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Review Article

Research Progress of Bovine Interferon Alpha (BoIFN-α) in Veterinary Science: A Review

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Abstract

Interferons (IFNs) are a class of cytokines with antiviral, antitumor, and immunoregulatory functions, and they have great potential for use in veterinary science. Bovine interferon alpha (BoIFN-α), as a crucial member of the type I IFN family, is essential for keeping Bovine population healthy and preventing diseases. In recent years, research on BoIFN-α has been increasing, covering aspects such as its structural characteristics, biological functions, and mechanisms of action. But in practical real-world application in clinical settings, BoIFN-α still has a variety of significant challenges, like keeping its clinical effectiveness stable and managing production costs. This review gives a thorough overview of the research on BoIFN-α, particularly its applications in antiviral, immune regulation, and disease treatment, and also introduces its clinical applications in the prevention and control of common viral, bacterial, and parasitic diseases in Bovine population. Additionally, the review discusses the challenges BoIFN-α faces in veterinary practice and future development directions, with the goal of giving solid theoretical support and practical advice for its in-depth research and widespread application in veterinary science.

Keywords: Antiviral, Bovine interferon alpha (BoIFN-α), Clinical application, Disease treatment, Immune regulation, Veterinary science

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Introduction

The bovine industry is crucial for global farming, impacting meat and dairy quality, but diseases threaten herds, causing production losses and economic damage [1]. Traditional disease control methods like vaccination and antibiotics have helped, but pathogen mutations and drug resistance necessitate new prevention strategies.

Interferons (IFNs) are crucial for the immune defense, recognized since 1957 for their antiviral, immunoregulatory, and antitumor effects [2,3]. Based on their structure, receptor specificity, and functional characteristics, IFNs can be classified into type I, II, and III. Among them, type I IFNs are the most diverse, including several subtypes such as IFN-α, IFN-β, and IFN-ω [3]. Bovine interferon alpha (BoIFN-α) rapidly triggers the immune response after pathogen infection, crucial for antiviral and immune regulation, protecting bovine health [4].

Significant progress in veterinary science on BoIFN-α includes its gene structure, protein characteristics, mechanisms, and clinical applications *(Fig. 1)*, showing its role in bovine antiviral immunity and potential in regulating immune responses and reducing diseases like respiratory issues and dermatitis [5].

A deeper understanding of BoIFN-α's biological characteristics and mechanisms is crucial for advancing bovine health and industry sustainability, with molecular biology research providing new evidence for improving bovine health and informing related fields [6].

This review systematically summarizes BoIFN-α research in veterinary medicine, covering its classification, structure,

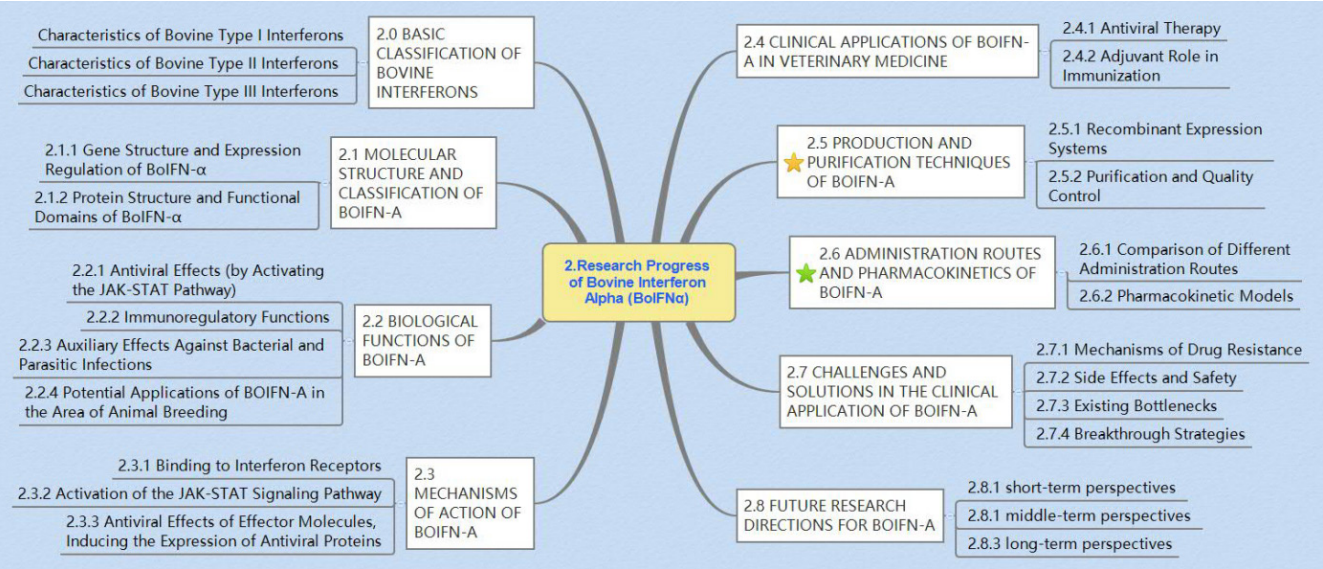

Fig 1. Study scheme on the research progress of Bovine Interferon Alpha (BoIFN-α) in veterinary science

functions, mechanisms, applications, production, challenges, and future directions to enhance its use in bovine herd health and livestock sustainability.

Basic Classification of Bovine Interferons

Bovine interferons (BoIFNs) are crucial for bovine immunity, classified into type I, II, and III, each with distinct gene families and functions. Type I (IFN-α, IFN-β, IFN-ω, IFN-τ, IFN-χ, IFN-ε, IFN-κ) mediates antiviral responses, while type II (IFN-γ) and type III (IFN-λ) are involved in immune response networks, aiding in disease prevention and control.

Characteristics of Bovine Type I IFNs

Type I IFNs, particularly IFN-α and IFN-β, vary in tissue expression and are regulated by viral infections, influencing their antiviral activity [7,8]. Bovine interferon-omega (BoIFN-ω), a type I IFN, has broad antiviral functions, inducing ISGs via the JAK (Janus kinase)-STAT (signal transducer and activator of transcription) pathway and showing potential as a therapeutic agent due to its efficacy in other species [9,10]. Bovine interferon-tau (BoIFN-τ) is vital for early pregnancy by promoting maternal recognition and endometrial receptivity through Interferon regulatory factor 1 (IRF1) and Leukemia inhibitory factor receptor (LIFR) expression, enhancing implantation, and regulating prostaglandin synthesis for pregnancy maintenance [11,12]. Bovine interferon-chi (BoIFN-χ) is a type I IFN with immune functions, enhancing innate immunity and promoting IFN production during viral infections via the JAK-STAT pathway [13]. Bovine interferon-epsilon (BoIFN-), cloned from bovine liver, has a specific gene structure, shows lower antiviral activity than BoIFN-α1, and is expressed in various organs, indicating its typical type I IFN properties and therapeutic potential [14]. Bovine interferon-kappa (BoIFN-κ) is a 215-amino acid protein with 63% similarity to human IFN-κ, showing type I IFN traits-like antiviral activity. It binds to the bovine type I IFN receptor, inducing Mx1, ISG15, and ISG56 transcription, and operates mainly via the JAK-STAT pathway [15]. The summary of Bovine interferon subtypes are listed in *Table 1*. Based on the retrieved PubMed literature, it is important to note that for Bovine Type I IFNs, all entries remain at the laboratory stage, and no specific trade names or commercially approved products are mentioned in the literature *(Table 2)*. Therefore, we clarified the regulatory status of all products are "experimental". This statement highlights the gap between Bovine Type I IFNs research and practical application, emphasizing challenges like production, stability, delivery, safety, and regulation.

Characteristics of Bovine Type II IFNs

Bovine Type II IFN, mainly IFN-γ, is produced by T cells and NK cells, enhancing immune responses through macrophage activation and antigen presentation via the STAT1 pathway, playing a crucial role in antiviral and immune regulation [19]. This literature highlights IFN-γ's key role in immune responses and its application in disease control, but does not address the STAT1 pathway.

Characteristics of Bovine Type III IFNs

Bovine Type III IFNs (IFN-λ) resemble type I IFNs but are limited to epithelial cells, aiding mucosal immunity. Research shows IFN-λ's antiviral activity against bovine viral diarrhea virus (BVDV), reducing viral load and symptoms, and it primarily induces local antiviral states, minimizing systemic inflammation, highlighting its

Table 1. Summary of bovine interferon subtypes

Parameter	BoIFN-α (Bovine IFN-alpha)	BoIFN-β (Bovine IFN-beta)	BoIFN-ω (Bovine IFN-omega)	BoIFN-τ (Bovine IFN-tau)	BoIFN-χ (Bovine IFN-chi)	BoIFN-ε (Bovine IFN-epsilon)	BoIFN-κ (Bovine IFN-kappa)	BoIFN-γ (Bovine IFN-gamma, Type II IFN)	BoIFN-λ (Bovine IFN-lambda, Type III IFN)
Subtype	Multigene family, multiple subtypes [16]	3 subtypes [17]	Different subtypes exist in species like cats and pigs [10,18]	At least 18 different BoIFN-τ cDNA variants	4 subtypes	Not specified	Likely 1 major subtype	Single gene	Multiple IFN-λ genes (e.g., λ1, λ2, λ3)
Source Tissue/Cell	Peripheral blood mononuclear cells (PBMC), lymphoblast cell lines	Bovine kidney cells (e.g., BK, CRIB cells)	Inferred from studies in pigs and cats, likely various cells	Trophoblast cells	Can be expressed in various cells (e.g., virus-infected cells)	Cloned from bovine liver genomic DNA	Gene amplified from bovine tissues, specific source not specified	Mainly produced by activated T cells and natural killer (NK) cells	Mucosal epithelial tissues, source include epithelial cells and dendritic cells
Amino Acid Number	Varies by subtype	Varies by subtype	195 amino acid residues (including 23-residue N-terminal signal peptide)	Varies by subtype, protein molecular weight approximately 23 kDa	Varies by subtype	193 amino acids (including 21-amino acid signal peptide) [14]	215 amino acids [15]	143 amino acids	200 amino acids. Includes 19-23-residue N-terminal signal peptide
Signaling Pathway	Activates JAK-STAT pathway via IFNAR receptor complex, induces ISG expression	Activates JAK-STAT pathway via common type I interferon receptor	Induces ISG expression via JAK-STAT pathway [10]	(Based on type I IFN characteristics) speculated to possibly act through JAK-STAT pathway	JAK-STAT pathway	Inferred as type I IFN, likely through JAK-STAT pathway	JAK-STAT signaling pathway	Activates JAK-STAT pathway via IFNGR receptor complex, promotes Th1 immune response [19]	Speculated BoIFN-λ function via JAK-STAT signaling pathway, its specificity determined by the IFNLR1 receptor.
Characteristics	1. Subtype expression differences; 2. Rigid expression patterns; 3. Activity differences among subtypes; 4. Potent, broad-spectrum antiviral activity, induce systemic responses; 5side effects like fever [19]	1. Virus-induced specificity;2. Promoter differences among subtypes; 3. Viral infection leads to immune evasion	1. Induces ISG expression; 2. Cross-species activity; 3. Affects miRNA composition in blood cell-derived extracellular vesicles, possesses novel immunomodulatory mechanisms [20]	1. Induces IRF1 and LIFR expression; 2. Reduces E-cadherin expression; 3. Enhances endometrial receptivity; 4. Regulates prostaglandin synthesis [11,12]	1. Highly sensitive to trypsin, but stable under pH and temperature changes; 2. Multigene family, possesses antiviral and antiproliferative activities	1. Antiviral activity lower than BoIFN-α; 2. Highly transcribed in EBK cells; 3. Constitutively expressed in liver, thymus, kidney, small intestine, testis, but not in heart [14]	1. Sensitive to trypsin; 2. Stable under pH and temperature changes; 3. Possesses antiviral and antiproliferative activities [15]	1. Core pro-inflammatory cytokine; 2. Primarily activates macrophages; 3. Involved in cellular immune responses.	1. Possesses antiviral activity; 2. Good safety profile; 3. Its receptor is mainly expressed on epithelial cells; 4. Involved in mucosal immune defense [21]
Function	1. Establishes systemic antiviral state; 2. Activates innate immune cells; 3. Key cytokine in defense against viral infections [7]	Mediates antiviral responses and plays roles in immune regulation [22]	1. Broad-spectrum antiviral; 2. Participates in immune regulation; 3. Has therapeutic potential [9,10]	Related to reproduction, including: 1. Maternal recognition; 2. Enhancing endometrial receptivity and embryo implantation; 3. Regulating prostaglandin synthesis to maintain pregnancy [23]	1. Antiviral and antiproliferative activities; 2. Enhances IFN production via positive feedback regulation (e.g., activates IFN-gulating prostaglandin s [13]	1. Antiviral activity; 2. Potential therapeutic agent	1. Antiviral activity: binding to bovine type I IFN receptor, can be blocked by specific antibodies; 2. Induces ISG gene transcription; 3. Activates promoters: including NF-κB, ISRE, and BoIFN-β promoter	1. Regulates adaptive immunity; 2. Activates macrophages; 3. Enhances antigen presentation [19]	1. Anti-infection; 2. Induces immune responses, promotes early production of bovine viral diarrhea virus (BVDV) neutralizing antibodies post-infection

Table 2. *Summary of commercial and experimental use of bovine type I IFNs*

Trade Name	Active Ingredient	Target Species	Indication	Formulation and Route of Administration	Common Dosage/ Regimen	Regulatory Status	Key References
No information	BoIFN-alpha 1-1	Swine population	Antiviral (transmissible gastroenteritis virus)	Recombinant protein, oral	1mg orally every 12 hours	Experimental	[24]
No information	rIFN-alpha	Bovine population	Antiviral diseases (e.g., viral infections)	Recombinant protein, expression system	No specific dosage information	Experimental	[25]
No information	BoIFN-omega	Bovine population	Antiviral (e.g., VSV, BEV)	Recombinant protein, yeast expression	No specific dosage information	Experimental	[26]
No information	BoIFN-epsilon	Bovine population	Antiviral	Recombinant protein, E. coli expression	No specific dosage information	Experimental	[14]
No information	BoIFN-kappa	Bovine population	Antiviral	Recombinant protein	No specific dosage information	Experimental	[15]

Based on the retrieved PubMed literature, the above table summarizes the commercial and experimental applications of Bovine Type I IFNs. It is important to note that all entries are derived from research contexts, and no specific trade names or commercially approved products are mentioned in the literature

importance in mucosal immunity [21]. The study had a small sample size (n=4 treatment, n=2 control), limiting its statistical power and generalizability, and focused on BVDV type-2, suggesting varying effects of IFN-λ with other strains.

Molecular Structure and Classification of BoIFN-A

Gene Structure and Expression Regulation of BoIFN-α

BoIFN-α is a key antiviral cytokine in cattle, with its gene on chromosome 1 consisting of 5 exons and 4 introns, coding for about 567 bp. There are at least 10 subtypes, showing 80%-95% sequence homology, possibly linked to bovine adaptation to pathogens [27,28].

Research indicates that BoIFN-α subtypes regulate immune responses via different pathways, offering insights into bovine immune systems [29]. The subtypes differ in antiviral, antibacterial, and immune regulation, impacting bovine herd health. BoIFN-α levels are affected by factors like viral infections, which significantly upregulate its expression, highlighting its role in antiviral responses [30].

Further research shows BoIFN-α levels are linked to the JAK-STAT pathway; using the BacMam system allows effective expression of BoIFN-α in cells to observe its viral replication inhibition [31]. The key to this process is that BoIFN-α induces ISG transcription, enhancing antiviral capacity and supporting its veterinary use.

Protein Structure and Functional Domains of BoIFN-α

The BoIFN-α precursor protein has 189-192 amino acids, a 23-amino acid signal peptide, and a mature form of 166-169 amino acids, weighing about 19 kDa with a pI of 5.0-6.5, featuring a structure of 5 anti-parallel α-helices (A-E) crucial for receptor binding [32].

Compared to IFN-α from other species, BoIFN-α has the highest amino acid homology with sheep IFN-α (about 85%), about 60% with human IFN-α, and about 70% with pig IFN-α. This species difference causes some specificity in BoIFN-α activity, yet there is cross-reactivity with related species [33].

BoIFN-α, a secreted glycoprotein, has a central α-helical structure and β-sheet segments, allowing it to bind to IFN receptors and trigger signal transduction pathways for its biological functions. In the structure of BoIFN-α, the receptor-binding domain is one of its important functional regions [34]. The domain composition affects BoIFN-α's receptor binding affinity and selectivity, influencing its biological effects through stable interactions and activation of signaling pathways that enhance antiviral and immune responses.

Additionally, the signaling domain of BoIFN-α is also crucial [35]. The signaling domain transduces external signals into cells, activating the JAK-STAT pathway after BoIFN-α binds to its receptor, which regulates cell proliferation, differentiation, and immune responses by recruiting and activating JAK enzymes firstly and then followed by activating STAT transcription factors that express specific genes involved in antiviral, antitumor, and immune regulation.

It is noteworthy that the glycosylation modifications of BoIFN-α also have a significant impact on its function [36-38]. Glycosylation not only affects its stability and biological activity but may also influence its binding ability to receptors [39,40]. By regulating the glycosylation pattern, the biological activity of BoIFN-α can be enhanced or reduced,

resulting in different effects in clinical applications [41-43]. Therefore, in-depth studies of the protein structure and functional domains of BoIFN-α are of great significance for understanding its role in veterinary science and developing new therapeutic approaches.

Biological Functions of BoIFN-A

Antiviral Effects (by Activating the JAK-STAT Pathway)

BoIFN-α is a crucial cytokine that plays a key role in antiviral immunity. Studies have shown that BoIFN-α significantly inhibits the replication of viruses by activating the JAK-STAT signaling pathway [44,45] *(Fig. 2)*. The JAK-STAT pathway is crucial for cytokine signaling, regulating immune responses; BoIFN-α activates it, through boosting ISG expression and enhancing antiviral capacity.

The specific process is as follows: after IFN-α binds to the IFN-α-receptor on the cell surface, it subsequently activates intracellular Janus kinases (Janus kinases 1(JAK1) and tyrosine-protein kinase 2 (TYK2)). The phosphorylation of JAK kinases leads to the activation of STAT proteins, causing STAT1 and STAT2 to be phosphorylated and form heterodimers, which bind to IRF9 to form "interferon-stimulated gene factor 3 (ISGF3)." ISGF3 enters the cell nucleus and binds to the "interferon-stimulated response element (ISRE)" in the promoter region of target genes, initiating downstream gene transcription. Ultimately, it regulates the expression of ISGs, thereby enhancing the cell's resistance to viruses.

Antiviral Effects of Effector Molecules, Inducing the Expression of Antiviral Proteins

BoIFN-α exerts significant antiviral effects in veterinary science by inducing the expression of antiviral proteins and regulating the immune responses of host cells [46] *(Fig. 3)*. Key effector molecules induced by BoIFN-α include: (1) 2',5'-oligoadenylate synthetase (OAS): catalyzes the production of 2',5'-oligoadenylate from ATP, activating RNase L to degrade viral RNA; (2) Protein kinase R (PKR): phosphorylates the eukaryotic translation initiation factor eIF2α, inhibiting viral protein synthesis; (3) Mx protein: prevents the release and replication of viral nucleic acids by binding to viral nucleocapsid proteins (e.g., inhibition of BVDV); (4) Interferon regulatory factors (IRF): further amplify immune signals and enhance antiviral responses.

Research indicates that BoIFN-α activates ISGs like MxA, OAS, and PKR, crucial for antiviral immunity; rBoIFN-α transduced via BacMam significantly inhibited virus replication and induced ISG transcription [31]. It was proved that BoIFN-α enhances antiviral response via

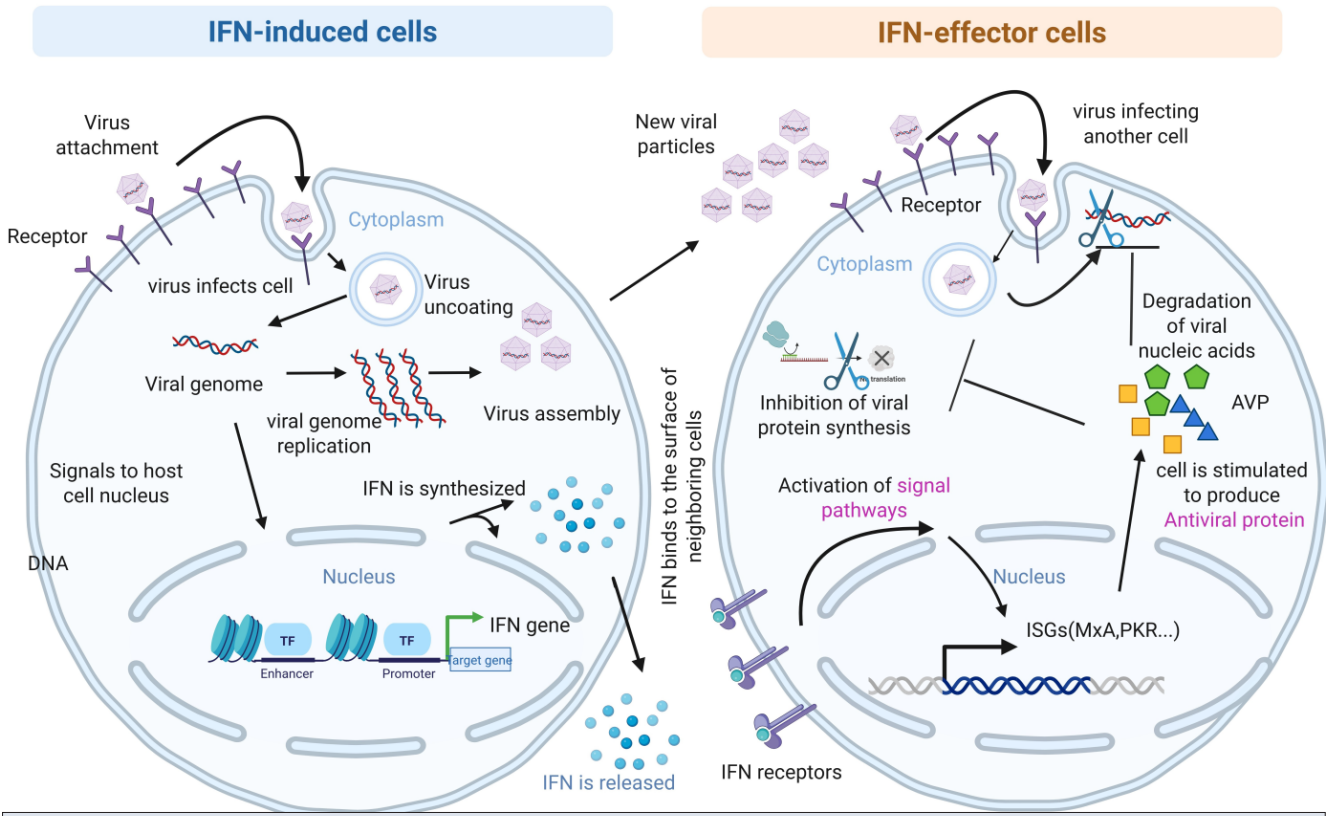

Fig 2. Presentation of the induction and effectiveness of interferon (IFN)

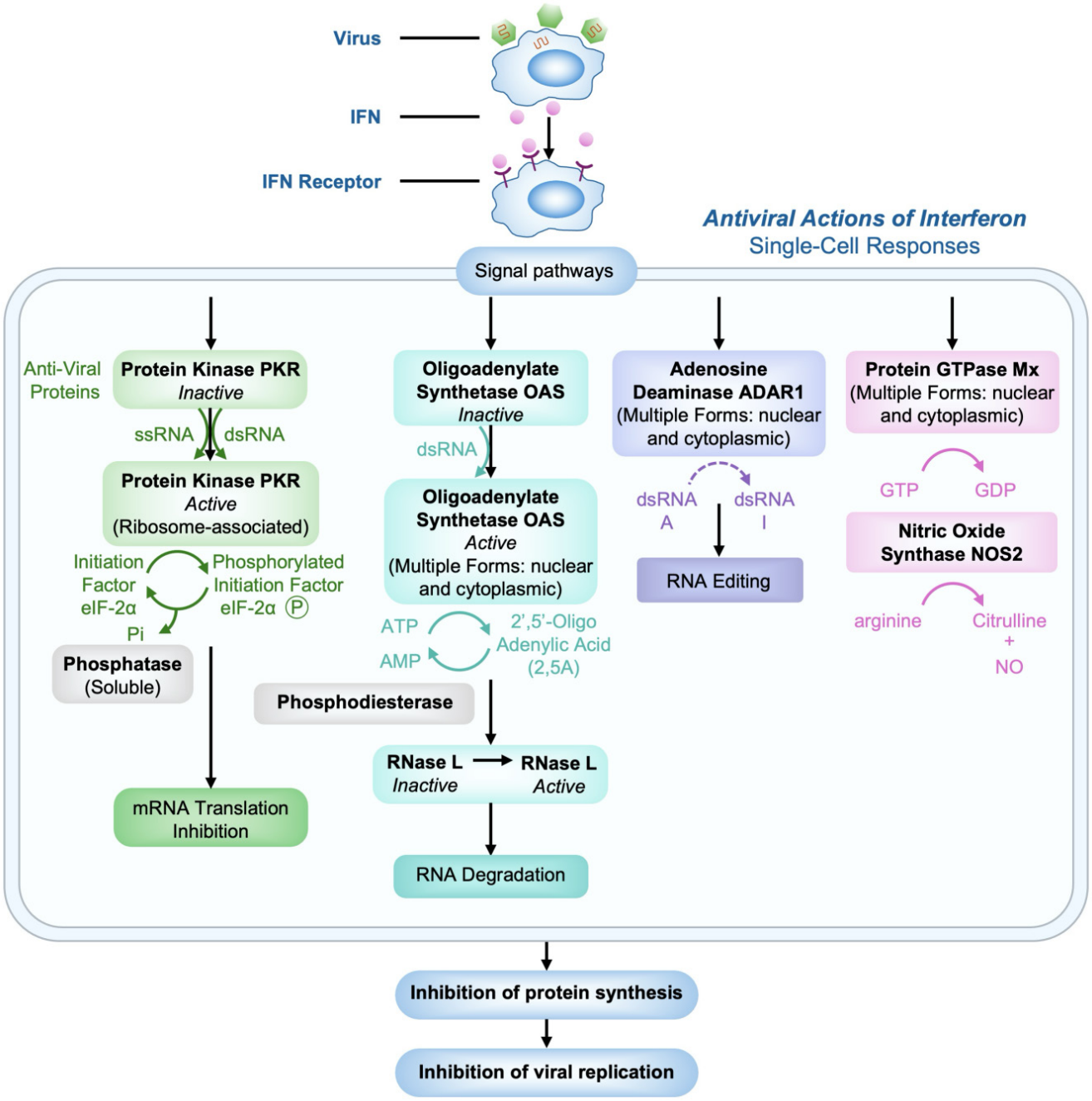

Fig 3. Functions of selected IFN-inducible proteins are critical. Among the IFN-induced proteins that affect virus multiplication within single cells are PKR kinase, the OAS synthetase family, the Mx protein GTPase family, and ADAR. These proteins appear to target viral nucleocapsids and inhibit RNA synthesis, thereby preventing viral replication

JAK-STAT, but the study lacks focus on long-term effects like viral adaptation or immune tolerance.

Additionally, IFNs have antiviral effects through various mechanisms; herbal extracts like matrine and icariin upregulate IFN-α and IFN-β, enhancing antiviral protein production and inhibiting BVDV replication *in vitro*, but more research is needed for "synergistic effect" conclusions [47].

BoIFN-α's antiviral mechanisms also include regulating host cell metabolism, with IFNs inhibiting viral infections by altering metabolic states. For example, SAMHD1 limits some RNA virus (such as Hepatitis C Virus and yellow fever virus) replication by regulating lipid synthesis, highlighting IFNs' antiviral effects [48]. However, the studied viruses are human viruses, while our BoIFN-α is bovine-derived, indicating potential functional differences in IFN and SAMHD1 across species.

Moreover, BoIFN-α is linked to interactions with other

cytokines. For example, ZAP protein, as an ISG, exhibits broad antiviral activity, and in cells lacking a robust type I IFN system, ZAP can also inhibit the replication of certain viruses [49]. However, Further studies are warranted to elucidate this IFN-alpha and -beta independent anti-Zika virus activity and involvement of ZAP.

Immunoregulatory Functions

BoIFN-α is important for immune regulation, enhancing NK cells and macrophages' activity, and boosting NK cells' ability to kill tumors and viruses-infected cells [50]. Besides, BoIFN-α boosts macrophage phagocytosis and bactericidal functions, crucial for innate immunity, and enhances cytokine secretion like tumor necrosis factor alpha (TNF-α) and IFN beta (IFN-β) in bovine infection models [51].

In adaptive immunity, BoIFN-α promotes Th1 responses and inhibits Th2, regulating immune balance by enhancing Th1 cell differentiation and cytokine production, activating T and B cells [52]. This regulatory effect is significant for fighting viral infections and tumors, as Th1 responses eliminate infected cells and tumors while suppressing excessive humoral responses, reducing allergy and autoimmune risks. Moreover, BoIFN-α has notable antitumor effects, such as inhibiting cell proliferation, suppressing angiogenesis, and boosting immune cell activity. One study showed it significantly inhibits tumor growth by upregulating tumor suppressor genes and downregulating oncogenes [53]. At the same time, BoIFN-α can induce apoptosis in tumor cells [54], and enhance the immune surveillance capacity of macrophages and NK cells against tumor cells, providing new ideas and potential applications for cancer immunotherapy.

Auxiliary Effects Against Bacterial and Parasitic Infections

The use of BoIFN-α in veterinary science is gaining attention for aiding in bacterial and parasitic infections by enhancing immune functions, crucial for combating diseases that affect bovine health and farming economics. In terms of combating bacterial infections, BoIFN-α has been found to significantly enhance the phagocytic ability of bovine macrophages against *Brucella* [55]. *Brucella* causes brucellosis in cattle, impacting reproduction and milk yield. BoIFN-α activates macrophages, boosting their ability to kill *Brucella* by enhancing activation, cytokine secretion, and antibacterial proteins. It also helps against calf diarrhea from *E. coli* [56]. *Escherichia coli* diarrhea in calves can cause high mortality, but BoIFN-α enhances local immunity and intestinal barrier functions, aiding infection control and informing prevention strategies. BoIFN-α shows promise against parasitic infections like bovine babesiosis and theileriosis, which cause anemia and reduced production performance, by boosting the host's immune response [57]. BoIFN-α enhances T cell activation and cytokine secretion, such as IFN-γ and TNF-α, improving bovine resistance to parasites.

Potential Applications of BOIFN-A in the Area of Animal Breeding

BoIFN-α improves animal reproduction by increasing pregnancy rates and regulating immunity, with sheep studies showing rates rising from 58% to 79% without teratogenic effects [58]. Additionally, BoIFN-α is linked to embryo signaling molecules like Bovine trophoblast protein-1 (bTP-1), which aids in maternal immune regulation to prevent embryo rejection [59]. Furthermore, BoIFN-α inhibits lymphocyte migration from lymph nodes but does not affect their entry into lymph nodes, supporting embryo implantation and pregnancy maintenance, suggesting its immunity may influence reproduction [60]. Overall, BoIFN-α improves pregnancy rates and immune responses in animals, but further research is needed for safety in species like cattle, requiring more clinical trials for livestock management.

Mechanisms of Action of Boifn-A

BoIFN-α functions through the "receptor-signaling pathway-effector molecules" axis, primarily activating the JAK-STAT pathway to express antiviral proteins [46].

Binding to IFN Receptors

BoIFN-α binds to type I IFN α receptors (IFNAR) on target cells, with IFNAR2 binding BoIFN-α and IFNAR1 involved in signaling; this high-affinity binding (Kd ~10^{-10} M) relies on BoIFN-α's α-helical structure, activating antiviral gene expression and highlighting its role in immunity through bioassays in MDBK cells that stimulate Toll-like receptors TLRs (e.g.TLR3, TLR7, and TLR8) and RIG-I-like receptors (RIG-I and MDA5) [61].

Moreover, the antiviral mechanism of BoIFN-α includes regulating host immune responses, not just direct effects [62]. BoIFN-α significantly increases during BVDV infection, enhancing antiviral factors and improving bovine resistance by upregulating cytokines like IFN-β, interleukin-1(IL-1), and TNF-α [47].

After binding to the IFN receptor, the activated IFN receptor signaling pathways induce ISG expression, crucial for antiviral, antitumor, and immunoregulatory responses, with BoIFN-α enhancing antiviral gene expression like Mx1, OAS1, and IFIT in host cells [63].

It is noteworthy that the effects of BoIFN-α on IFN receptors depend on factors like the host's genetics and the virus type, indicating the need to consider immune status and viral circumstances for effective treatment

strategies. Future research should investigate BoIFN-α's efficacy against various pathogens for improved veterinary options.

Clinical Applications of BoIFN-A in Veterinary Medicine

Antiviral Therapy

Treatment of Bovine Viral Diarrhea Virus (BVDV) Infection: BVDV is a harmful virus in cattle, causing symptoms like calf diarrhea, respiratory issues, and abortions, leading to economic losses [64].

Research shows that BoIFN-α can significantly inhibit the replication of different biotypes of BVDV (cytopathic and non-cytopathic) [47], and its antiviral effect is time-dependent and dose-dependent. In the early stages of infection (0-20 h), BoIFN-α maximally inhibits BVDV RNA synthesis in the early stages of infection (0-20 h), reducing viral load significantly without relying on the PKR pathway, indicating alternative antiviral mechanisms [65]. Experimental results show rBoIFN-α significantly inhibits BVDV replication in Madin Darby Bovine Kidney (MDBK) cells, indicating its antiviral potential [4]. Additionally, rBoIFN-α boosts antiviral capacity by activating immune cells and inducing interferon-stimulated genes (ISGs) like ISG15 and OAS1, lowering viral load and disease severity [4].

In scientific experiments, BoIFN-α significantly relieved BVDV symptoms in cattle, reducing viral load and improving CD_4^+ and CD_8^+ T cell function [66].

Prevention and Treatment of Infectious Bovine Rhinotracheitis Virus (IBRV): Infectious Bovine Rhinotracheitis Virus (IBRV) is a common virus in the bovine respiratory tract, which early infection damages the respiratory mucosal barrier, leading to decreased immune defense and susceptibility to secondary bacterial infections [67]. The early use of BoIFN-α in IBR infection boosts antiviral responses in respiratory cells and immune cells, strengthening local immune barriers and lowering viral replication.Studies indicate BoIFN-α activates ISGs in respiratory mucosa, boosting NK cells and macrophages to enhance immune clearance of IBR virus [68]. Meanwhile, The combination of BoIFN-α and the IBR vaccine enhances immune protection and lowers viral transmission risk [69].

Prevention of Bovine Respiratory Disease (BRD): Akiyama et al.[6] described a clinical trial testing the effect of recombinant bovine interferon alpha 1 (rBoIFN-α1) on the incidence of bovine respiratory disease (BRD). The experiment with 60 bulls showed that rBoIFN-α1 injections significantly reduced respiratory disease symptoms, with lower incidence, recurrence rate, and average days of illness, and slightly higher weight gain, leading to the conclusion that it may prevent BRD.

Treatment of Foot-and-Mouth Disease Virus (FMDV), Bovine Rotavirus (BRV), Coronaviruses (CoV), and Influenza Virus (IFV) Infections: Regarding Foot-and-Mouth Disease Virus (FMDV), BoIFN-α has inhibits effects by regulating immune responses and promoting antibody production [70]. BoIFN-α activates the IFN pathway, inhibiting the virus and boosting immune response for better resistance.

Regarding Bovine Rotavirus (BRV), BoIFN-α treatment positively affects calf diarrhea, alleviating the condition and promoting recovery [71,72].

Regarding Coronaviruses (CoV), Recombinant bovine interferon-alpha I1 (rBoIFN-α) is a sustained release treatment for bovine respiratory disease, indicating broad antiviral potential, but its effect on coronaviruses is not mentioned in the literature [69].

Regarding the Influenza Virus (IFV), BoIFN-α inhibits viral replication by impairing early protein synthesis in bovine cells, unlike BoIFN-γ [44]. The antiviral effect relates to two Mx-related proteins from BoIFN-α, akin to the resistance against influenza in mice [44].

Adjunctive Therapy for Bovine Leukemia Virus (BLV) Infection: BLV infection is a chronic disease causing lymphocyte proliferation and immune dysfunction, potentially leading to leukemia or lymphoma [73]. Currently, there is no cure, so management focuses on controlling the virus and regulating immunity.

BoIFN-α enhances T cell and NK cell recovery [66], boosts cytokine production, reduces viral load, inhibits replication, and improves immune function against BLV.

BoIFN-α can enhance conventional vaccines in managing BLV-infected cattle, alleviating symptoms, improving health indicators, and extending lifespan while strengthening immune responses.

Adjuvant Role in Immunization

BoIFN-α is a key immunoregulatory factor studied in veterinary science, particularly as a vaccine adjuvant, enhancing immunogenicity by promoting cytokine secretion, boosting antibody production, and regulating T cell activity, thus improving vaccine efficacy. Traditional adjuvants like aluminum salts stimulate immune responses but have limitations and side effects, making the search for new adjuvants crucial. BoIFN-α enhances vaccine immunogenicity by inducing strong cell-mediated and humoral immune responses, as shown in a study where it increased antibody levels and T cell responses with the foot-and-mouth disease vaccine [74].

More importantly, Vaccines with BoIFN-α enhance

specific T cell responses, increasing IFN-γ-positive CD_8^+ lymphocytes, offering new insights for foot-and-mouth disease vaccine research and application. Optimizing adjuvants may improve vaccine efficacy and safety for sustainable farming.

Production and Purification Techniques of BoIFN-A

Recombinant Expression Systems

The choice of expression system is vital for producing recombinant BoIFN-α, with options like Escherichia coli, yeast, and mammalian cells, each having pros and cons. *Escherichia coli* is fast and cost-effective for large-scale production but lacks post-translational modifications [32], potentially impairing protein folding and antiviral activity [31]. Yeast, a eukaryotic system, allows some modifications and is suitable for medium-scale production, but may still struggle with glycosylation and stability issues. Mammalian cell systems like HEK293T better mimic *in vivo* conditions, enabling crucial post-translational modifications for BoIFN-α activity. Baculovirus-mediated gene transfer (BacMam) effectively expresses BoIFN-α in these cells, yielding a biologically active product with antiviral properties, showing promise in veterinary science [31]. Researchers optimize BoIFN-α yield and activity through strategies like media optimization, culture condition adjustments, and genetic engineering, enhancing protein effectiveness and safety for clinical use.

Purification and Quality Control

Purification and quality control of BoIFN-α are vital for its biological activity and safety, with methods like affinity and ion exchange chromatography influencing the final product's characteristics. Affinity chromatography uses specific binding to enhance purification [32], often employing antibodies against IFN-α as ligands [75]. Ion exchange chromatography separates proteins based on charge at varying pH levels, using appropriate resins for effective purification.

Activity testing uses bioactivity assays like MTT, cell proliferation, or ELISA to assess BoIFN-α's effects on cells, showing its role in immune responses against BVDV by upregulating gene expression [75]. Stability assessments ensure product effectiveness during storage, with research indicating BoIFN-α's long stability under proper conditions, supporting its clinical potential.

Administration Routes and Pharmacokinetics of BoIFN-A

Comparison of Different Administration Routes

The choice of administration routes for BoIFN-α in veterinary science is vital for treatment efficacy. Subcutaneous injections provide slower release and sustained immune activation, while intramuscular injections offer quicker delivery and higher bioavailability [76], though they may cause more local reactions. Nebulized inhalation delivers BoIFN-α directly to the respiratory tract, minimizing systemic side effects and enhancing local immunity, with good tolerance and bioavailability in small animals, but requires further validation for large animals [77]. The half-life varies by administration route, with nebulized inhalation achieving higher initial concentrations but shorter half-life, impacting its use in chronic diseases [78]. Thus, careful selection of administration routes and frequencies is crucial for maximizing therapeutic effects.

Pharmacokinetic Models

BoIFN-α is a key immune regulator in bovine population with significant pharmacokinetic traits essential for clinical use. It quickly enters the bloodstream post-injection, maintaining high concentrations influenced by administration route, dosage, and bovine physiological state [79]. It is widely distributed, mainly in immune organs like the liver, spleen, and lungs, which relates to its immune regulation function [76]. Metabolized by the liver and excreted by the kidneys, BoIFN-α has a short biological half-life, limiting long-term efficacy [79]. Thus, dosing regimens must account for its rapid clearance, possibly requiring frequent doses or sustained-release formulations [78], while individual bovine differences may necessitate tailored clinical applications.

The dose-response relationship of BoIFN-α is vital in drug research and requires systematic clinical trials, as it significantly influences cattle's immune response; low doses enhance antiviral capacity while high doses may cause immunosuppression [80]. Establishing this relationship involves controlled experiments to monitor immune indicators like cytokines and clinical symptoms, aiding in determining optimal dosages and providing evidence for clinical applications [81].

Challenges and Solutions in the Clinical Application of BoIFN-A

Mechanisms of Drug Resistance

In the context of viral infections, working procedures of drug resistance mainly manifest in two aspects: the emergence of viral escape mutants and adaptive changes in host immune responses.

Firstly, the emergence of viral escape mutants involves viruses altering their antigenic epitopes through mutations to evade the host immune system. Research shows BoIFN-α is vital in regulating immune responses,

particularly for CD_8^+ T cell responses against viral infections [50]. Besides, studies on CpHV-1 (Caprine herpesvirus type 1) infection have found that this virus can regulate immune genes within host cells, thereby affecting the host's immune response [82]. This phenomenon is crucial in viral transmission and persistent infections, showcasing virus-host interactions.

Secondly, adaptive changes in host immune responses involve adjustments to the immune system due to viral infections, with research indicating significant changes in immune gene expression, including ISG upregulation crucial for antiviral immunity [82]. Studies show that heat-inactivated *Mycobacterium* (HIMB) and P22PI protein complexes stimulate the innate immune response, increasing cytokine expression and enhancing pathogen defense [83]. This adaptive change enhances the host's defense against infections and may create lasting "trained immunity".

Side Effects and Safety

BoIFN-α has therapeutic effects in veterinary science but also presents side effects and safety concerns, necessitating careful monitoring. Common side effects include fever and leukopenia, which can affect bovine health and performance. Long-term use is generally safe at proper doses, with studies showing good tolerance in cattle when monitored effectively.

However, the use of BoIFN-α must adhere to dosage guidelines to prevent misuse and side effects, while veterinarians should tailor treatment plans based on the health and disease of the bovine population, adjusting them through regular monitoring.

Existing Bottlenecks

BoIFN-α faces significant clinical application bottlenecks, primarily due to its short half-life, which complicates maintaining effective body concentrations and reduces therapeutic efficacy [61], necessitating frequent dosing that may lower compliance from patients and veterinarians.

BoIFN-α production costs are much higher than other drugs, hindering its use, especially in economically disadvantaged areas where affordability is a concern [84].

The administration of BoIFN-α via injection faces challenges like animal cooperation and stress, complicating large-scale operations [50]. Oral administration research is still exploratory and lacks a mature regimen.

BoIFN-α can trigger immunogenic reactions, posing a significant risk; exogenous proteins may cause antibody production, reducing IFN efficacy and leading to immune-related diseases, complicating clinical management [85].

Breakthrough Strategies

The use of gene editing (such as CRISPR/Cas9) in BoIFN-α research enhances its activity and production, optimizing expression systems for higher yields and aiding vaccine and drug development [86].

Multi-omics research is crucial for understanding BoIFN-α by integrating genomic, transcriptomic, proteomic, and metabolomic data, revealing its effects on viral infections and host responses, and aiding in new therapeutic strategies and personalized medicine [87].

The development of new formulations is key for improving BoIFN-α's clinical use, with researchers investigating drug delivery systems like nanoparticles, liposomes, and hydrogels to enhance IFNs' stability and bioavailability, protect against degradation, and enable targeted release, thus improving therapeutic effects and sustaining antiviral responses, particularly in veterinary medicine [86].

Combination therapy with BoIFN-α and other antivirals or immune modulators shows promise in enhancing efficacy, improving host resistance, reducing viral replication, and minimizing drug resistance, requiring personalization for different pathogens [87].

Personalized dosing regimens for BoIFN-α enhance treatment efficacy by adjusting dosage and frequency based on individual immune responses, pathogen types, and resistance characteristics, relying on detailed patient assessments [88].

Future Research Directions for BoIFN-α

Short-term Perspectives

The development of BoIFN-α derivatives advances veterinary science by enhancing activity and stability through genetic engineering, with BacMam systems effectively expressing recombinant BoIFN-α in mammalian cells against viruses like bovine parainfluenza virus 3 (BPIV3) and BVDV [31]. This process boosts BoIFN-α expression and antiviral effects both *in vitro* and *in vivo*, showing genetic engineering's effectiveness.

Middle-term Perspectives

PEGylation attaches PEG chains to biomolecules, improving pharmacokinetics and biocompatibility; in BoIFN-α development, it enhances stability, extends half-life, reduces immune clearance, and boosts therapeutic effects while minimizing immunogenicity and allergic reactions, crucial for veterinary medicine safety [89].

Long-term Perspectives

BoIFN-α shows potential in veterinary science, particularly in its synergistic effects when combined with antiviral drugs or vaccines. Research indicates that IFNs play an important role in enhancing the immune response, improving the

efficacy of antiviral drugs, and enhancing vaccine immune responses. This combination therapy strategy enhances the overall therapeutic effects by boosting the body's antiviral response, making the synergistic effects of IFNs with antiviral drugs or vaccines more pronounced.

In co-infections, IFNs offer unique benefits by enhancing immune responses against multiple pathogens, as shown in studies with pigs infected by porcine epidemic diarrhea virus (PEDV) and porcine delta coronavirus (PDCoV), where IFN-α improved disease severity and immune response [90].

Moreover, research on canines has shown that in the treatment of canine vascular sarcoma, the combined use of IFNs with chemotherapy drugs showed better efficacy, enhancing the immune system's ability to recognize and eliminate tumors [91]. This combination therapy strategy not only reduces recurrence rates but also provides new treatment options for clinical practice.

Conclusion

The prospects of BoIFN-α in veterinary science are promising, particularly in antiviral and immune regulation, showing great potential in tackling various viral infections and offering novel ideas for antiviral treatments. However, research on BoIFN-α's antiviral effects and applications varies, with some studies showing effective virus inhibition while others highlight species and method differences, necessitating consideration of experimental conditions.

Future research should focus on optimizing BoIFN-α production and administration methods for better clinical use and personalized plans to enhance effectiveness and reduce side effects. Systematic clinical trials will evaluate its safety and efficacy for veterinary applications.

At the same time, exploring BoIFN-α's potential for new treatments is vital, as combining it with vaccines and antiviral drugs may enhance clinical outcomes and improve animal immunity against diseases.

In summary, BoIFN-α, as a biologic with significant therapeutic potential, has promising development prospects in veterinary science. By examining molecular, clinical, and epidemiological research perspectives, we can gain a deeper understanding of BoIFN-α's role in antiviral activity and immune regulation. Future research should focus on enhancing BoIFN-α's clinical applications. This will facilitate its broader adoption in the veterinary field, helping to address the continuously evolving challenges posed by infectious and immune-related diseases. We can only unlock BoIFN-α's true potential through ongoing exploration and validation, which will provide more effective solutions for animal health and welfare.

Highlight Keypoints

1. BoIFN-α, as a key cytokine, can effectively inhibit virus replication, activate host immune responses, and protect the health of bovine population.

2. The gene structure, protein characteristics, signaling pathways, antiviral activities, and immunoregulatory functions of BoIFN-α were analyzed. It shows significant effects in antiviral, immune regulation, antitumor, and assisting in anti-bacterial and anti-parasitic infections.

3. The effects of BoIFN-α in clinical treatment, vaccine adjuvant applications, and production purification technologies were evaluated. The clinical application potential of BoIFN-α is enormous, but it needs to overcome limitations such as stability, production costs, and drug delivery methods.

4. The pharmacokinetic characteristics and safety issues of BoIFN-α were discussed.

5. Future research for BoIFN-α should focus on optimizing production technology, developing new drug delivery methods, and creating personalized therapy plans to promote the widespread application of BoIFN-α in veterinary medicine and the sustainable development of the bovine industry.

Declarations

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References

1. **Capper JL, Williams P:** Investing in health to improve the sustainability of cattle production in the United Kingdom: A narrative review. *Vet J*, 296-297:105988, 2023. DOI: 10.1016/j.tvjl.2023.105988

2. **Battistini A, Affabris E, Fiorucci G, Coccia EM, Romeo G, Marziali G, Rossi GB:** Spectrum of biological activity of interferons. *Ann Ist Super Sanita,* 26 (3-4): 227-253, 1990.
3. **Zhang J, Wang Y, Sun N, Zou B, Wang Z, Yin H, Xie J, Xia B, Sun N:** miR-30a enhanced RIG-I-mediated type I interferon antiviral response by targeting USP14. *Microbiol Spectr,* 13 (8):e0018825, 2025. DOI: 10.1128/spectrum.00188-25
4. **Yin H, Li S, Chai C, Zhang F, Ma Y, Wu Y, Fu C, Diao Y, Zhou Y, Zhang J, Niu R, Wang W:** Biological activity of recombinant bovine IFN-alpha and inhibitory effect on BVDV *in vitro*. *Microb Pathog,* 181:106155, 2023. DOI: 10.1016/j.micpath.2023.106155
5. **Cortes JA, Hendrick S, Janzen E, Pajor EA, Orsel K:** Economic impact of digital dermatitis, foot rot, and bovine respiratory disease in feedlot cattle. *Transl Anim Sci,* 5 (2):txab076, 2021. DOI: 10.1093/tas/txab076
6. **Akiyama K, Sugii S, Hirota Y:** A clinical trial of recombinant bovine interferon alpha 1 for the control of bovine respiratory disease in calves. *J Vet Med Sci,* 55 (3): 449-452, 1993. DOI: 10.1292/jvms.55.449
7. **Schanen C, Chieux V, Lobert PE, Harvey J, Hober D:** Correlation between the anti-virus-induced cytopathic effect activity of interferon-alpha subtypes and induction of MxA protein *in vitro*. *Microbiol Immunol,* 50 (1): 19-24, 2006. DOI: 10.1111/j.1348-0421.2006.tb03766.x
8. **Greenway AL, Overall ML, Sattayasai N, Rowley MJ, Hertzog PJ, McMullen GL, Cheetham BF, Marzuki S:** Selective production of interferon-alpha subtypes by cultured peripheral blood mononuclear cells and lymphoblastoid cell lines. *Immunology,* 75 (1): 182-188, 1992.
9. **Li SF, Zhao FR, Shao JJ, Xie YL, Chang HY, Zhang YG:** Interferon-omega: Current status in clinical applications. *Int Immunopharmacol,* 52, 253-260, 2017. DOI: 10.1016/j.intimp.2017.08.028
10. **Li SF, Zhao FR, Gong MJ, Shao JJ, Xie YL, Chang HY, Zhang YG:** Antiviral activity of porcine interferon omega 7 against foot-and-mouth disease virus *in vitro. J Med Virol,* 91 (2): 208-214, 2019. DOI: 10.1002/jmv.25272
11. **Ma B, Cui H, Wang X, Feng W, Zhang J, Chen N, Umar T, Zhou H, Liu W, Feng X, Deng G:** IFNT-induced IRF1 enhances bovine endometrial receptivity by transactivating LIFR. *J Reprod Immunol,* 163:104212, 2024. DOI: 10.1016/j.jri.2024.104212
12. **Wang X, Chen C, Wang L, Su Y, Li B, Xiao L, Lin Z, Sheng X, Qi X, Ni H, Guo Y:** Specific activation of embryonic IFNAR1 and endometrial IFNAR2 induced by embryonic IFNtau directs normal uterine fate for bovine early implantation. *J Reprod Immunol,* 153:103677, 2022. DOI: 10.1016/j.jri.2022.103677
13. **Guo Y, Song Z, Li C, Yu Y, Dai H, Luo X, Wang Y, Wang J, Gao M:** A novel type-I interferon family, bovine interferon-chi, is involved in positive-feedback regulation of interferon production. *Front Immunol,* 11:528854, 2020. DOI: 10.3389/fimmu.2020.528854
14. **Guo Y, Gao M, Bao J, Luo X, Liu Y, An D, Zhang H, Ma B, Wang J:** Molecular cloning and characterization of a novel bovine IFN-epsilon. *Gene,* 558 (1): 25-30, 2015. DOI: 10.1016/j.gene.2014.12.031
15. **Guo Y, An D, Liu Y, Bao J, Luo X, Cheng X, Wang Y, Gao M, Wang J:** Characterization and signaling pathway analysis of interferon-kappa in bovine. *Dev Comp Immunol,* 67, 213-220, 2017. DOI: 10.1016/j.dci.2016.09.018
16. **Szubin R, Chang WL, Greasby T, Beckett L, Baumgarth N:** Rigid interferon-alpha subtype responses of human plasmacytoid dendritic cells. *J Interferon Cytokine Res,* 28 (12): 749-763, 2008. DOI: 10.1089/jir.2008.0037
17. **da Silva LF, Jones C:** Infection of cultured bovine cells with bovine herpesvirus 1 (BHV-1) or Sendai virus induces different beta interferon subtypes. *Virus Res,* 157 (1): 54-60, 2011. DOI: 10.1016/j.virusres.2011.02.004
18. **Wang X, Li F, Han M, Jia S, Wang L, Qiao X, Jiang Y, Cui W, Tang L, Li Y, Xu YG:** Cloning, prokaryotic soluble expression, and analysis of antiviral activity of two novel feline IFN-omega proteins. *Viruses,* 12 (3), 2020. DOI: 10.3390/v12030335
19. **Romero MP, Chang YM, Brunton LA, Parry J, Prosser A, Upton P, Drewe JA:** Assessing the potential impact of applying a higher sensitivity test to selected cattle populations for the control of bovine tuberculosis in England. *Prev Vet Med,* 219:106004, 2023. DOI: 10.1016/j.prevetmed.2023.106004
20. **Yang M, Xu G, Zhang J, Guo Z, Liang C, Li Y, Wang L, Zhou Y, Ru Y, Li J, Wang X, Sun Y:** Correlation between microRNA by extracellular vesicle mediated and antiviral effects of interferon omega in feline peripheral blood. *J Interferon Cytokine Res,* 44 (3): 124-134, 2024. DOI: 10.1089/jir.2023.0174
21. **Quintana ME, Cardoso NP, Pereyra R, Barone LJ, Barrionuevo FM, Mansilla FC, Turco CS, Capozzo AV:** Interferon lambda protects cattle against bovine viral diarrhea virus infection. *Vet Immunol Immunopathol,* 230:110145, 2020. DOI: 10.1016/j.vetimm.2020.110145
22. **da Silva LF, Gaudreault N, Jones C:** Cytoplasmic localized infected cell protein 0 (bICP0) encoded by bovine herpesvirus 1 inhibits beta interferon promoter activity and reduces IRF3 (interferon response factor 3) protein levels. *Virus Res,* 160 (1-2): 143-149, 2011. DOI: 10.1016/j.virusres.2011.06.003
23. **Maldonado MBC, Cabrini DC, de Oliveira Bezerra L, Rolniche LCM, de Castro Lourenco V, Feltrin IR, Morelli KG, Mendes AF, Rocha CC, Bridi A, Pugliesi G, Nogueira MFG, Membrive CMB:** Conjugated linoleic acid supplementation: Insights into prostaglandin synthesis and *in vitro* embryonic development. *Anim Reprod Sci,* 281: 107990, 2025. DOI: 10.1016/j.anireprosci.2025.107990
24. **MacLachlan NJ, Anderson KP:** Effect of recombinant DNA-derived bovine alpha-1 interferon on transmissible gastroenteritis virus infection in swine. *Am J Vet Res,* 47 (5): 1149-1152, 1986.
25. **Shi X, Xia C, Pan B, Wang M:** Interferon-alpha genes from Bos and Bubalus bubalus. *Anim Biotechnol,* 17 (1): 59-72, 2006. DOI: 10.1080/10495390500461104
26. **Gao M, Guo Y, Luo X, Du J, Wang Y, Cao C, Wang J, Han W:** Design, biological activity and signaling pathway of bovine consensus omega interferon expressed in *Pichia pastoris*. *Mol Immunol,* 106, 46-52, 2019. DOI: 10.1016/j.molimm.2018.12.004
27. **Velan B, Cohen S, Grosfeld H, Leitner M, Shafferman A:** Bovine interferon alpha genes. Structure and expression. *J Biol Chem,* 260 (9): 5498-5504, 1985. DOI: 10.1016/S0021-9258(18)89050-0
28. **Velan B, Cohen S, Grosfeld H, Shalita Z, Shafferman A:** Cloning of a bovine interferon-alpha gene subfamily and comparisons between genetically engineered and leukocyte bovine interferons. *Dev Biol Stand,* 60, 355-369, 1985.
29. **Ambrus JL Sr, Dembinski W, Chadha K, Ambrus JL Jr, Chadha KC:** Resistance to interferons. *Discov Med,* 4 (23): 310-314, 2004.
30. **Van Wyk B, Snider M, Scruten E, van Drunen Littel-van den Hurk S, Napper S:** Induction of functional interferon alpha and gamma responses during acute infection of cattle with non-cytopathic bovine viral diarrhea virus. *Vet Microbiol,* 195, 104-114, 2016. DOI: 10.1016/j.vetmic.2016.09.015
31. **Fan Z, Hou Y, Liu Y, Zhao J, Wang Y, Fu C, Wang S, Wang J, Guo Y, Gao M:** Blocking virus infection with BacMam virus delivery bovine interferon-alpha gene. *Virulence,* 15 (1):2435372, 2024. DOI: 10.1080/21505594.2024.2435372
32. **Yu HY, Liu J, He ZY, Zhou W, Xia BB, Wang M, Chen J, Wang ML, Jiang GT, Zhao J:** Soluble expression, rapid purification and antiviral activity of recimbinant bovine interferon-α in *Escherichia coli. Appl Biochem Microbiol,* 56 (2): 154-163, 2020. DOI: 10.1134/s0003683820020143
33. **Martinod S, Maurer RR, Siegenthaler B, Gerber C, Hansen PJ:** The effects of recombinant bovine interferon-alpha on fertility in ewes. *Theriogenology,* 36 (2): 231-239, 1991. DOI: 10.1016/0093-691x(91)90382-n
34. **Cutrone EC, Langer JA:** Identification of critical residues in bovine IFNAR-1 responsible for interferon binding. *J Biol Chem,* 276 (20): 17140-17148, 2001. DOI: 10.1074/jbc.M009663200
35. **Hu R, Bekisz J, Hayes M, Audet S, Beeler J, Petricoin E, Zoon K:**

Divergence of binding, signaling, and biological responses to recombinant human hybrid IFN. *J Immunol,* 163 (2): 854-860, 1999.

36. **Prabhu SK, Yang Q, Tong X, Wang LX:** Exploring a combined *Escherichia coli*-based glycosylation and *in vitro* transglycosylation approach for expression of glycosylated interferon alpha. *Bioorg Med Chem,* 33:116037, 2021. DOI: 10.1016/j.bmc.2021.116037
37. **Adolf GR, Kalsner I, Ahorn H, Maurer-Fogy I, Cantell K:** Natural human interferon-alpha 2 is O-glycosylated. *Biochem J,* 276 (2): 511-518, 1991. DOI: 10.1042/bj2760511
38. **Shirono H, Koga J, Uemura H, Matsuo A:** Identification of glycosylated subtypes of interferon-alpha produced by human leukocytes. *Biosci Biotechnol Biochem,* 58 (9): 1714-1715, 1994. DOI: 10.1271/bbb.58.1714
39. **Pigny P, Berault A, Dewailly D, Boersma A:** Glycoprotein hormones, glycosylation and biological activity. *Ann Biol Clin (Paris),* 50 (8): 557-564, 1992.
40. **Young KH, Buhi WC, Horseman N, Davis J, Kraeling R, Linzer D, Bazer FW:** Biological activities of glycosylated and nonglycosylated porcine prolactin. *Mol Cell Endocrinol,* 71 (2): 155-162, 1990. DOI: 10.1016/0303-7207(90)90252-4
41. **Rossmann C, Sharp N, Allen G, Gewert D:** Expression and purification of recombinant, glycosylated human interferon alpha 2b in murine myeloma NSo cells. *Protein Expr Purif,* 7 (4): 335-342, 1996. DOI: 10.1006/prep.1996.0050
42. **Katla S, Yoganand KNR, Hingane S, Ranjith Kumar CT, Anand B, Sivaprakasam S:** Novel glycosylated human interferon alpha 2b expressed in glycoengineered *Pichia pastoris* and its biological activity: N-linked glycoengineering approach. *Enzyme Microb Technol,* 128, 49-58, 2019. DOI: 10.1016/j.enzmictec.2019.05.007
43. **Gugliotta A, Ceaglio N, Raud B, Forno G, Mauro L, Kratje R, Oggero M:** Glycosylation and antiproliferative activity of hyper-glycosylated IFN-alpha2 potentiate HEK293 cells as biofactories. *Eur J Pharm Biopharm,* 112, 119-131, 2017. DOI: 10.1016/j.ejpb.2016.11.012
44. **Horisberger MA:** The action of recombinant bovine interferons on influenza virus replication correlates with the induction of two Mx-related proteins in bovine cells. *Virology,* 162 (1): 181-186, 1988. DOI: 10.1016/0042-6822(88)90407-2
45. **Li J, Kemper T, Broering R, Chen J, Yuan Z, Wang X, Lu M:** Interferon alpha induces cellular autophagy and modulates hepatitis B virus replication. *Front Cell Infect Microbiol,* 12:804011, 2022. DOI: 10.3389/fcimb.2022.804011
46. **Samuel CE:** Antiviral actions of interferons. *Clin Microbiol Rev,* 14 (4): 778-809, 2001. DOI: 10.1128/CMR.14.4.778-809.2001
47. **Cai D, Shen Z, Tian B, Chen J, Zhang Y, Shen L, Wang Y, Ma X, Zuo Z:** Matrine and icariin can inhibit bovine viral diarrhoea virus replication by promoting type I interferon response *in vitro*. *J Vet Res,* 68 (1): 35-44, 2024. DOI: 10.2478/jvetres-2024-0013
48. **An N, Ge Q, Shao H, Li Q, Guo F, Liang C, Li X, Yi D, Yang L, Cen S:** Interferon-inducible SAMHD1 restricts viral replication through downregulation of lipid synthesis. *Front Immunol,* 13:1007718, 2022. DOI: 10.3389/fimmu.2022.1007718
49. **Le NPK, Singh PP, Sabir AJ, Trus I, Karniychuk U:** Endogenous ZAP is associated with altered Zika virus infection phenotype. *Virol J,* 21 (1):285, 2024. DOI: 10.1186/s12985-024-02557-x
50. **Peters SO, Hussain T, Adenaike AS, Hazzard J, Morenikeji OB, De Donato M, Paul S, Babar M, Yakubu A, Imumorin IG:** Evolutionary pattern of interferon alpha genes in bovidae and genetic diversity of IFNAA in the bovine genome. *Front Immunol,* 11:580412, 2020. DOI: 10.3389/fimmu.2020.580412
51. **Ishikawa S, Miyazawa M, Tanaka C, Uesawa R, Nishizawa J, Uemura R, Kobayashi I, Hobo S:** Interferon gamma, lipopolysaccharide, and modified-live viral vaccines stimulation alter the mRNA expression of tumor necrosis factor alpha, inducible nitric oxide synthase, and interferon beta in bovine alveolar macrophages. *Vet Immunol Immunopathol,* 244:110378, 2022. DOI: 10.1016/j.vetimm.2021.110378
52. **Frovoll TL, Lybeck K, Lund H, Makvandi-Nejad S, Grimholt U, das Neves CG, Tryland M, Nymo IH, Klevar S:** Measuring cytokines in Eurasian tundra reindeer *(Rangifer tarandus tarandus)* with a bovine bead-based multiplex immunoassay and real-time PCR. *Acta Vet Scand,* 67 (1):34, 2025. DOI: 10.1186/s13028-025-00819-4
53. **Meng X, Chen Y, Macip S, Leppard K:** PML-II regulates ERK and AKT signal activation and IFNalpha-induced cell death. *Cell Commun Signal,* 19 (1):70, 2021. DOI: 10.1186/s12964-021-00756-5
54. **Saleiro D, Kosciuczuk EM, Fischietti M, Perez RE, Yang GS, Eckerdt F, Beauchamp EM, Hou Y, Wang Q, Weinberg RS, Fish EN, Yue F, Hoffman R, Platanias LC:** Targeting CHAF1B enhances IFN activity against myeloproliferative neoplasm cells. *Cancer Res Commun,* 3 (5): 943-951, 2023. DOI: 10.1158/2767-9764.CRC-23-0010
55. **Skyberg JA, Thornburg T, Rollins M, Huarte E, Jutila MA, Pascual DW:** Murine and bovine gammadelta T cells enhance innate immunity against *Brucella abortus* infections. *PLoS One,* 6 (7):e21978, 2011. DOI: 10.1371/journal.pone.0021978
56. **Jin Y, Jia Z, Cai Q, Sun Y, Liu Z:** Escherichia coli infection activates the production of IFN-alpha and IFN-beta via the JAK1/STAT1/2 signaling pathway in lung cells. *Amino Acids,* 53 (10): 1609-1622, 2021. DOI: 10.1007/s00726-021-03077-6
57. **Gonzalez-Navajas JM, Lee J, David M, Raz E:** Immunomodulatory functions of type I interferons. *Nat Rev Immunol,* 12 (2): 125-135, 2012. DOI: 10.1038/nri3133
58. **Schalue-Francis TK, Farin PW, Cross JC, Keisler D, Roberts RM:** Effect of injected bovine interferon-alpha I1 on estrous cycle length and pregnancy success in sheep. *J Reprod Fertil,* 91 (1): 347-356, 1991. DOI: 10.1530/jrf.0.0910347
59. **Imakawa K, Hansen TR, Malathy PV, Anthony RV, Polites HG, Marotti KR, Roberts RM:** Molecular cloning and characterization of complementary deoxyribonucleic acids corresponding to bovine trophoblast protein-1: A comparison with ovine trophoblast protein-1 and bovine interferon-alpha II. *Mol Endocrinol,* 3 (1): 127-139, 1989. DOI: 10.1210/mend-3-1-127
60. **Kalaaji AN, Abernethy NJ, McCullough K, Hay JB:** Recombinant bovine interferon-alpha I 1 inhibits the migration of lymphocytes from lymph nodes but not into lymph nodes. *Reg Immunol,* 1 (1): 56-61, 1988.
61. **Lion J, Meyer G, Ducatez M, Foucras G, Lion A:** Development and assessment of a new bioassay for accurate quantification of Type I interferons induced by bovine respiratory viruses. *J Immunol Methods,* 504: 113256, 2022. DOI: 10.1016/j.jim.2022.113256
62. **Ohmann HB, Gilchrist JE, Babiuk LA:** Effect of recombinant DNA-produced bovine interferon alpha (BoIFN-alpha 1) on the interaction between bovine alveolar macrophages and bovine herpesvirus type 1. *J Gen Virol,* 65 (9): 1487-1495, 1984. DOI: 10.1099/0022-1317-65-9-1487
63. **Geng Y, Jiang C, Yang H, Xia Q, Xu X, Yang K, Yuan X, Chen J, Chen Y, Chen X, Zhang L, Hu C, Guo A:** Construction of an IFNAR1 knockout MDBK cell line using CRISPR/Cas9 and its effect on bovine virus replication. *Front Immunol,* 15:1404649, 2024. DOI: 10.3389/fimmu.2024.1404649
64. **Newcomer BW:** 75 years of bovine viral diarrhea virus: Current status and future applications of the use of directed antivirals. *Antiviral Res,* 196:105205, 2021. DOI: 10.1016/j.antiviral.2021.105205
65. **Elsheikh AA, Braun LJ, Mansour SMG, Orabi A, Alqahtani AS, Benfield DA, Chase CCL:** The effect of human interferon alpha on replication of different bovine viral diarrhea virus strains. *Acta Virol,* 63 (3): 261-269, 2019. DOI: 10.4149/av_2019_303
66. **Wang Y, Swiecki M, Cella M, Alber G, Schreiber RD, Gilfillan S, Colonna M:** Timing and magnitude of type I interferon responses by distinct sensors impact CD8 T cell exhaustion and chronic viral infection. *Cell Host Microbe,* 11 (6): 631-642, 2012. DOI: 10.1016/j.chom.2012.05.003
67. **Kabelik V, Horyna B, Trunkat J:** Corticoid activation of infectious bovine rhinotracheitis virus--infectious pustular vulvovaginitis virus (IBR-IPV). *Vet Med (Praha),* 21 (8): 449-460, 1976.

68. **Osman R, Gonzalez-Cano P, Brownlie R, Griebel PJ:** Induction of interferon and interferon-induced antiviral effector genes following a primary bovine herpesvirus-1 (BHV-1) respiratory infection. *J Gen Virol,* 98 (7): 1831-1842, 2017. DOI: 10.1099/jgv.0.000825

69. **Hughes HP, Rossow S, Campos M, Rossi-Campos A, Janssen S, Godson DL, Daflon B, Voirol MJ, Gerber C, Babiuk LA:** A slow release formulation for recombinant bovine interferon alpha I-1. *Antiviral Res,* 23 (1): 33-44, 1994. DOI: 10.1016/0166-3542(94)90031-0

70. **Chinsangaram J, Piccone ME, Grubman MJ:** Ability of foot-and-mouth disease virus to form plaques in cell culture is associated with suppression of alpha/beta interferon. *J Virol,* 73 (12): 9891-9898, 1999. DOI: 10.1128/JVI.73.12.9891-9898.1999

71. **Uddin Ahmed N, Khair A, Hassan J, Khan M, Rahman A, Hoque W, Rahman M, Kobayashi N, Ward MP, Alam MM:** Risk factors for bovine rotavirus infection and genotyping of bovine rotavirus in diarrheic calves in Bangladesh. *PLoS One,* 17 (2):e0264577, 2022. DOI: 10.1371/journal.pone.0264577

72. **Chaplin PJ, Entrican G, Gelder KI, Collins RA:** Cloning and biologic activities of a bovine interferon-alpha isolated from the epithelium of a rotavirus-infected calf. *J Interferon Cytokine Res,* 16 (1): 25-30, 1996. DOI: 10.1089/jir.1996.16.25

73. **Benitez OJ, Norby B, Bartlett PC, Maeroff JE, Grooms DL:** Impact of bovine leukemia virus infection on beef cow longevity. *Prev Vet Med,* 181:105055, 2020. DOI: 10.1016/j.prevetmed.2020.105055

74. **Bidart J, Kornuta C, Gammella M, Gnazzo V, Soria I, Langellotti C, Mongini C, Galarza R, Calvinho L, Lupi G, Quattrocchi V, Marcipar I, Zamorano P:** A new cage-like particle adjuvant enhances protection of foot-and-mouth disease vaccine. *Front Vet Sci,* 7:396, 2020. DOI: 10.3389/fvets.2020.00396

75. **Gonzalez Altamiranda EA, Arias ME, Kaiser GG, Mucci NC, Odeon AC, Felmer RN:** Upregulation of interferon-alpha gene in bovine embryos produced *in vitro* in response to experimental infection with noncytophatic bovine-viral-diarrhea virus. *Mol Biol Rep,* 47 (12): 9959-9965, 2020. DOI: 10.1007/s11033-020-05958-7

76. **Mueller RS, Hartmann K:** Interferon therapies in small animals. *Vet J,* 271:105648, 2021. DOI: 10.1016/j.tvjl.2021.105648

77. **Martabano BB, Dow S, Chow L, Williams MMV, Mack MK, Bellone R, Wotman KL:** Intralesional interferon alpha-2b as a novel treatment for periocular squamous cell carcinoma in horses. *PLoS One,* 19 (2): e0297366, 2024. DOI: 10.1371/journal.pone.0297366

78. **Rwandamuriye FX, Evans CW, Wylie B, Norret M, Vitali B, Ho D, Nguyen D, Roper EA, Wang T, Hepburn MS, Sanderson RW, Pfirrmann M, Fear VS, Forbes CA, Wyatt K, Ryan AL, Johns TG, Phillips MB, Hodder R, Leslie C, Kennedy BF, Zemek RM, Iyer KS, Lesterhuis WJ:** A surgically optimized intraoperative poly(I:C)-releasing hydrogel prevents cancer recurrence. *Cell Rep Med,* 4 (7):101113, 2023. DOI: 10.1016/j.xcrm.2023.101113

79. **Yu HY, Zhao Y, Li SQ, Fu XL, Zhou W, Xia BB, Chen J, Zhao J, Wang ML:** Pharmacokinetic studies of the recombinant bovine interferon-alpha in cattle. *Kafkas Univ Vet Fak Derg,* 25 (1): 17-23, 2019. DOI: 10.9775/kvfd.2018.20133

80. **Zhu X, Fan B, Song S, Gao J, Zhou J, Zhao Y, Guo W, Zhang X, Qian J, Zhu M, Li J, Li Y, Guo R, Fan H, Dong H, Zhang X, Li B:** Transcriptomic and antiviral analyses of PoIFN-Delta5 against porcine enteric viruses in porcine intestinal epithelial cells. *Vet Microbiol,* 280:109718, 2023. DOI: 10.1016/j.vetmic.2023.109718

81. **Pinard CJ, Stegelmeier AA, Bridle BW, Mutsaers AJ, Wood RD, Wood GA, Woods JP, Hocker SE:** Evaluation of lymphocyte-specific programmed cell death protein 1 receptor expression and cytokines in blood and urine in canine urothelial carcinoma patients. *Vet Comp Oncol,* 20 (2): 427-436, 2022. DOI: 10.1111/vco.12788

82. **Hao F, Xie X, Liu M, Mao L, Li W, Na W:** Transcriptome and proteomic analysis reveals up-regulation of innate immunity-related genes expression in caprine herpesvirus 1 infected Madin Darby bovine kidney cells. *Viruses,* 13 (7):1293, 2021. DOI: 10.3390/v13071293

83. **Agullo-Ros I, Burucua MM, Cheuquepan FA, Dominguez M, Sevilla IA, Martinez R, Pla N, Risalde MA, Marin MS:** Heat-inactivated *Mycobacterium bovis* and P22PI protein immunocomplex: Two candidates for use as immunostimulants of innate immune response. *Vet Microbiol,* 305:110527, 2025. DOI: 10.1016/j.vetmic.2025.110527

84. **Yu HY, Gao DM, Zhou W, Xia BB, He ZY, Wu B, Jiang MZ, Wang ML, Zhao J:** Acute and sub-chronic toxicity study of recombinant bovine interferon alpha in rodents. *J Vet Res,* 65 (2): 183-192, 2021. DOI: 10.2478/jvetres-2021-0023

85. **Abdelbaky HH, Shimoda N, Akthar I, Nakamura S, Hasan MH, Ushio N, Miyamoto A, Nishikawa Y:** *In vitro* regulation of gene expression of pregnancy-associated proteins and cytokines in bovine endometrial epithelial cells and bovine trophoblastic cells by infection with *Neospora caninum*. *Parasitol Int,* 101:102898, 2024. DOI: 10.1016/j.parint.2024.102898

86. **Pedroso-Santana S, Lamazares Arcia E, Fleitas-Salazar N, Gancino Guevara M, Mansilla R, Gomez-Gaete C, Altamirano C, Fernandez K, Ruiz A, Toledo Alonso JR:** Polymeric nanoencapsulation of alpha interferon increases drug bioavailability and induces a sustained antiviral response *in vivo*. *Mater Sci Eng C Mater Biol Appl,* 116:111260, 2020. DOI: 10.1016/j.msec.2020.111260

87. **Ma Z, Bai J, Jiang C, Zhu H, Liu D, Pan M, Wang X, Pi J, Jiang P, Liu X:** Tegument protein UL21 of alpha-herpesvirus inhibits the innate immunity by triggering CGAS degradation through TOLLIP-mediated selective autophagy. *Autophagy,* 19 (5): 1512-1532, 2023. DOI: 10.1080/15548627.2022.2139921

88. **Jobe F, Simpson J, Hawes P, Guzman E, Bailey D:** Respiratory syncytial virus sequesters NF-kappaB subunit p65 to cytoplasmic inclusion bodies to inhibit innate immune signaling. *J Virol,* 94 (22):e01380-20, 2020. DOI: 10.1128/JVI.01380-20

89. **Zhang JW, Lai RM, Wang LF, Wang SL, Xue HX, Li C, Zheng ZZ, Li J, Zhu YY, Zeng DW, Chen J, Ou QS, Chen TB, Xun Z, Jiang JJ, Zheng Q:** Varied immune responses of HBV-specific B cells in patients undergoing pegylated interferon-alpha treatment for chronic hepatitis B. *J Hepatol,* 81 (6): 960-970, 2024. DOI: 10.1016/j.jhep.2024.06.033

90. **Saeng-Chuto K, Madapong A, Kaeoket K, Pineyro PE, Tantituvanont A, Nilubol D:** Coinfection of porcine deltacoronavirus and porcine epidemic diarrhea virus increases disease severity, cell trophism and earlier upregulation of IFN-alpha and IL12. *Sci Rep,* 11 (1):3040, 2021. DOI: 10.1038/s41598-021-82738-8

91. **Konduri V, Halpert MM, Baig YC, Coronado R, Rodgers JR, Levitt JM, Cerroni B, Piscoya S, Wilson N, DiBernardi L, Omarbekov Z, Seelhoff L, Ravi V, Douglass L, Decker WK:** Dendritic cell vaccination plus low-dose doxorubicin for the treatment of spontaneous canine hemangiosarcoma. *Cancer Gene Ther,* 26 (9-10): 282-291, 2019. DOI: 10.1038/s41417-019-0080-3