Indian Journal of Biochemistry & Biophysics
Vol. 61, April 2024, pp. 232-240
DOI: 10.56042/ijbb.v61i4.1230

Cloning, phylogeny and three dimensional structure analysis of the gene encoding Interferon-ε of Sheep (*Ovis aries*)

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Received 08 May 2023; *revised 06 February 2024*

Interferon (IFN) epsilon (ε) has been identified as an important protein of interest, which plays a major role in innate immune system of mammals. Indian Avishaan sheep is well known for its twinning and triplet traits. Hence, the present study has been undertaken with the aim to characterize Indian Avishaan sheep IFNε, although reports found on O*vis aries* IFN-epsilon and Arabian Camel IFN-epsilon. The cDNA of type I interferon *viz*., Interferon-ε from the liver of Avishaan sheep has been amplified by PCR and subsequently cloned for sequence analysis. Sequence analysis revealed that Avishaan sheep shared 100 % both at nucleotide and amino acid levels with that of Rambouillet sheep. With other artiodactyls, the range of identity of Avishhan sheep was 85- 99 % and 78- 98 %, at nucleotide and amino acid levels, respectively. Perissodactyls shared 84-85 % and 77-78 % identity at nucleotide and amino acid levels, respectively, with that of Avishaan sheep from India. Three dimensional structure of Interferon-ε protein of Avishaan sheep was also discussed. Phylogenetic analysis based on amino acid sequences indicated the close relationship in Interferon-ε gene between sheep and other artiodactyls. The structural and functional specific residues noticed across amino acid sequences shows specificity towards orthologs, which needs further characterization.

Keywords: Bioinformatic analysis, Interferon-ε, Liver, Sheep, Three dimensional structure

Interferons (IFN) are basically soluble proteins discovered by Isaacs and Lindenmann during 1957 and known for their inhibitory effects on viruses in cell culture[1]. According to Vilcek[2] and Pestika & his team[3], these antiviral proteins are of three types: type I (IFN-I), type II (IFN-II), and type III (IFN-III). Pestika & his team[3] reviewed that IFN- α subtypes, IFN- β, IFN-ε, IFN- κ & IFN- ω are classified under Type I IFN, whereas Type II IFN consists of IFN – γ only. Vilcek[2] stated that there are three IFN- λ molecules, IFN- λ 1, IFN- λ 2, and IFN- λ 3) within Type III IFN. McNab and his team[4] stated that within the innate immune system of mammals, the members of type I interferon (IFN-I) family were the first line of defence against viruses and other dreadful microorganisms.

It is an unique feature that when compared to other type-I IFNs, one could find the constitutive expression of IFN ε in the lung, brain, small intestine, and reproductive tissue; therefore, it is speculated that IFN ε would have the ability to protect the reproductive tract from viral infections or assist in the early development of placenta in mammalian species[5,6].

Recently, Abdel-Fattah and his team[7] studied the cytotoxic effects of IFN ε of the Dromedarian camel (*Camelus dromedarius*) on two breast cancer cell lines MDA-MB-231 and MCF-7. Interestingly, IFN ε was able to arrest cell survival in a dose dependent manner as observed by MTT assay, morphological changes and apoptosis assay in both cell lines.

The female reproductive tract could be effectively protected against bacterial and viral infections,

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especially from HIV-1 infection by IFN ε[8]. Afsar and Afsar[9] quoted that in COVID-19 infections among human beings, IFN ε could be responsible of decreased mortality in females, when compared to males. The natural reservoirs for SARS-CoV-2-like CoVs are the Pangolins[10]. In Pangolin species, the single copy intronless IFNε gene becomes pseudogenised[11] and this could be the attribute for the immune responses of Pangolins against viral infections including COVID-19[9].

In Livestock species, bovine and canine IFN-ε are known to express strong antiviral activity on both homologous and heterologous animal cells *in vitro*[12,13]. Fischer and his team[14] reported that the endometrium of mares was having the constitutive expression of IFN-ε, which is getting upregulated during the luteal phase. Guo and his team[15] validated that antiviral effects of the recombinant Ovine IFN-ε could inhibit the replication of Vesicular Stomatitis Virus and Bovine Viral Diarrhoea Virus.

Among the small ruminants, sheep are invariably utilized as a large animal model for a broad range of human immunological and physiological implications including asthma. Further, owing to their large size and placid nature, sheep is the animal of choice for the optimization of invasive techniques, which are being commonly performed in human transplantation immunology. Sheep are also being used to study for studying many of the unanswered clinical issues of human beings[16].

Further, Entrican and his team[17] also proposed that when compared to mice, sheep could be the ideal biomedical models for studying reproductive physiology and pathogens infecting respiratory system of human beings. Therefore, it is imperative to explore the immune system of sheep for the development of diagnostics and therapeutics against human pathogens. In this direction, while searching for Interferon-ε gene of Sheep in the literature, the baseline information about this important type I interferon was scanty.

The prolific cross, *i.e.*, Avishaan sheep, was developed and released by ICAR-Central Sheep & Wool Research Institute, Avikanagar, India, during January 2016 & is a composite cross having 12.5% Garole, 37.5% Malpura and 50% Patanwadi blood. Avishaan is further known for the possession of its twinning (49 %) and triplet (2%) traits.

Since Avishaan sheep is well known for bearing more than one foetus, as a first step, it is important to investigate its innate immune responses, afforded particularly by IFN-ε within the reproductive tract of the said prolific ovine breed. Therefore, the present study is proposed with the aim of establishing the baseline information about the nucleotide and its corresponding amino acid sequences for Interferon-ε gene of Avishaan Sheep.

Keeping the aforementioned information in view, the present study was carried out with the objective to clone and characterize the full length of IFN-ε of Avishaan sheep. The deduced amino acid sequences were then used for phylogenetic relationship analyses by comparing them with published sequences from other mammalian animal species.

Materials and Methods

Collection of Sheep liver, RNA isolation and Reverse transcription- PCR

All animal experiments were performed according to protocols approved by the institutional committee for use and care of animals at ICAR- Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India.

A male sheep around two years of age (Avishan breed) was slaughtered at Livestock products Technology Division of ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India on 15th February 2020. From this animal, the liver tissue was collected in Trizol™ reagent and brought to the laboratory in ice. Subsequently, liver tissue containing Trizol™ reagent was ground in a mortar with the help of pestle using liquid nitrogen. The resultant ground tissue in the form of powder was used for the isolation of total cellular RNA using FAVOURPREP™ Blood/ cultured cells kit as per the manufacturer's instructions. The quality and quantity of the RNA isolated from liver tissues were determined by Quawell Nanodrop UV-Vis Spectrophotometer (Q5000).

An aliquot of the total RNA (5 µg) was reverse-transcribed using iScript cDNA synthesis kit (BioRad, USA) in a 20 µL volume reaction mixture according to the manufacturer's instructions.

Polymerase chain reaction

Reaction volumes for the PCR of 50 µL were used and contained 5 µL of 10X buffer with 15 mM $MgCl_2$, 10 mM of each dNTPs, 100 pmol of each oligonucleotide primer, 100 ng of cDNA sample and 3U Taq DNA polymerase . The primers used for the amplification of Interferon-ε gene of Sheep ; The forward primer, OAIFNE-F (29 mer): 5′ ATGA TTAACAAGGCTTTCTTTGAAATTG 3′ and reverse primer, OAIFNE-R (28 mer): 5′ TCAAGTTTC

CATGCCCTGTTCACTCAGC 3′. The primer sequences were designed based on Interferon-ε gene sequences of Sheep reported in NCBI Accession No.XM_012127127.2.

The reaction mixture was subjected to initial denaturation of the template at 95°C for 3 min in a thermal cycler (BioRad, USA). Cycling conditions for PCR were 35 cycles of 60 s at 94°C, 60 s at 55°C and 60 s at 72°C, followed by a final extension for 10 min at 72°C. The total cellular RNA isolated from the blood of sheep was included as a negative control in the PCR.

Cloning and sequencing of Interferon-ε gene of Sheep

Resultant PCR products were separated on 1.2% agarose gels containing ethidium bromide (10 mg/ml), and visualised under UV light. The PCR products were purified using QIA quick Gel Extraction Kit and cloned into pJET1.2/blunt cloning vector (Thermo Scientific clone JET PCR Cloning kit, Catalog No. #K1231, K#1232) using Sticky end protocol according to manufacturer's instructions. The plasmids were transformed into *Escherichia coli* DH5α. Colonies harbouring the recombinant plasmid were inoculated into LB (Luria Bertani) broth and incubated at 37°C overnight with horizontal shaking. The plasmid DNA was extracted from culture using QI Aprep Spin Miniprep Kit. The recombinant plasmids were confirmed by PCR using the primer set; pJET1.2 forward sequencing primer: 5′- CGACTCACTAT AGGGAGAGCGGC-3′ and pJET1.2 reverse sequencing primer 5′- AAGAACATCGATTTTCC ATGGCAG-3′. The sequencing of three positive clones was carried out in both directions using Sanger sequencing method by M/s. Xcelris Labs Limited, Ahmedabad, Gujarat, India and the firm also used the above said vector specific primer set for the sequencing experiments.

Sequence, three dimensional structure and phylogenetic analysis

Using BLAST (Biological Local Alignment Search Tool) software of NCBI[18] the nucleotide sequences provided by M/s. Xcelris Labs Limited, Ahmedabad, Gujarat, India, were analysed. Upon BLAST search, the top most sequences displaying 100 % alignment with the nucleotide sequences of the present study were Interferon-ε gene of Sheep reported in NCBI Accession No.XM_012127127.2 and eventually, the corresponding amino acid sequences of Interferon-ε gene of Sheep were deduced using the same BLAST software. The determined nucleotide sequences of Interferon-ε gene of Avishaan sheep from India were then submitted to GenBank and the accession No. MT151626 was obtained.

Further, the resultant nucleotide and amino acid sequences of Interferon-ε gene of Avishaan sheep from India were then assembled and drawn the alignment with that of 22 mammalian animal species published earlier in the GenBank (Table 1) using sequence

Table 1 — Percent nucleotide (Nt) and amino acid (aa) identity of IFNε gene of Avishaan sheep with other mammalian species

Sl. No.	Animal species	Mammalian order	NCBI Accession No.	Nt (%)	aa (%)
1	*Ovis aries* (Avishaan Sheep- India)	Artiodactyla	MT151626	-	-
2	*Ovis aries* (Rambouillet sheep)	Artiodactyla	XM_012127127.2	100	100
3	*Capra hircus*	Artiodactyla	XM_013965868.2	99	98
4	*Bos taurus*	Artiodactyla	XM_005209901.4	97	95
5	*Bos indicus*	Artiodactyla	XM_019965656.1	97	95
6	*Bos mutus*	Artiodactyla	XM_005887858.1	97	95
7	*Bison bison bison*	Artiodactyla	XM_010853312.1	97	95
8	*Bubalus bubalis*	Artiodactyla	XM_006054569.2	96	94
9	*Odocoileus virginianus texanus*	Artiodactyla	XM_020904911.1	96	94
10	*Balaenoptera acutorostrata scammoni*	Artiodactyla	XM_007176821.1	91	85
11	*Sus scrofa*	Artiodactyla	NM_001105310.1	89	85
12	*Vicugna pacos*	Artiodactyla	XM_031677013.1	86	79
13	*Camelus ferus*	Artiodactyla	XM_032477603.1	86	78
14	*Camelus bactrianus*	Artiodactyla	XM_010946008.1	86	78
15	*Camelus dromedarius*	Artiodactyla	XM_031449624.1	85	78
16	*Equus caballus*	Perissodactyla	XM_005605033.3	85	78
17	*Equus przewalskii*	Perissodactyla	XM_008531931.1	85	78
18	*Equus asinus*	Perissodactyla	XM_014831282.1	84	77
19	*Rhinolophus ferrumequinum*	Chiroptera	XM_033124095.1	84	79
20	*Hipposideros armiger*	Chiroptera	XM_019629430.1	84	79
21	*Felis catus*	Carnivora	NM_001278829.1	83	76
22	*Canis lupus familiaris*	Carnivora	XM_022425479.1	83	73
23	*Oryctolagus cuniculus*	Lagomorpha	XM_017348927.1	77	65

alignment software Clustal X 2.1[19]. Pairwise nucleotide and amino acid sequence identities among the 23 mammalian animal species were also computed using Clustal X 2.1. The signature motifs in Interferon-ε protein of Avishaan sheep were identified as per the reports published earlier[20, 21].

A three dimensional model structure of Interferon Epsilon protein of *Ovis aries* was constructed using Modeller algorithm[22]. A template structure was chosen by employing NCBI-BLAST algorithm against NCBI-PDB protein structure-sequence database. The built 3D model structure was validated by employing Procheck[23].

The phylogenetic tree was constructed by using MEGA X software[24] and the evolutionary history was inferred by the Maximum Likelihood method and JTT matrix-based model[25].

Results and Discussion

Avishaan sheep developed by ICAR-Central Sheep & Wool Research Institute, Avikanagar, India, is known for its prolificacy (triplet yielder). It is imperative to study the immune status of an animal, which is known for augmented productivity. In this way, as a first step, an attempt was made to study type I interferons of Avishhan sheep.

Among type I interferons, Interferon-ε is the most essential in the protection of the female reproductive tract from bacterial and viral infections[8] as well as in the assistance of placental development in mammals[5,6].

Therefore, in order to generate the baseline information about Interferon-ε of Avishaan sheep, amplification of the said type I IFN, from the liver tissue of sheep, subsequent cloning of the amplified DNA fragment into *E.coli* based vector, sequencing of the recombinant plasmid and final analysis of the relatedness of determined nucleotide sequences of IFN-ε of Avishaan sheep with that of other mammalian species were carried out in the present study.

For the first time, analysis of the complete nucleotide sequences of IFN-ε of Avishaan sheep was carried out. The deduced amino acid sequences of the gene encoding IFN - ε of Avishaan sheep from India, were compared to that of 22 different mammalian species available in the NCBI database (Fig. 1). Similar to earlier reports on sheep IFNε[15], the Avishhan sheep IFNε cDNA also contained a 582-bp open reading frame encoding a protein of 193 amino acids with an estimated molecular weight of 22.680 kDa. A signature motif, "YFQRIHDYLESQDY SSCAW" (description- PS00252, Interferon alpha, beta and delta family signature) is found from the position No. 147 to 165 of IFN - ε protein of Avishaan sheep.

The Ovine Interferon Tau protein (PDB ID: 1B5L) was obtained as the close homologue of IFN-ε of *Ovis aries*, which was having the sequence identity of 39%. As none of the interferon epsilon crystal structure is available, Ovine Interferon Tau's X-ray crystal structure with the resolution of 2.1 A^{o} has been found to be a close homolog and considered as the template structure. The pairwise sequence alignment has been constructed by employing Clustal algorithm and the alignment has been edited using Jalview tool[29]. The pairwise sequence identity between template and target sequence is 39% (Fig. 2).

A three-dimensional model structure has been predicted by employing Modeller algorithm[22] (Fig. 3). Three have been five α-helices, labelled from A to E. The residues falling in the helix regions have been highlighted on the sequences of IFN-ε of *Ovis aries* within the pairwise alignment. The three conserved cysteine residues, Cys53, Cys163 and Cys175 in the type 1 Interferon were mapped on this model structure (Fig. 3). Moreover, a disulphide bond found between Cys53 and Cys163 is highlighted, which is predicted to be involved in stabilizing the 3D structure of the protein (Fig. 4).

As expected among IFN homologs, like those found in the ovine IFNε[15], cameline IFNε[7], human IFNλ[26,] human interferon β[27] and rabbit interferon-γ[28], Avishaan sheep IFNε also contained the three conserved cysteine residues at the positions of 53, 163 and 175 (Fig. 5).

Further, the amino acid residues Ser38, Glu112, and Ile167 are also conserved in Avishaan sheep IFNε, as seen among other species of type I INFε, as described earlier[7] (Fig. 5).

While comparing the sequences of camel IFNε protein[7], Avishaan sheep IFNε protein, out of seven potential glycation sites, has retained the similar amino acid residues in four putative glycation sites, which include 59NFLL, 90NLFR, 139NLRL, and 173NRCL (Fig. 5). In other three glycation sites, each one is having a change in one amino acid residue: 3NKAF, 35NQES and 43NKLQ, instead of 3NKPF, 35NRES and 43NKLR, as predicted in putative camel IFNε. Among the mutated glycation sites, two of them

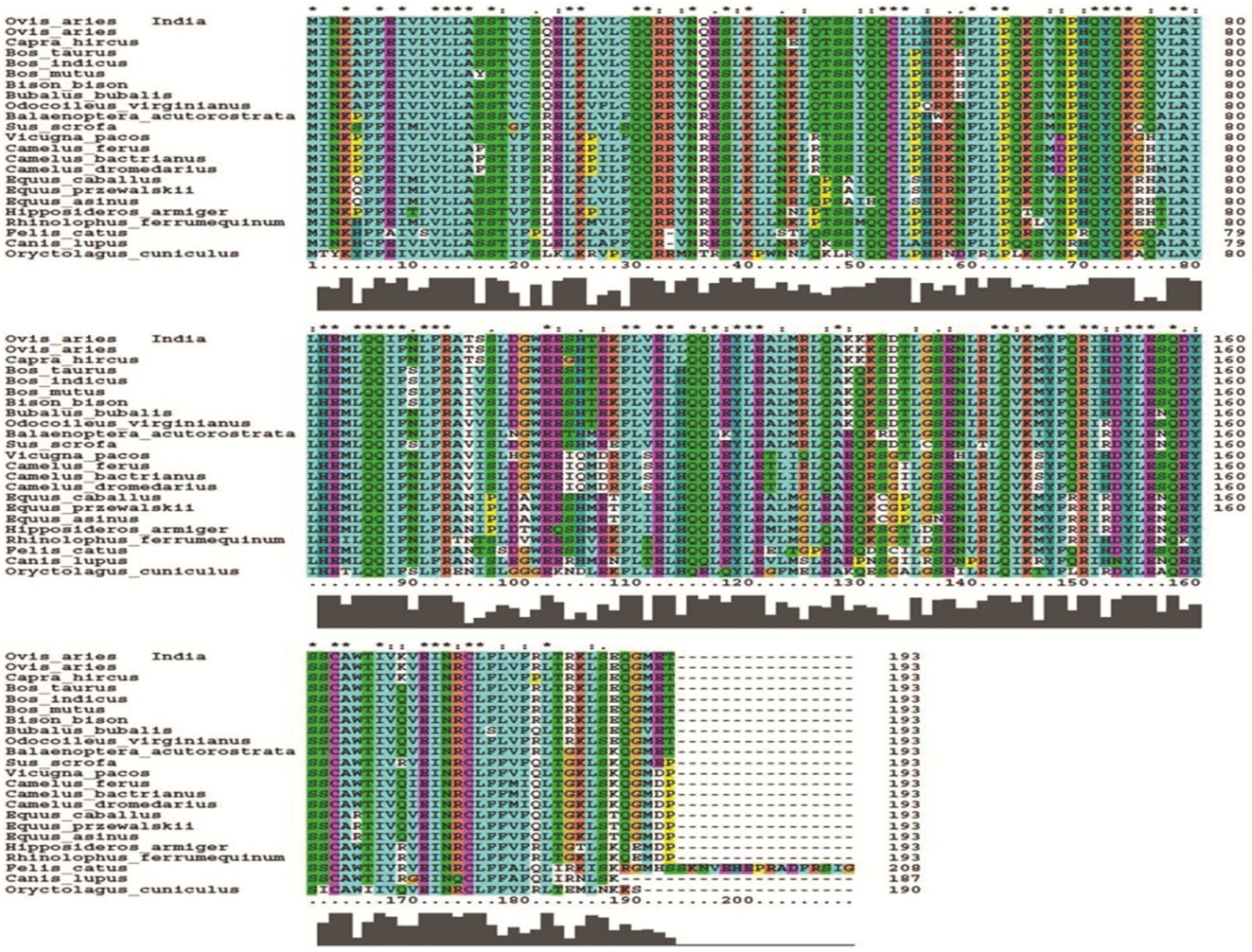

Fig. 1 — Alignment of amino acid sequences of IFNε gene of Avishaan sheep with other mammalian species

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IFNe   MINKAFFEIVLVLLASSTVCSQELKLVLCQQRRVNQESLKLLNKLQTSSIQQCLLHRKNF 60
1B5L   -----------------------CYLSRKLMLDARENLKLLDRMNRLSPHSCLQDRKDF 36
                                :  :     :*.****:::  * :.** .**:*
                                B                     C
IFNe   LLPQKSVNPHQYQKGQVLAILHEMLQQIFNLFRATSSLDGWEESHTEKFLVELLQQLEYL 120
1B5L   GLPQEMVEGDQLQKDQAFPVLYEMLQQSFNLFYTEHSSAAWDTTLLEQLCTGLQQQLDHL 96
        ***: *: .* **.*.:.:*:***** **** :  *  .*: :  *:: . * ***::*
                                D                     E
IFNe   EALMRLQAKKKSDTLGSENLRLQVKMYFQRIHDYLESQDYSSCAWTIVKVEINRCLFLVF 180
1B5L   DTCRGM------------DPIVTVKKYFQGIYDYLQEKGYSDCAWEIVRVEMMRALTVST 144
       ::   :            :  : ** *** *:***:.:.**.*** **:**: *.* :

IFNe   RLTRKLSEQGMET 193
1B5L   TLQKRLTK----- 152
        * ::*::
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Fig. 2 — Model-template alignment of amino acid residues of *Ovis aries* IFNε and *Ovis aries* IFN-tau

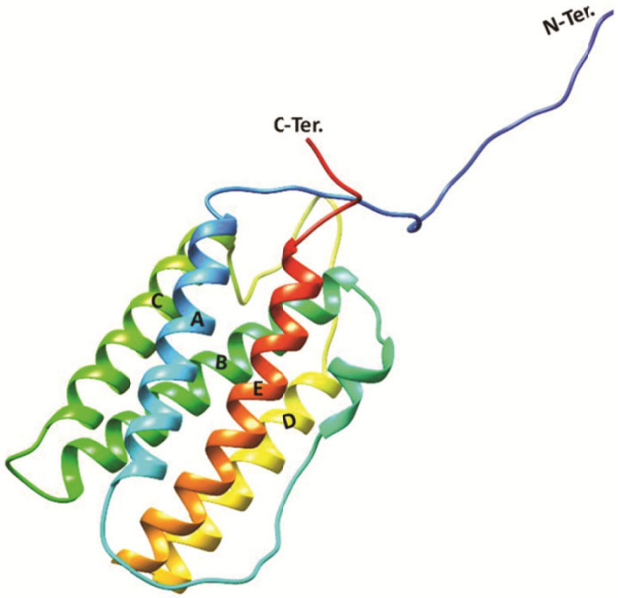

Fig. 3 — Predicted 3D structure model of *Ovis aries* IFNε protein. Fiv alpha helices are labeled as A to E.

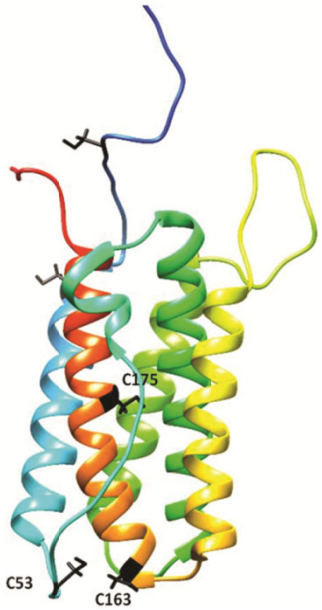

Fig. 4 — 3D structure of *Ovis aries* IFNε protein showing three conserved cysteine residues and disulphide bonds

1	Met	Ile	Asn	Lys	Ala	Phe	Phe	Glu	Ile	Val	Leu	Val	Leu	Leu	Ala	Ser	Ser	Thr	Val	Cys	Ser	Gln	22
1	M	I	N	K	A	F	F	E	I	V	L	V	L	L	A	S	S	T	V	C	S	Q	22
23	Glu	Leu	Lys	Leu	Val	Leu	Cys	Gln	Gln	Arg	Arg	Val	Asn	Gln	Glu	Ser	Leu	Lys	Leu	Leu	Asn	Lys	44
23	E	L	K	L	V	L	C	Q	Q	R	R	V	N	Q	E	S	L	K	L	L	N	K	44
45	Leu	Gln	Thr	Ser	Ser	Ile	Gln	Gln	Cys	Leu	Leu	His	Arg	Lys	Asn	Phe	Leu	Leu	Pro	Gln	Lys	Ser	66
45	L	Q	T	S	S	I	Q	Q	C	L	L	H	R	K	N	F	L	L	P	Q	K	S	66
67	Val	Asn	Pro	His	Gln	Tyr	Gln	Lys	Gly	Gln	Val	Leu	Ala	Ile	Leu	His	Glu	Met	Leu	Gln	Gln	Ile	88
67	V	N	P	H	Q	Y	Q	K	G	Q	V	L	A	I	L	H	E	M	L	Q	Q	I	88
89	Phe	Asn	Leu	Phe	Arg	Ala	Thr	Ser	Ser	Leu	Asp	Gly	Trp	Glu	Glu	Ser	His	Thr	Glu	Lys	Phe	Leu	110
89	F	N	L	F	R	A	T	S	S	L	D	G	W	E	E	S	H	T	E	K	F	L	110
111	Val	Glu	Leu	Leu	Gln	Gln	Leu	Glu	Tyr	Leu	Glu	Ala	Leu	Met	Arg	Leu	Gln	Ala	Lys	Lys	Lys	Ser	132
111	V	E	L	L	Q	Q	L	E	Y	L	E	A	L	M	R	L	Q	A	K	K	K	S	132
133	Asp	Thr	Leu	Gly	Ser	Glu	Asn	Leu	Arg	Leu	Gln	Val	Lys	Met	Tyr	Phe	Gln	Arg	Ile	His	Asp	Tyr	154
133	D	T	L	G	S	E	N	L	R	L	Q	V	K	M	Y	F	Q	R	I	H	D	Y	154
155	Leu	Glu	Ser	Gln	Asp	Tyr	Ser	Ser	Cys	Ala	Trp	Thr	Ile	Val	Lys	Val	Glu	Ile	Asn	Arg	Cys	Leu	176
155	L	E	S	Q	D	Y	S	S	C	A	W	T	I	V	K	V	E	I	N	R	C	L	176
177	Phe	Leu	Val	Phe	Arg	Leu	Thr	Arg	Lys	Leu	Ser	Glu	Gln	Gly	Met	Glu	Thr	*					193
177	F	L	V	F	R	L	T	R	K	L	S	E	Q	G	M	E	T	*					193

Fig. 5 — Deduced amino acid encoding region of Avishaan sheep IFNε. Important amino acid residues and regions include: ↔: Residues contact to N-Acetyl-2-Deoxy; ↑: Residue contact to Zn^{+2}; *: Three conserved cysteine residues at the positions of 53, 163 and 175; ↓: Residues conserved in type I INFε species - Ser38, Glu112, and Ile167; : four putative glycation sites (out of seven potential glycation sites in Camel IFNε) retained by Avishhan sheep

are showing Glutamine to Arginine replacement. Such glycation sites could be involved in the protection to IFNε protein against proteases-mediated hydrolysis as well as in the process of folding, oligomerization, and stability of the protein.

In addition,, the predicted amino acid residues for N-Acetyl-2-Deoxy-2-Amino-Galactose (ligand) binding in Camel are Arg131 & Ser132 but in sheep, they are Lys131 & Ser132, where the basic amino acid Arginine at position 131 is replaced by another basic amino acid, *i.e.*, Lysine (Fig. 5) in Avishaan sheep IFNε protein.

Another feature, Zinc (metal) ion binding residue Gln143 in Camel interferon ε protein is found to be conserved in sheep interferon ε too (Fig. 5).

The earlier reports on putative IFNAR-1 and IFNAR-2 binding domains of Camel IFNε protein have been characterized by the possession of eight amino acid residues, C29, Q30, R33, Q36, E37, K40, N43, & K44 and thirteen amino acid residues, L54, L55, H56, R57, K58, N59, F60, L61, P63, Q64, K65, Q71, & Y72, respectively[7].

However, the IFNAR-1 binding domain of Avishaan sheep IFNε protein is different from that of Camel IFNε protein in the context that the former possesses C29 & Q36, whereas in the latter, they are F29 & R36. Similarly, IFNAR-2 binding domain of sheep IFNε protein also contains a change in only one amino acid residue, *i.e.*, at position 55, it is Lysine, instead of Proline in case of camel IFNε protein[7]. The IFNAR1 binding domain (Residue numbers 29, 30, 33, 36, 37, 40, 43 and 44) falls in the alphaA helix region in IFN epsilon of Avishaan sheep (Fig. 6A). The IFNAR2 binding domain (Residue numbers 54-61, 63-65, 71 and 72) falls in between alphaA helix and alphaB helix, which is majorly occupied by the coil or loop region and a small helix region in IFN epsilon of Avishaan sheep (Fig. 6B). As per the earlier published reports, this small helix region is marked as alpha B in case of IFN epsilon of Camel[7]; however, which is not being marked for helix region in case of sheep[15]. In the present study, the said helix region of IFN epsilon protein model of sheep is also not labelled, although a small helix region is found in the characterized IFNAR2 binding domain.

Sequence analysis involving 23 sequences (Fig. 1) revealed that the gene encoding interferon ε of Avishaan sheep from India shared 100 % sequence identity both at nucleotide and amino acid levels with that of Rambouillet sheep. With other artiodactyls, the range of identity of Avishhan sheep was 89- 99 % and 85-98 %, at nucleotide and amino acid levels, respectively. However, within the Artiodactyls, IFN-ε protein of *Bos taurus* and *Bos indicus* have shown 100% sequence identity. In case of Tylopods, the range of identity of Avishhan sheep at nucleotide level was 85- 86 %, whereas at amino acid level, the range of identity was 78-79 %. As noticed in Artiodactyls, IFN-ε protein sequence of *Camelus ferus* shows 100% sequence identity with IFN-ε of *Camelus bactrianus* in Tylopods. Perissodactyls shared 84-85 % and 77-78 % identity at nucleotide and amino acid levels, respectively, with that of Avishaan sheep from India (Table 1). *Equus caballus* IFN-ε protein shows 100% sequence identity with *Equus przewalskii* IFN-ε protein in perissodactyls.

Arnason and Janke[30] reported that in recent years, phylogenetic analysis could extensively be used for the estimation of the genetic relatedness among mammalian species.

Phylogenetic relationship was constructed employing MEGA-X on the multiple sequence alignment of interferon-ε protein sequences of 23 species. Phylogenetic analysis based on amino acid sequences indicated the close relationship between sheep and other artiodactyls (Fig. 7). However, it is also interesting to note that a separate branch formed by IFN-ε of sheep, which indicates that functional specific residues may vary between these groups although they are closely related. These results could be correlated with the previously published Phylogenetic analysis[7]. The white-tailed deer

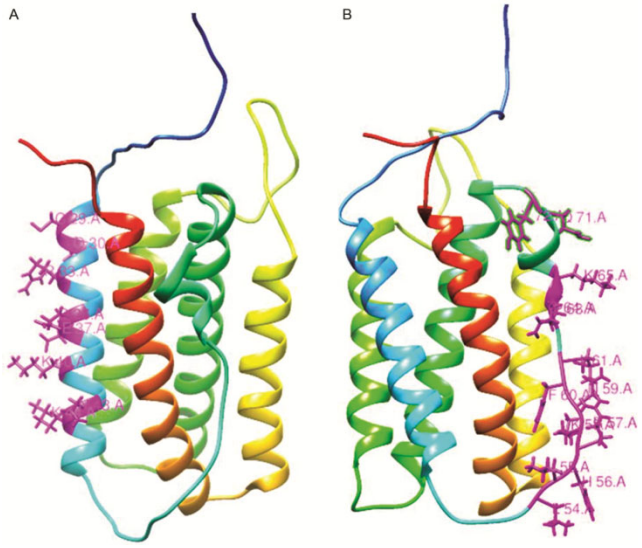

Fig. 6 — 3D structure of *Ovis aries* IFNε protein showing (A) IFNAR-1; and (B) IFNAR-2 binding domain of Avishaan sheep IFNε protein

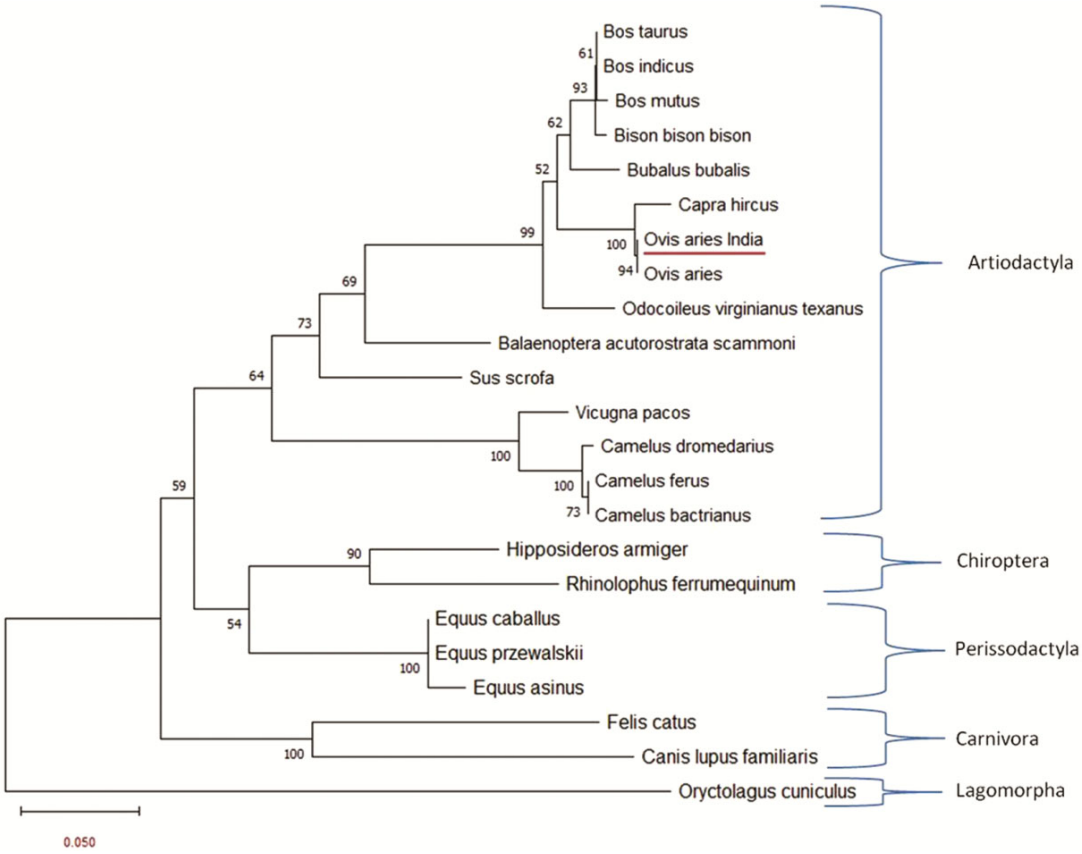

Fig. 7 — The evolutionary relationship between the species was conducted for Interferon-ε gene in MEGA X employing Neighbour-Joining distance based algorithm

(*Odocoileus virginianus*) is closely related to sheep and cattle. Similarly, the minke whale (*Balaenoptera acutorostrata scammoni*) is closely related with sheep, goat and cattle than with swine (*Sus scrofa*). The camel IFN-ε is branched out separately, though they are closely related with sheep and cattle, which is comparable with the earlier published results[7]. The relationship noticed in the phylogenetic tree shows that how they are related and deviated from each other, which clearly indicates the differences in their amino acids that are responsible for maintaining the structural and functional properties pertaining to that species.

Conclusion

The complete analysis of nucleotide and amino acid sequences of IFN-ε of Avishaan sheep from India was carried out for the first time. Similar to other IFN homologs, three cysteine residues at the positions of 53, 163 and 175 are also conserved in IFN-ε of Avishaan sheep. The obtained sequence information would be useful for the generation of Type-I interferon based therapeutics for sheep and other mammalian species.

Acknowledgement

The authors gratefully acknowledged the facilities and fund rendered by the Director, ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan for carrying out the study. The authors are also thankful to the Head, Animal Genetics & Breeding Division, ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, who had given the permission to avail the lab facilities. The financial support provided by Indian Council of Agricultural Research to carry out this study is gratefully acknowledged.

Conflict of interest

All authors declare no conflict of interest.

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