites for each amino acid sequence in all studied animals carried out using programs for Prediction of Glycosysylation sites in Eukaryotics Proteins.

(http://crdd.osdd.net/raghava/glycoep/submit.html) and (http://www.cbs.dtu.dk/services/NetOGlyc-4.0/output.php)

RESULTS AND DISCUSSION

Sequence analysis

The Glycosylation-dependent Cell Adhesion Molecule 1 (*Glycam*1) gene was amplified from DNA and mRNA samples of Egyptian *B. bubalis*, *B. taurus*, *O. aries*, *E. caballus* and *S. scrofa*. DNA lengths were 2846, 2905, 2893 bp, 2296 bp and 2455 bp while the mRNA lengths were 640, 650, 628 bp, 643 bp and 625 bp for *B. bubalis*, *B. taurus*, *O. aries*, *E. caballus* and *S. scrofa*, respectively. The sequences were submitted to Genbank and have been assigned with accession numbers for *B. bubalis* (DNA LC522942, mRNA LC522943 and protein BCB25101), *B. taurus* (DNA LC534833, mRNA LC534834 and protein BCB97910), *O. aries* (DNA LC522944, mRNA LC522945 and protein BCB25103), *E. caballus* (DNA LC522952, mRNA LC522953 and protein BCB25112) and *S. scrofa* (DNA LC522946, mRNA LC522947 and protein BCB25106). The genomic structure of *Glycam*1 gene encoding *Glycam1* for these animal species revealed that the coding region is divided into four exons and three introns, also the sequences characterized with very short exon II, which was surrounded by two long intron sequences (Table 2 and Fig. 1).

As the molecular genetics is a significant tool for characterization of genes and identification of genetic variations of individuals (20). In this study the data reported demonstrated that the Egyptian *B. bubalis*, *B. taurus*, *O. aries*, *E. caballus* and *S. scrofa Glycam1* gene is similar to those encoding glycophorin, *CD34*, and *MUCl* in that the coding region is divided into a number of exons, some of which correlate with functional domains of the glycoprotein. *Glycam1* gene is similar in structure to *Glycam1* gene on human (21), bovine (22), murine (23) and caprine (24). Various studies on *Glycam1* gene revealed results agree with our results that the gene consists of four exons and three introns, and the approx. full-length gene is 2.5 kb, all introns are in the triplet encoding between the first and second nts. The length and number of exons and the position of the introns in the mouse and bovine genes are therefore identical (24). The selected sequence alignments (DNA or mRNA) that gave more than 70% identity or similarity indicated the relationship between the aligned sequences (25).

Sequence comparison between *Glycam1*-DNA genomic sequences from the all five animal species and *Glycam1* gene of other species showed that, the five animals shared similarity ranged from 71-98% with *B. taurus* (X83391), *C. hircus* (AJ249733), *C. dromedarius* (AJ131714), as shown in phylogenetic tree (Fig. 2) and percent identity matrix of DNA multiple sequence alignment (Table 3).

The length variation of the DNA gene of *Glycam1* within and between species could be due to evolution and differentiation. By comparison with known sequences, many longitudinal variations caused by insertions and deletions resulting in variation of amino acids within species have been found (26).

The alignments of selected sequences (DNA or mRNA) which gave more than 70% identity or similarity indicated that the aligned sequences are related (25). So, *Glycam1* gene DNA or mRNA sequences from *E. caballus* and *S. scrofa* related to each other and to the *Glycam1* gene DNA or mRNA from other species including *B. bubalis*, *B. taurus*, *O. aries* and *C. hircus*. However, the results showed high difference with *Glycam1* of *M. musculus* and *R. norvegicus*.

The sequence alignments of the five animals *Glycam1*-mRNA sequences and *Glycam1*-mRNA gene of other species showed that the five animals shared similarity ranged from 71-99% with *B. bubalis* (XM006063685), *B. taurus* (NM174828), *C. hircus* (NM001285626), *O. aries* (MH917952) and *C. dromedarius* (XM010994081) as shown in phylogenetic tree (Fig. 3) and multiple sequence alignment (Table 4).

The results of mRNA multiple sequence alignment from the Egyptian *B. bubalis*, *B. taurus*, *O. aries*, *E. caballus* and *S. scrofa Glycam1* gene with other species related to the pervious results as nucleotide sequence of bovine *Glycam1*-cDNA length is 642 bp, which derived from a lactating cow's mammary gland. The bovine *Glycam1*-cDNA showed sequence similarity (57% and 55%) to the mouse and rat *Glycam1*-cDNAs, respectively (22).

Six frame translations of amino acid sequences from the five animals were conducted from each genomic sequence. Translation of all the coding region of the five species reported the presence of 154 amino acids for both of *O. aries* and *S. scrofa* while 153 amino acids in *B. bubalis*, *B. taurus* and *E. caballus*. These sequences which are the most aligned frames were linked with the relative gene's amino acid sequences reported using Clustal Omega program. For the amino acid alignment the results showed that the five animals shared similarity ranged from 64-97% with *B. bubalis* (XP006063747), *B. taurus* (NP777253), *O. aries* (QCE31183**)**, *C. hircus* (NP001272555), *C. dromedarius* (CAB53389) as shown in phylogenetic tree (Fig. 4) and percent identity matrix of amino acid multiple sequence alignment (Table 5).

The results of Glycam1 amino acid multiple sequence alignment agree with the results reported by Groenen study on bovine Glycam1 as bovine Glycam1 amino acid demonstrated an overall similarity of only 41% with mouse and 40% with rate, although there was 83 and 81% similarity between two specific protein regions, respectively. The bovine amino acid also showed similarity (55%) to a small protein isolated from camel milk whey fraction (22).

The results of Glycam1 amino acid alignments revealed that *E. caballus* more related to *S. scrofa* then *C. dromedarius*, *B. bubalis*, *B. taurus*, *O. aries*, *C. hircus* than any other species (*R. norvegicus* and *M. musculus*) according to CLUSTALW pairwise comparison which illuminate similarities and differences in amino acid sequences. Similarity percentages more than 50% indicates that the two sequences are likely to share one or more functional domains whereas more than 30% indicates that the two sequences are likely related whereas. However, if the similarity is less than 20% it indicates that the two sequences are not likely to be functionally related (25).

Chromosomal assignment

Modern advances in biology, such as functioning of chromosome apparatus and knowledge of the structure, support phylogenetic research. Cytogenetic techniques are an essential tool in mammals' phylogeny and systematics (27). The analysis of fine chromosomal

structures is commonly used in evolutionary genetics. In the homology study of chromosomes and chromosomal regions, there is an approach in several different mammalian species with comparison in their striation variations (28). In this study, the UPGMA phylogenetic tree from Glycam1 gene sequences of amino acids, DNA and mRNA in the various organisms showed that each of the Egyptian (*B. bubalis*, *B. taurus* and *O. aries*) was appeared closer to each other, and with *Glycam1* gene of *C. hircus* than that of *H. sapiens*, *C. dromedarius*, *R. norvegicus* and *M. musculus,* also *E. caballus*, *S. scrofa* and *C. dromedarius* were appeared closer to each other, and with *Glycam1* gene of *H. sapiens* than that of *C. hircus*, *B. taurus*, *R. norvegicus* and *M. musculus*. This clustering based on both *Glycam1* gene nucleotide and amino acid sequences clearly shows the phylogenetic interrelationship between these species and is also generally consistent with established species relationships. This tree was calculated using MrBayes (29), using a previously calculated multi-sequence gene family (30).

In this study chromosomal assignment of the investigated genes were made on molecular basis. DNA sequence from *B. bubalis, B. taurus*, *O. aries E. caballus* and *S. scrofa* aligned with their genomes to assign *Glycam1* gene sequences by using the BLAST program. The results of these alignments represent *B. bubalis* (LC522942) sequence on chromosome 4 (BBU4), *B. bubalis* breed Mediterranean chromosome 4, ASM312139v1 (NC037548.1), ranging from 94822286 to 94825131, *B. taurus* (LC534833) sequence on chromosome 5 (BTA5), *B. taurus* isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 5, ARS-UCD1.2 (NC037332.1) ranging from 25478966 to 25481870, *O. aries* (LC522944) sequence on chromosome 3 (OAR3), *O. aries* strain OAR USU Benz2616 breed Rambouillet chromosome 3, Oar rambouillet v1.0 (NC040254.1) ranging from 141501925 to 141504817, *E. caballus* (LC522952) sequence on chromosome 6 (ECA6), *E. caballus* isolate Twilight breed thoroughbred chromosome 6, EquCab3.0 (NC009149.3) ranging from 72278655 to 72280950 and *S. scrofa* (LC522946) sequence on chromosome 5 (SSC5), *S. scrofa* isolate TJ Tabasco breed Duroc chromosome 5, Sscrofa11.1 (NC010447.5) ranging from 19787979 to 19790433.

Many technological advances had donated to the generation of these data which increased the capability to find and isolate genes that led to genetic diseases and/or had a significant in economic importance of production traits of livestock (31).

The results of this study revealed that chromosomal localization of *Glycam1* gene investigated to be on *E. caballus* chromosome 6 (ECA6) and *S. scrofa* chromosome 5 (SSC5). Our results in agreement with the results of the *Glycam1* sequences from caprine, bovine and ovine were determined and they were mapped by fluorescent *in situ* hybridization (FISH) on cattle (BTA5q21), sheep (OAR3q21), goat (CHI5q21) (24) and murine chromosome 15 (23). Therefore, our results confirmed the comparative mapping data indicating chromosomal conservation among *H. sapiens* chromosome 12 (HSA12), *B. taurus* chromosome 5 (BTA5) (32) and *O. aries* chromosome 3 (OAR3) (27,33).

In these closely related species that belong to the Bovidae family, *B. bubalis, B. taurus* and *O. aries* have chromosome band homology, which has a high chromosome conservation degree between its members and where bi-armed autosomes are produced by centric fusions of acrocentric autosomes (34). The results of this study in agreement with comparative mapping data indicating conservation among *B. bubalis* chromosome 4 (BBU4) (34), *B. taurus* chromosome 5 (BTA5) (32,35) and *O. aries* chromosome 3 (OAR3) (27,33). Also, in agreement with our results, the results of the cDNA and gene sequences from caprine, bovine and ovine were determined and they were mapped by FISH on goat (CHI5q21), cattle (BTA5q21) and sheep (OAR3q21) (24). Also, the *Glycam1* gene has been mapped to chromosome 15 in mouse by segregation analyses (23).

Physicochemical properties analysis

The results in Table 6 revealed that the molecular weight of Glycam1 peptides was calculated and ranged from 16839.84-17151.60 Da, theoretical isoelectric points ranged from 5.46-6.22, instability index ranged from 40.63-62.87, aliphatic index ranged from 67.71-95.69 and grand average of hydropathicity ranged from -842- -0.414 in all animal species under study (Table 6). Peptide was expected to be stable when the index of instability was below 40. Otherwise, peptide was supposed to be unstable. Its aliphatic index indicated the peptide heat stability. The higher aliphatic index means higher stability in heat. GRAVY predicted the hydrophobicity and hydrophilicity of peptide. When the GRAVY value was plus, the peptide was hydrophobic; otherwise, it was hydrophilic (36–38).

The domain structure of Glycam1 peptide was determined using Swiss-Model server and Uniprot database. The results in Table 7 showed that the signal peptide cleavage site was 18-19 (SLA-IL) in glycam1 peptides of the five animal species. The domain position was varied in the animal species under study, with 20-154 in *O. aries* and *S. scrofa*, 20-153 in *B. bubalis* and *E. caballus* and 19-152 in *B. taurus*. The results also showed that glycosylation sites and their types in our study, where the five animals contained many O-linked glycosylation as Ser and Thr sites with rich in Ser sites. On the other hand, there is no N-linked glycosylation sites except in *B. taurus* where one site detected as Asn at position 95 (Table 7). Glycosylation is one of the most common post-translational modifications (PTMs) of proteins, which plays a variety of crucial roles in many cellular functions such as structural, ligand-binding, subcellular recognition and signaling cell-cell adhesion (39). Glycosylation are classified to O-linked glycans are attached to the hydroxyl group of serine (Ser) or threonine (Thr) side chains (40). However, N-linked glycans are attached to the amide nitrogen of asparagine (Asn) side chains in the consensus sequences Asn-Xaa-Ser or Asn-Xaa-Thr, where Xaa represents any amino acid residue except proline (Pro) (41).

The secondary structure of Gylcam1 peptide was predicted. The helices constitute 69.9-82.4% of the secondary structure of the five animal species under study, where strand units were 15.5-33.3% (Table 8). The results revealed that Glycam1 peptide are folded in 2-5 helical unites and 5-7 loops in their mucin domain. The fourth helical peptide in *B. bubalis*, *B. taurus* and *E. caballus,* the third in *S. scrofa* and the second in *O. aries* were the longest (115-143, 115-143, 117-137, 116-144 and 116-144, respectively) (Table 8).

CONCLUSION

The coding region of *Glycam*1 gene is divided into four exons and three introns, also the sequences characterized with very short exon II, which was surrounded by two long intron sequences. Translation of all the coding

region of the five species reported the presence of 154 amino acids for both of *Ovis aries* and *S. scrofa* while 153 amino acids in *B. bubalis*, *B. taurus* and *E. caballus*. Glycam1 peptide was unstable, heat stable and hydrophilic. The Glycam1 peptide are folded in 2-5 helical unites and 5-7 loops in their mucin domain. The fourth helical peptide in *B. bubalis*, *B. taurus* and *E. caballus,* the third in *S. scrofa* and the second in *O. aries* were the longest.

CONFLICT OF INTEREST

The Authors have declared no conflict of interest.

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Table 1: Primers sequence used in this study.

Species	Size bp		Sequence	Size	Tm
DNA Sequence					
Bubalus bubalis	2846	Forward	CCTCCTCAGCAGCACCAAG	19	62.13
		Reverse	TTGGGATGCAATGCTTTAAT	20	58.11
Bos taurus	2905	Forward	CCTCCTCACCAGCACCAA	18	60.84
		Reverse	TTGGGATGCAATGCTTTAAT	20	58.11
Ovis aries	2893	Forward	CACCATGAAATTCCTCTGCGT	21	58.91
		Reverse	TACAAAGCCAACACAGAGCC	20	58.40
Sus scrofa	2458	Forward	CCTCTGCAGCACCAAGCAT	19	62.56
		Reverse	TTGGGACGCAACGCTTTAAT	20	62.72
Equus caballus	2297	Forward	CCTCAGCAACGCTAAGCAG	19	59.90
		Reverse	TTGGGATGAGACACTTTAATAAGAA	25	58.34
mRNA Sequence					
Bubalus bubalis	602	Forward	CCTCCTCAGCAGCACCAAG	18	62.13
		Reverse	TTGGGATGCAATGCTTTAAT	20	58.11
Bos taurus	640	Forward	CCTTCCTTCCTGGTGCTACA	19	60.84
		Reverse	TTGGGATGCAATGCTTTAAT	20	58.11
Ovis aries	627	Forward	ACCATGAAATTCCTCTGCGTCT	22	62.24
		Reverse	CCTTCCTTCCTGGTGCTACA	20	60.25
Sus scrofa	625	Forward	CCTCTGCAGCACCAAGCAT	19	62.56
		Reverse	TTGGGACGCAACGCTTTAAT	20	62.72
Equus caballus	643	Forward	CCTCAGCAACGCTAAGCAG	19	59.90
		Reverse	TTGGGATGAGACACTTTAATAAGAA	25	58.34

Table 2: DNA sequence of *Glycam1* gene containing the coding exons and introns for *Bubalus bubalis*, *Bos taurus, Ovis aries, Equus caballus* and *Sus scrofa.*

Species	Exon 1	Intron 1	Exon 2	Intron 2	Exon 3	Intron 3	Exon 4	DNA	mRNA	cds	Amino acid
Bubalus bubalis **(LC522942)**	60-123 (64 bp)	124-1081 (958 bp)	1082-1126 (45 bp)	1127-1886 (760 bp)	1887- 2108 (222 bp)	2109-2596 (488 bp)	2597- 2846 (131 bp)	2846 bp	640 bp	462 bp	154 aa
Bos taurus **(LC534833)**	60-123 (64 bp)	124-1095 (972 bp)	1096-1140 (45 bp)	1141-1928 (788 bp)	1929-2150 (222 bp)	2151-2655 (505 bp)	2656-2783 (131 bp)	2905 bp	650 bp	462 bp	154 aa
Ovis aries **(LC522944)**	4-68 (64 bp)	69-1044 (976 bp)	1045-1089 (45 bp)	1090-1885 (796 bp)	1886-2110 (225 bp)	2111-2603 (493 bp)	2604-2734 (131 bp)	2893 bp	628 bp	465 bp	155 aa
Equus caballus **(LC522952)**	58-121 (64 bp)	121-805 (684 bp)	806-850 (45 bp)	851-1575 (725 bp)	1576-1846 (271 bp)	1847-2090 (244 bp)	2091-2172 (82 bp)	2296 bp	643 bp	462 bp	154 aa
Sus scrofa **(LC522946)**	63-126 (64 bp)	69-1044 (630 bp)	757-801 (45 bp)	1763-1983 (961bp)	1763-1983 (222 bp)	2111-2603 (239bp)	2224-2353 (131bp)	2455 bp	625 bp	465 bp	155 aa

Table 3: Percent identity matrix of *Glycam1*-DNA gene multiple sequence alignment for the *Bubalus bubalis*, *Bos taurus, Ovis aries, Equus caballus* and *Sus scrofa* with the other sequences of similar genes from different species published in GenBank database.

Species	*M. musculus*	*R. norvegicus*	*C. dromedaries*	*B. bubalis*	*B. taurus 1*	*B. taurus 2*	*O. aries*	*C. hircus*	*E. caballus*	*S. scrofa*
M. musculus	100.00	86.47	52.01	53.30	52.38	52.38	52.70	52.32	56.19	54.39
R. norvegicus		100.00	53.13	53.34	52.71	52.71	53.34	53.02	55.23	54.52
C. dromedaries			100.00	71.53	71.71	71.71	71.47	70.95	71.85	71.87
B. bubalis				100.00	96.54	96.54	94.24	93.75	59.15	61.72
B. taurus 1					100.00	100.00	93.86	93.46	59.31	59.33
B. taurus 2						100.00	93.86	93.46	59.31	59.33
O. aries							100.00	97.82	60.30	62.21
C. hircus								100.00	60.18	59.23
E. caballus									100.0	71.92
S. scrofa										100.0

M. musculus: Mus musculus (D16108), *R. norvegicus: Rattus norvegicus* (NC005106), *H. sapiens: Homo sapiens* (AC238711), *C. dromedaries: Camelus dromedaries* (AJ131714), *B. bubalis: Bubalus bubalis* (LC522942), *B. taurus 1: Bos taurus* (LC534833), *B. taurus 2: Bos taurus* (X83391), *O. aries: Ovis aries* (LC522944), *C. hircus: Capra hircus* (AJ249733), *E. caballus: Equus caballus* (LC522952), *S. scrofa: Sus scrofa* (LC522946).

Table 4: Percent identity matrix of *Glycam1*-mRNA gene multiple sequence alignment for the *Bubalus bubalis*, *Bos taurus* and *Ovis aries, Equus caballus* and *Sus scrofa* with the other sequences of similar genes from different species published in GenBank database.

Species	*M. musculus*	*R. norvegicus*	*C. dromedarius*	*O. aries 1*	*O. aries 2*	*C. hircus*	*B. taurus 1*	*B. taurus 2*	*B. bubalis 1*	*B. bubalis 2*	*E. caballus*	*S. scrofa*
M. musculus	100.00	89.04	61.87	60.04	61.87	61.65	62.96	62.83	61.53	61.82	62.99	61.18
R. norvegicus		100.00	63.00	61.75	62.41	61.30	62.36	62.36	61.64	61.64	62.61	61.16
C. dromedarius			100.00	79.59	79.78	79.26	80.41	80.41	80.88	80.88	74.04	80.23
O. aries 1				100.00	98.92	97.35	91.98	93.45	95.21	95.21	71.33	77.42
O. aries 2					100.00	99.57	95.45	95.45	96.10	96.10	71.33	77.42
C. hircus						100.00	95.42	95.36	95.31	95.33	72.51	76.09
B. taurus 1							100.00	100.00	96.88	96.94	72.22	76.59
B. taurus 2								100.00	96.88	96.94	72.22	76.59
B. bubalis 1									100.00	100.00	72.54	77.56
B. bubalis 2										100.00	72.54	77.56
E. caballus											100.00	74.05
S. scrofa												100.00

M. musculus: *Mus musculus* (NM001289587), *R. norvegicus*: *Rattus norvegicus* (NM012794), *C. dromedarius*: *Camelus dromedarius* (XM010994081), *O. aries 1*: *Ovis aries* (LC522945), *O. aries 2*: *Ovis aries* (MH917952), *C. hircus*: *Capra hircus* (NM001285626), *B. taurus 1*: *Bos taurus* (NM174828), *B. taurus 2*: *Bos taurus* (LC534834), *B. bubalis 1*: *Bubalus bubalis* (XM006063685), *B. bubalis 2*: *Bubalus bubalis* (LC522943), *E. caballus*: *Equus caballus* (LC522953), *S. scrofa*: *Sus scrofa* (LC522947).

Table 5: Percent identity matrix of multiple GLYCAM1 amino acid sequence alignment for the *Bubalus bubalis*, *Bos taurus* and *Ovis aries*, *Equus caballus* and *Sus scrofa* with the other sequences from different species published in GenBank database.

Species	*M. musculus*	*R. norvegicus*	*C. Dromedarius*	*C. hircus*	*O. aries 1*	*O. aries 2*	*B. taurus 1*	*B. taurus 2*	*B. bubalis 1*	*B. bubalis 2*	*E. caballus*	*S. scrofa*
M. musculus	100.00	71.92	41.54	40.00	40.69	41.38	40.28	40.28	40.97	40.97	62.99	61.18
R. norvegicus		100.00	40.00	41.43	42.14	42.86	40.29	40.29	40.29	40.29	62.61	61.16
C. dromedarius			100.00	64.75	66.19	64.75	63.77	63.77	65.94	65.94	74.04	80.23
C. hircus				100.00	98.70	97.40	89.54	89.54	90.85	90.85	72.51	76.09
O. aries 1					100.00	97.40	89.54	89.54	90.85	90.85	71.33	77.42
O. aries 2						100.00	89.54	89.54	91.50	91.50	71.33	77.42
B. taurus 1							100.00	100.00	95.42	95.42	72.22	76.59
B. taurus 2								100.00	95.42	95.42	72.22	76.59
B. bubalis 1									100.00	100.00	72.24	77.56
B. bubalis 2										100.00	72.24	77.56
E. caballus											100.00	74.05
S. scrofa												100.00

M. musculus: *Mus musculus* (BAA03682), *R. norvegicus*: *Rattus norvegicus* (NP036926), *C. dromedarius*: *Camelus dromedarius* (CAB53389), *C. hircus*: *Capra hircus* (NP001272555), *O. aries 1*: *Ovis aries* (QCE31183), *O. aries 2*: *Ovis aries* (BCB25103), *B. taurus 1*: *Bos taurus* (NP777253), *B. taurus 2*: *Bos taurus* (BCB97910), *B. bubalis 1*: *Bubalus bubalis* (XP006063747), *B. bubalis 2*: *Bubalus bubalis* (BCB25101), *E. caballus*: *Equus caballus* (BCB25112), *S. scrofa*: *Sus scrofa* (BCB25106).

Table 6: Physicochemical properties of Glycam1 peptide in the five animal species (*Bubalus bubalis, Bos taurus, Ovis aries, Sus scrofa* and *Equus caballus*)

Species	M. wt. (Da)	pI*	GRAVY*	Half-life			II*	AI*
				Mammalian reticulocytes	Yeast	*E. coli*		
Bubalus bubalis	17057.39	5.55	-0.470	30	>20	>10	40.63	95.69
Bos taurus	17151.60	6.22	-0.449	30	>20	>10	45.77	95.03
Ovis aries	17055.54	5.46	-0.414	30	>20	>10	47.66	97.60
Sus scrofa	16955.28	5.77	-0.455	30	>20	>10	43.83	82.40
Equus caballus	16839.84	5.84	-0.842	30	>20	>10	62.87	67.71

*M. wt.: Molecular weight, pI: Theoretical Isoelectric point, II: Instability index, AI: Aliphatic index, GRAVY: Grand average of hydropathicity.

Table 7: Profile of GLYCAM1 protein in the five animal species (*Bubalus bubalis, Bos taurus, Ovis aries, Sus scrofa* and *Equus caballus*)

Species	Domain					Glycosylation				
		SP*	Others			N-linked			O-linked	
						aa*	Position		aa*	Position
B. bubalis	20-153	0.9997	0.0003	18-19 (SLA-IL)	0.8621	-	-		Ser	37, 56, 72, 73, 100, 109, 112
									Thr	103, 104, 107, 113, 114
B. taurus	20-152	0.9999	0.0001	18-19 (SLA-IL)	0.8691	Asn	95		Ser	37, 56, 72, 73, 78, 86, 100, 109, 112
									Thr	97, 103, 104, 107, 113, 114
O. aries	20-154	0.9999	0.0001	18-19 (SLA-IL)	0.8698	-	-		Ser	57, 59, 73, 98, 101, 110, 113
									Thr	104, 105, 114, 115
S. scrofa	20-154	0.9999	0.0001	18-19 (SLA-VL)	0.9151	-	-		Ser	14, 37, 73, 80, 93, 97, 101, 115
									Thr	76, 104, 105, 108, 113, 114
E. caballus	20-153	0.9998	0.0002	18-19 (SLA-IL)	0.8912	-	-		Ser	31, 37, 53, 57, 87, 93
									Thr	34, 88, 104, 105

*SP: Signal peptide, CSP: Cleavage Site Position, aa: Amino acid

Table 8: Profile of the domain of Glycam1 peptide in the five animal species (*Bubalus bubalis, Bos taurus, Ovis aries, Sus scrofa* and *Equus caballus*)

Species	Domain	H-number	H-length	L-number	Strands	Helix (%)	β-sheet (%)	Turns (%)
Bubalus bubalis	19-153	α-helix 1	26-29	6	1	82.4	34.6	14.4
		α-helix 2	36-40					
		α-helix 3	59-62					
		α-helix 4	115-143					
Bos taurus	19-152	α-helix 1	36-40	6	1	77.8	33.3	15.0
		α-helix 2	48-51					
		α-helix 3	59-62					
		α-helix 4	115-143					
Ovis aries	19-154	α-helix 1	60-63	5	2	74.7	20.0	13.0
		α-helix 2	116-144					
Sus scrofa	19-154	α-helix 1	59-62	6	2	80.5	24.7	18.2
		α-helix 2	88-92					
		α-helix 3	116-144					
Equus caballus	19-153	α-helix 1	36-40	7	1	69.9	15.7	17.0
		α-helix 2	49-52					
		α-helix 3	60-63					
		α-helix 4	117-137					
		α-helix 5	140-143					

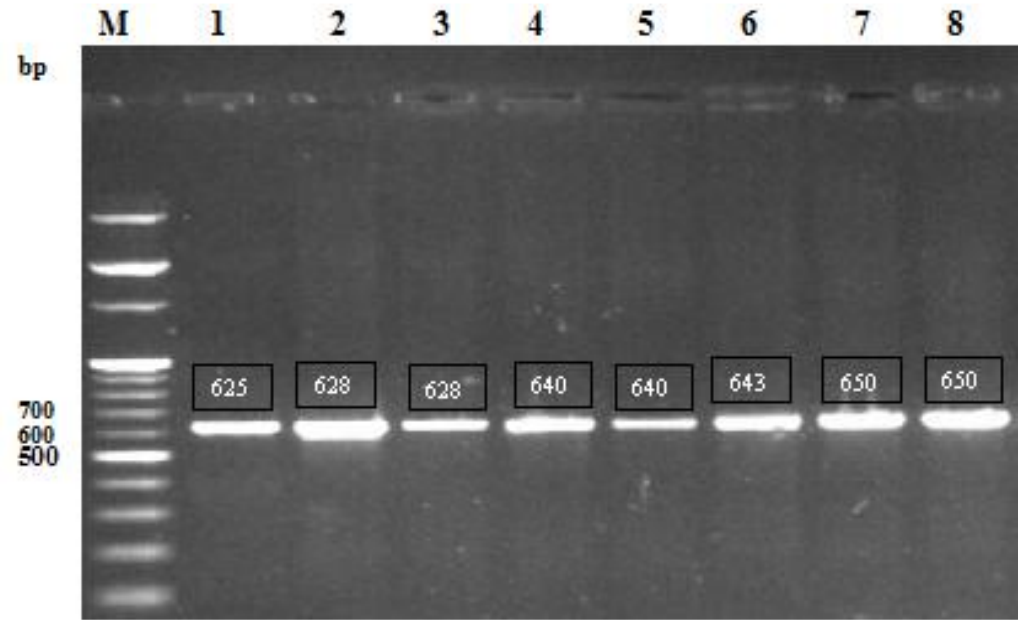

Figure 1: Gel electrophoresis image for PCR product from mRNA of: 1, *Sus scrofa* (blood), 2, *Ovis aries* (tissue), 3, *Ovis aries* (blood), 4, *Bubalus bubalis* (tissue), 5, *Bubalus bubalis* (blood), 6, *Equus caballus* (blood), 7, *Bos taurus* (tissue), 8, *Bos taurus* (blood) and M, marker.

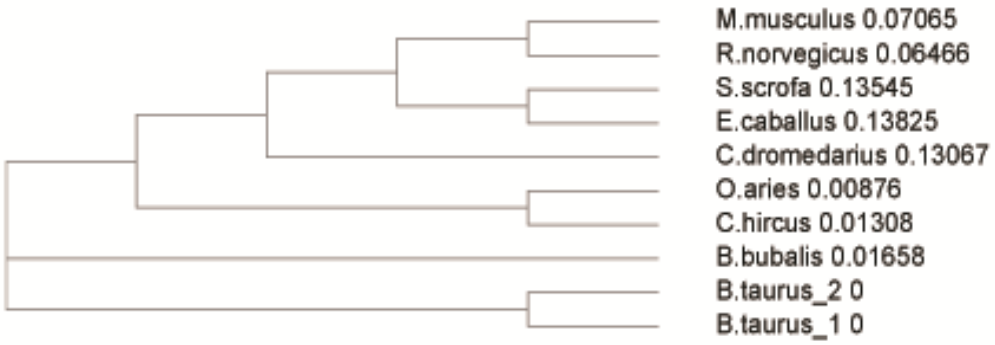

Figure 2: Phylogenetic tree of the multiple DNA sequence alignment of the Egyptian *Bubalus bubalis*, *Bos taurus, Ovis aries, Equus caballus* and *Sus scrofa Glycam1* gene in other organisms that are available in the GenBank database.

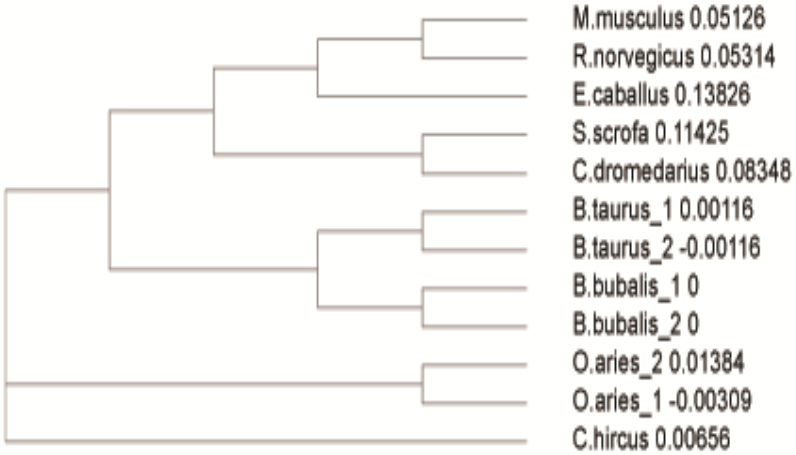

Figure 3: Phylogenetic tree of the multiple mRNA sequence alignment of the Egyptian *Bubalus bubalis*, *Bos taurus, Ovis aries, Equus caballus* and *Sus scrofa Glycam1* gene in other organisms that are available in the GenBank database.

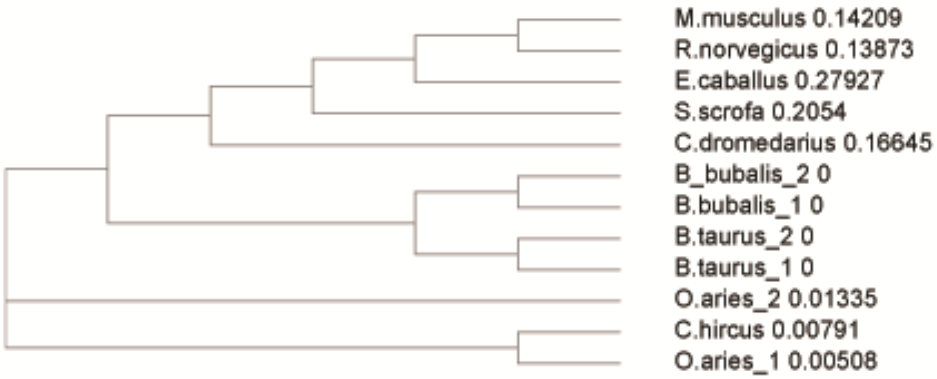

Figure 4: Phylogenetic tree of the multiple amino acid sequence alignment of the Egyptian *Bubalus bubalis*, *Bos taurus, Ovis aries, Equus caballus* and *Sus scrofa* Glycam1 protein in other organisms that are available in the GenBank database.