

## Review Article

## microRNA (miRNA) Therapeutics: From Gene Regulation to Clinical Applications and Future Directions

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### Abstract

MicroRNAs (miRNAs) are endogenous, small non-coding RNAs that function as critical post-transcriptional regulators of gene expression, influencing virtually every cellular process. This review provides a comprehensive analysis of the miRNA therapeutics field, beginning with the fundamental biology of miRNA biogenesis and their mechanisms of action in gene silencing. We detail the two primary therapeutic strategies: miRNA inhibition using antisense oligonucleotides (antagomirs) and miRNA replacement using synthetic miRNA mimics. The review critically examines the significant delivery challenges and evaluates current delivery platforms including lipid nanoparticles and GalNAc conjugates (van Rooij & Kauppinen, 2014; Rupaimoole & Slack, 2017). We survey the expanding therapeutic landscape in human medicine, focusing on oncology, cardiovascular, and metabolic diseases (Hanna, Hossain, & Kocerha, 2019). A dedicated section explores the burgeoning field of miRNA applications in veterinary medicine, particularly in cattle and water buffalo, where miRNAs offer revolutionary tools for enhancing production traits (milk yield, meat quality), improving reproductive efficiency, and combating endemic diseases like mastitis and foot-and-mouth disease (Miretti et al., 2013; Ioannidis & Donadeu, 2016). Finally, we discuss challenges in specificity and delivery, while outlining future directions including species-specific design, combination therapies, and the integration of miRNA therapeutics into precision livestock farming. The dual progress in human and veterinary applications underscores miRNAs' potential as transformative therapeutic agents across species.

**Keywords:** microRNA, miRNA therapeutics, antagomir, miRNA mimic, veterinary medicine, livestock, cattle, water buffalo, reproduction, mastitis, gene regulation.

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### 1. Introduction

#### 1.1. The Discovery and Fundamental Role of miRNAs

The discovery of microRNAs (miRNAs) revolutionized our understanding of gene regulation. The first miRNA, *lin-4*, was identified in *C. elegans* in 1993 by Ambros and colleagues, who found it regulated developmental timing by base-pairing with the 3' untranslated region (UTR) of the *lin-14* mRNA (Lee, Feinbaum, & Ambros, 1993). This was followed by the discovery of *let-7*, which revealed the conservation of miRNA mechanisms across species (Pasquinelli et al., 2000). miRNAs are now recognized as a vast class of endogenous, small (~22 nucleotide) non-coding RNAs that function as post-transcriptional regulators. They typically bind to complementary sequences in the 3' UTRs of target mRNAs,

leading to translational repression or mRNA degradation, thereby fine-tuning the expression of numerous genes involved in development, differentiation, proliferation, and apoptosis (Bartel, 2004).

### 1.2. miRNAs in Human and Animal Disease: The Therapeutic Rationale

Dysregulation of miRNA expression is a hallmark of many human and animal diseases. In human cancer, specific miRNAs can function as oncogenes (oncomiRs) when overexpressed (e.g., miR-21, miR-155) or as tumor suppressors when downregulated (e.g., let-7, miR-34a) (Croce, 2009). In livestock, miRNAs regulate economically critical traits: lactation, muscle development, reproduction, and immune response (Miretti et al., 2013). Aberrant miRNA profiles are documented in bovine mastitis, parasitic infections, and metabolic disorders. This disease- and trait-associated dysregulation presents a clear therapeutic opportunity: to inhibit overexpressed pathogenic miRNAs or to restore the levels of deficient, beneficial miRNAs to improve health and productivity.

### 1.3. The Emergence of miRNA-Targeted Therapeutics Across Species

The concept of targeting miRNAs therapeutically gained traction in the mid-2000s with studies showing chemically modified antisense oligonucleotides (antagomirs) could silence endogenous miRNAs in vivo (Krützfeldt et al., 2005). The field has since evolved with advances in oligonucleotide chemistry and delivery. While human applications have led the way, veterinary applications are rapidly emerging, driven by the high economic value of livestock and the need for sustainable, precision-based interventions to replace broad-spectrum antibiotics and hormones (Ioannidis & Donadeu, 2016).

### 1.4. Scope and Aims of This Review

This article provides a comprehensive overview of miRNA-based therapeutics for both human and veterinary medicine. We first elucidate the conserved biology of miRNA biogenesis and function. We then detail therapeutic strategies and delivery systems. The review surveys major human clinical applications and dedicates a significant section to the promising and distinct applications in cattle and water buffalo. We conclude by examining cross-species challenges and future directions in miRNA drug development.

## 2. Biology of miRNAs: Biogenesis, Mechanism, and Function

### 2.1. Canonical miRNA Biogenesis Pathway

miRNA production is a multi-step, tightly regulated process:

1. **Transcription:** miRNAs are primarily transcribed by RNA polymerase II as long primary transcripts (pri-miRNAs), which can be several kilobases long and contain hairpin structures (Lee et al., 2004).
2. **Nuclear Processing:** The microprocessor complex, consisting of the RNase III enzyme Drosha and its cofactor DGCR8, cleaves the pri-miRNA in the nucleus to release a ~70 nucleotide precursor miRNA (pre-miRNA) (Han et al., 2004).

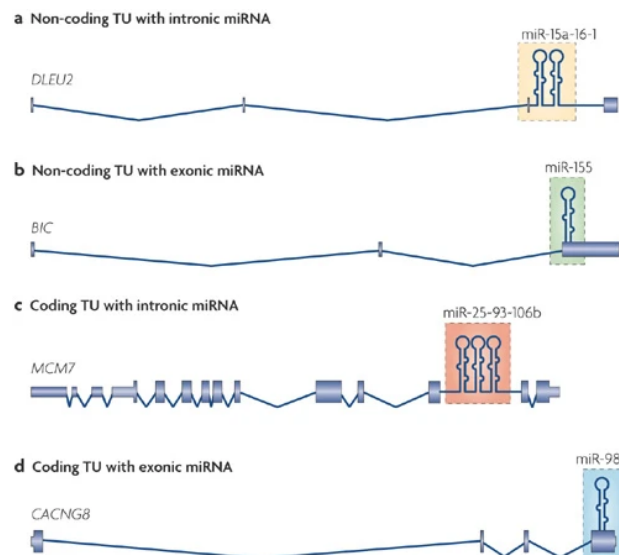
3. **Nuclear Export:** The pre-miRNA is exported to the cytoplasm by Exportin-5 (Yi, Qin, Macara, & Cullen, 2003).
4. **Cytoplasmic Processing:** The cytoplasmic RNase III enzyme Dicer cleaves the pre-miRNA hairpin, producing an ~22 nucleotide miRNA duplex (Bernstein, Caudy, Hammond, & Hannon, 2001).
5. **Loading into RISC:** One strand of the duplex (the guide strand) is loaded into the RNA-induced silencing complex (RISC), whose core component is an Argonaute (Ago) protein. The complementary passenger strand is degraded (Hutvagner & Zamore, 2002).

## 2.2. Mechanism of Gene Silencing

The miRNA-guided RISC complex silences gene expression primarily through two complementary mechanisms:

- **Translational Repression:** The miRNA-RISC complex binds to partially complementary sites in the 3' UTR of target mRNAs and inhibits translation (Pillai et al., 2005).
- **mRNA Destabilization:** miRNA binding often leads to deadenylation and subsequent degradation of the target mRNA (Wu, Fan, & Belasco, 2006).

A single miRNA can regulate hundreds of mRNAs, creating complex regulatory networks (Krek et al., 2005).



**Figure 1. MicroRNA biogenesis and mechanism of action.** (A) Canonical miRNA biogenesis pathway involving sequential processing by Drosha and Dicer complexes. (B) Non-canonical biogenesis pathways including mirtron and Dicer-independent routes. (C) Chemical structures of common oligonucleotide modifications used in therapeutic antagomirs and mimics. (D) Schematic of miRNA-mRNA interaction highlighting the critical seed region (nucleotides 2–8) and resulting translational repression/deadenylation. Figure 1. Schematic

created using [BioRender.com](https://BioRender.com), incorporating design concepts from Ha & Kim (2014) and Bartel (2004).

### 3. Therapeutic Strategies and Oligonucleotide Chemistry

#### 3.1. miRNA Inhibition: Targeting Overexpressed miRNAs

The goal is to sequester a pathogenic, overexpressed miRNA using single-stranded antisense oligonucleotides (ASOs). Key chemical modifications enhance stability and binding affinity:

- **Locked Nucleic Acid (LNA):** A bicyclic RNA analogue that confers exceptionally high affinity for complementary RNA and nuclease resistance. LNA-modified “antimiRs” are a leading platform (Elmén et al., 2008).
- **Phosphorothioate (PS) Backbone:** Replacement of a non-bridging oxygen with sulfur increases resistance to nucleases and extends circulation time (Geary, Norris, Yu, & Bennett, 2015).

#### 3.2. miRNA Replacement: Restoring Lost Function

This strategy uses synthetic, double-stranded RNA molecules that mimic endogenous miRNAs to restore the activity of a downregulated beneficial miRNA. The design is challenging as modifications must not interfere with RISC loading (Bader, Brown, & Winkler, 2010).

### 4. Delivery Systems: Translating Oligonucleotides into Drugs

#### 4.1. Delivery Requirements: Inhibitors vs. Mimics

- **miRNA Inhibitors (Antagomirs):** These single-stranded, chemically modified ASOs often achieve tissue uptake (especially in liver) without complex formulations following systemic administration.
- **miRNA Mimics:** These double-stranded RNAs require delivery vehicles (e.g., LNPs) to protect from degradation, facilitate uptake, and enable endosomal escape (Bader et al., 2010).

#### 4.2. Delivery Platforms

- **Naked/Oligonucleotide Conjugates:** Conjugation to N-acetylgalactosamine (GalNAc) enables receptor-mediated uptake by hepatocytes, achieving efficient liver targeting (Prakash et al., 2014).
- **Lipid Nanoparticles (LNPs):** The leading platform for systemic delivery of miRNA mimics, with tunable tissue tropism (Kulkarni et al., 2021).
- **Viral Vectors:** Adeno-associated viruses (AAVs) can provide long-term expression of miRNA modulators, relevant for chronic conditions (Brown & Naldini, 2009).

### 5. Therapeutic Applications in Human Medicine

#### 5.1. Oncology

Oncology represents the most active area.

- **miRNA Inhibition:** Targeting miR-155 (Cobomarsen/MRG-106) showed promising activity in cutaneous T-cell lymphoma trials (Seto et al., 2018).
- **miRNA Replacement:** An LNP-formulated miR-34a mimic (MRX34) entered trials but was terminated due to immune-related toxicity, highlighting delivery challenges (Beg et al., 2017).

### 5.2. Cardiovascular Diseases

- **Heart Failure:** An LNA-antimiR targeting miR-132 (CDR132L) improved cardiac function in a Phase Ib trial for heart failure (Taubel et al., 2021).
- **Fibrosis:** Inhibition of miR-21 showed efficacy in preclinical models of cardiac fibrosis (Thum et al., 2008).

### 5.3. Metabolic and Liver Diseases

- **Hepatitis C Virus (HCV):** Miravirsin, an LNA-antimiR-122, demonstrated potent antiviral activity in Phase II trials, validating the miRNA inhibition concept (Janssen et al., 2013).

## 6. Applications in Veterinary Medicine: Focus on Cattle and Water Buffalo

The application of miRNA therapeutics in livestock, particularly cattle (*Bos taurus*, *Bos indicus*) and water buffalo (*Bubalus bubalis*), represents a frontier in precision veterinary medicine with significant economic and welfare implications. These species are crucial for global dairy and meat production, and miRNA modulation offers non-hormonal, non-antibiotic strategies to enhance productivity and health.

### 6.1. miRNA Biology in Ruminants

Ruminant miRNA biology shares core conservation with humans but exhibits species- and tissue-specific expression patterns critical for unique physiological adaptations like rumination and lactation. Extensive miRNA profiling has identified key regulators in mammary gland, muscle, ovary, and immune cells (Miretti et al., 2013; Ioannidis & Donadeu, 2016).

### 6.2. Enhancing Production Traits

- **Lactation and Milk Production:** miRNAs are master regulators of mammary gland development and lactation. bta-miR-148a is highly expressed in bovine milk and mammary tissue, targeting genes like PTEN and IRS1 to promote mammary epithelial cell proliferation and milk synthesis (Li et al., 2012). bta-miR-152 and bta-miR-26a regulate lipid metabolism and milk fat synthesis. Therapeutic delivery of miRNA mimics targeting these pathways (e.g., via intramammary infusion) could potentially enhance milk yield and composition (fat, protein content).
- **Meat Quality and Muscle Development:** miRNAs control myogenesis and muscle fiber type. bta-miR-1, bta-miR-133a, and bta-miR-206 (the “myomiR” family) are critical for skeletal muscle development and regeneration. bta-miR-27b regulates

adipogenesis in intramuscular fat (marbling), a key determinant of meat quality. Modulation of these miRNAs in developing calves could optimize growth efficiency and carcass traits (Sun et al., 2019).

### 6.3. Improving Reproductive Efficiency

Reproductive efficiency is a major economic driver in livestock.

- **Ovarian Function and Embryo Development:** miRNAs like the let-7 family, bta-miR-21, and bta-miR-145 are dynamically expressed during oocyte maturation, folliculogenesis, and early embryogenesis. They target genes involved in cell cycle progression and steroidogenesis (Tesfaye et al., 2009). miRNA-based interventions could improve oocyte quality, synchronization of estrus, and embryo survival rates.
- **Pregnancy Recognition and Maintenance:** In cattle, interferon-tau (IFNT) is the signal for maternal recognition of pregnancy. miRNAs such as bta-miR-98 regulate the endometrial response to IFNT. Modulating these miRNAs could improve conception rates and reduce early embryonic loss (Bauersachs et al., 2009).

### 6.4. Combating Infectious and Metabolic Diseases

- **Mastitis:** Bovine mastitis, an inflammatory disease of the mammary gland, is the most costly disease in dairy farming. Pro-inflammatory miRNAs like bta-miR-21, bta-miR-146a, and bta-miR-223 are upregulated during *E. coli* or *S. aureus* mastitis, regulating the TLR4/NF- $\kappa$ B pathway (Lawless et al., 2014). Local administration of antagomirs against these miRNAs (e.g., via topical or intramammary routes) could dampen detrimental inflammation, reduce tissue damage, and improve recovery, potentially reducing antibiotic use.
- **Foot-and-Mouth Disease (FMD):** FMD virus (FMDV) infection alters host miRNA profiles. Host miRNAs like bta-miR-17-5p and bta-miR-125b can target viral genomes or regulate antiviral immune responses (Stenfeldt et al., 2014). miRNA mimics or inhibitors could serve as novel antiviral agents or immunomodulators alongside vaccination.
- **Parasitic Infections:** bta-miR-15b/16 are upregulated in *Fasciola hepatica* (liver fluke) infection and may modulate host immune evasion (Toet, Piedrafita, & Spithill, 2014). Targeting these pathways could enhance parasite clearance.
- **Metabolic Disorders:** During the transition period, dairy cows are prone to ketosis and fatty liver disease. miRNAs like bta-miR-33a (regulating fatty acid oxidation) and bta-miR-122 (linked to hepatic lipid metabolism) are dysregulated. Hepatic-targeted miRNA modulation could help maintain metabolic homeostasis (Zhang et al., 2016).

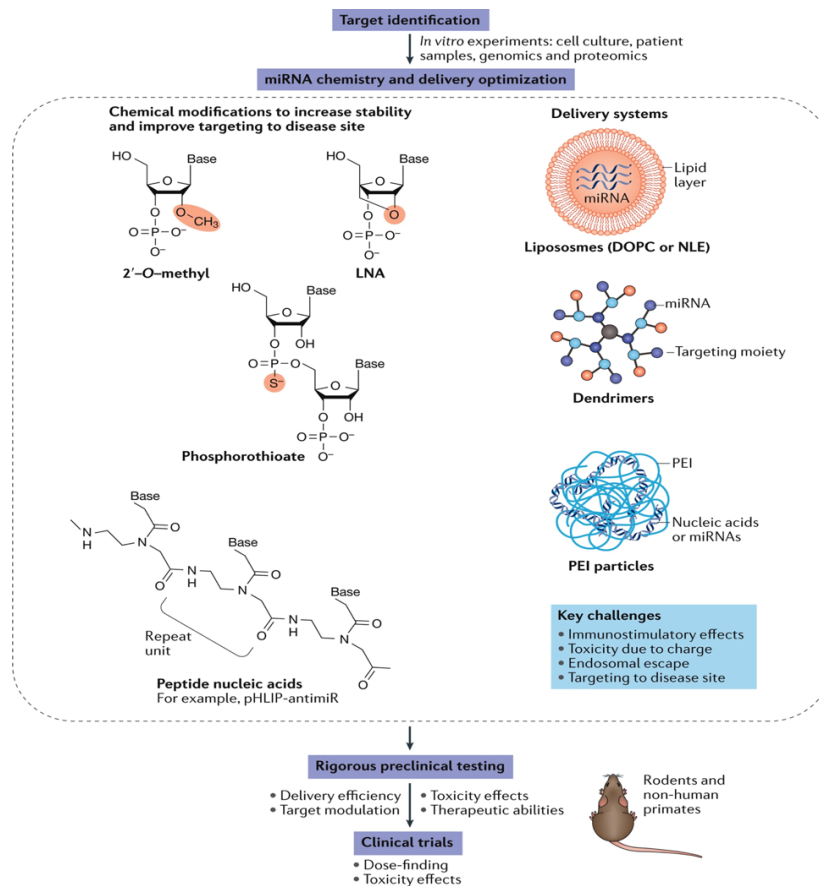
### 6.5. Delivery Challenges and Strategies in Livestock

Delivery in large animals presents unique challenges and opportunities:

- **Local vs. Systemic Administration:** For udder (mastitis) or reproductive tract applications, local administration (intramammary, intrauterine) minimizes systemic exposure and cost. For metabolic or growth effects, systemic delivery is needed.
- **Formulation for Oral Delivery:** Given routine management practices, oral delivery via feed or rumen-protected supplements is highly desirable but challenging due to RNase degradation in the gastrointestinal tract. Nanoparticle formulations resistant to rumen and abomasal conditions are under investigation.
- **Species-Specific Design:** miRNA sequences, while often conserved, may have species-specific variants. Oligonucleotides must be designed against the specific *Bos taurus* or *Bubalus bubalis* miRNA sequence for optimal efficacy.
- **Economic Viability:** Any therapeutic must be low-cost per dose to be viable for livestock. This favors simple chemistries (e.g., unconjugated antagomirs) and efficient delivery routes.

#### 6.6. Current Status and Future Outlook

Most applications are currently at the preclinical validation stage in animal models. Promising in vivo data includes the use of antagomir-21 to attenuate murine mastitis models, suggesting translatability. The future will involve developing commercially viable, approved delivery platforms and conducting efficacy trials in target species. Regulatory pathways for miRNA-based zootechnical additives or veterinary therapeutics are yet to be fully defined but are essential for translation.



**Figure 2. Cross-species applications of miRNA therapeutics.** (A) miRNA inhibition using antagonists (e.g., LNA-anti-miR-155 for lymphoma) and replacement using mimics (e.g., miR-34a for cancer). (B) Tissue-specific delivery strategies including GalNAc conjugates for hepatocytes and LNPs for extrahepatic targets. (C) Veterinary applications in cattle: intramammary antagonists for mastitis, systemic mimics for enhancing milk production, and reproductive interventions. (D) Comparative table of homologous miRNA targets and their therapeutic contexts in humans and livestock. Figure 2. Schematic created using [BioRender.com](https://www.biorender.com), incorporating design concepts from Rupaimoole & Slack (2017).

## 7. Challenges and Future Perspectives

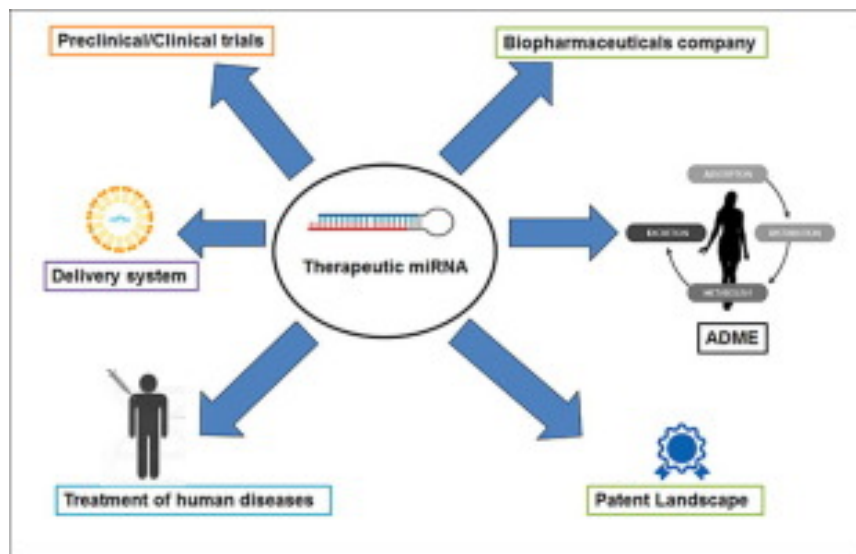
### 7.1. Cross-Species Challenges

- **Delivery Efficiency and Specificity:** Achieving effective, targeted delivery, especially of mimics and to extrahepatic tissues, remains paramount. Off-target effects require careful management in both humans and animals.
- **Immunogenicity and Safety:** RNA oligonucleotides and delivery vehicles can trigger innate immune responses. Long-term safety of modulating complex gene networks, especially in food-producing animals, requires extensive study (Robbins, Judge, & MacLachlan, 2009).

- **Economic and Regulatory Hurdles (Veterinary):** Developing cost-effective formulations and establishing clear regulatory guidelines for miRNA-based products in livestock are significant barriers.

## 7.2. Future Directions

- **Precision Livestock Farming:** Integration of miRNA diagnostics (miRNA biomarkers in milk, blood) with therapeutics for real-time health and productivity management (Ioannidis & Donadeu, 2016).
- **Species-Tailored Therapeutics:** Development of species-specific oligonucleotide libraries and delivery systems optimized for bovine/water buffalo physiology.
- **Combination Therapies:** Rational combinations of miRNA therapeutics with vaccines, probiotics, or low-dose antibiotics to enhance disease resistance and reduce drug resistance.
- **Gene Editing Synergy:** Using CRISPR/Cas to create genetic knock-ins of miRNA sponges or knockouts of miRNA genes in breeding lines for permanent trait improvement, complementing transient therapeutic approaches.
- **One Health Approach:** Lessons from veterinary applications (e.g., delivery in large animals, local administration) can inform human therapeutic strategies, and vice versa.



**Figure 3. Clinical development pipeline and challenges for miRNA therapeutics.** (A) Current pipeline showing developmental stage of leading miRNA-targeting candidates in human medicine. (B) Bubble chart representing clinical trials by disease area and phase. (C) Key veterinary candidates in preclinical development for livestock applications. (D) Analysis of clinical trial outcomes highlighting major challenges in delivery (46%), immunotoxicity (27%), and lack of efficacy (18%) based on published data from 2010–2023. Figure 3.

Schematic created using [BioRender.com](https://BioRender.com), incorporating design concepts from Chakraborty et al. (2021).

### Conclusion

The miRNA therapeutic field stands at a compelling crossroads, with validated mechanisms and growing clinical experience in humans, and transformative potential in veterinary medicine. In cattle and water buffalo, miRNA-based strategies offer a path toward sustainable intensification of livestock production by enabling precise enhancement of desirable traits and targeted combat against costly diseases, potentially reducing reliance on antibiotics and hormones. While substantial challenges in delivery, safety, and commercial viability persist, the convergence of advances in RNA biology, nanotechnology, and genomics is accelerating progress. The ongoing exploration of miRNAs across species not only promises novel therapeutics but also deepens our fundamental understanding of comparative gene regulation. The next decade will likely witness the first approved miRNA therapeutics for human disease and the translation of these platforms into innovative tools for improving animal health, welfare, and productivity, contributing to global food security.

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