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Take the Example of Mammary Gland: Unveiling Potential Strategies for piRNAs in Regulating Development and Disease in Domestic Animals

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Posted Date: 2 May 2025

doi: 10.20944/preprints202505.0021.v1

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Take the Example of Mammary Gland: Unveiling Potential Strategies for piRNAs in Regulating Development and Disease in Domestic Animals

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Simple Summary: PIWI-interacting RNAs (piRNAs) have been demonstrated to maintain transposons silencing and regulate reproductive development and disease progression by binding to PIWI proteins. However, research on piRNAs in livestock animals is still in its infancy, with only preliminary studies available regarding their roles in the reproduction of swine, bovine, and ovine species. Given the regulatory mechanisms of piRNAs in mammary cancer and inflammation, as well as the established roles of other non-coding RNAs (ncRNAs) in mammary development, this paper focuses on the dairy cow mammary system to explore the potential functions of piRNAs in mammary development and mastitis. This review offers a novel perspective on mammary gland biology in livestock and provides a theoretical foundation for understanding the role of piRNAs in mammary gland development and associated diseases, representing significant value for both basic research and practical applications.

Abstract: PiRNAs, a specialized subclass of ncRNAs characterized by their 26–31 nucleotides (nt) length, function through specific interactions with PIWI proteins to form dynamic regulatory complexes that modulate gene expression. As a significant constituent of ncRNAs, piRNAs exhibit distinctive biogenesis mechanisms and regulatory features that underscore their unique functional roles. Current research on piRNAs is predominantly centered on transposons silencing, reproductive development, and the regulation of disease processes. Research on piRNAs in livestock animals remains in its infancy, with only preliminary studies investigating their roles in the reproductive development of pigs, cows, and sheep. It is hypothesized that piRNAs may play an important role in regulating mammary gland development and inflammation in dairy cows, owing to their regulatory properties and the established roles of other ncRNAs in this process. In this review, we present recent advances in the study of piRNAs relating to mammary cancer and inflammation. Using the dairy cow mammary gland as a case study, we examine the potential role of piRNAs in mammary gland development and mastitis regulation at both the regulatory and molecular levels. This review provides a theoretical foundation for the further elucidation of piRNA roles in mammary gland development and related diseases in livestock animals. Additionally, piRNAs may reveal novel regulatory pathways that can enhance livestock production efficiency.

Keywords: piRNAs; mammary development; mastitis; cow

1. Introduction

The livestock farming industry confronts a dual challenge amid global population growth and an increasing demand for food. This dual challenge entails enhancing production efficiency and adopting green, circular development practices. Consequently, this imposes increased demands on fundamental aspects such as genetic enhancement, disease prevention and control, and reproductive efficiency. In this context, epigenetic regulatory mechanisms have emerged as a promising research field with the potential to alleviate production bottlenecks in livestock, owing to their crucial roles in gene expression regulation and transgenerational inheritance [1]. The impact of epigenetic regulatory networks on key phenotypic traits—including growth performance, stress tolerance, and reproductive efficiency—in livestock is well documented. This regulation is mediated through DNA methylation, histone modifications, and ncRNAs, whose significance in livestock is increasingly recognized [2,3].Therefore, a comprehensive understanding of ncRNA involvement in livestock farming may provide a nuanced perspective on their potential future applications in this sector. NcRNAs include a variety of forms, such as microRNAs (miRNAs), small interfering RNAs (siRNAs), piRNAs, and long non-coding RNAs (lncRNAs), among others. Of particular note is the discovery of piRNAs in 2006 [4–8], which are now recognized for their pivotal role in gonadal tissues, especially in maintaining genomic stability by suppressing transposable elements. Moreover, piRNAs have been shown to regulate protein synthesis by binding to PIWI proteins [9,10]. Recently, the significance of piRNAs in cancer research, particularly in breast cancer, has gained increasing recognition. These non-coding small RNAs are emerging as potential prognostic markers and therapeutic targets for breast cancer [11,12]. However, the role of piRNAs in mammary gland development remains incompletely understood. Based on rational analysis, we have proposed a series of hypotheses suggesting that piRNAs may play a role in mammary gland development. We have also briefly discussed the prospects for applying piRNAs in domestic animals. In this paper, we present a systematic review of the production, characteristics, functions, and roles of piRNAs in inflammation and mammary cancer, and propose a hypothesis regarding their potential roles in mammary gland development and mastitis. Additionally, we preliminarily address the potential applications of piRNAs in domestic animals. Furthermore, we propose new strategies for studying growth, development, and disease regulation in domestic animals.

2. The Process of Generating piRNAs

As a class of ncRNAs, piRNAs exhibit a unique biogenesis pathway that encompasses both primary processing and secondary amplification. Notably, piRNA biogenesis occurs independently of the Dicer enzyme. Primary piRNAs are characterized by a single-stranded linear structure featuring a 5'-monophosphate and a 2'-O-methyl modification at the 3' end [13]. In contrast, secondary piRNAs display a 10 nt complementarity at their 5' ends with primary piRNAs [14]. This characteristic underpins the efficient repression of transposons by the piRNA pathway.

2.1. Primary Processing

Primary processing represents a crucial stage in piRNA biogenesis. The primary sources of piRNAs are piRNA clusters enriched in transposons and other repetitive sequences in the genome [13], additionally, a subset of piRNAs originates from non-cluster regions, including lncRNAs, transposons transcripts, and antisense of protein-coding genes [15]. Primary processing of piRNAs is a complex process that involves multiple proteins, whose identities vary across species (Table 1). In Drosophila, the Rhino-Deadlock-Cutoff (RDC) complex, located in the nucleus, plays a pivotal role in recognizing heterochromatin regions. Subsequently, the RDC complex recruits Moonshiner protein, activates RNA polymerase II (Pol II), and initiates the transcription of piRNA clusters from both genomic strands. These processes culminate in the production of either sense or antisense piRNA precursors (pre-piRNA) [16]. The pre-piRNA is subsequently cleaved by the Zucchini (Zuc) protein, typically at a uracil-enriched region, resulting in piRNAs with a strong 5' uracil bias (1U bias) [17]. The cleaved precursor RNAs are trimmed by the Trimmer protein to yield single-stranded, linear piRNAs with a final length of 26–31 nt, thereby forming primary piRNAs [18–21].

Table 1. Proteins involved in the generation of piRNAs in different species.

Species	5'-terminal Enzyme	3'-terminal Enzyme	Key PIWI protein	Reference
Nematodes	Uncertainty	Uncertainty	Plasticity-related gene 1 (PRG-1)、plasticity-related gene 2 (PRG-2)	[22]
Zebrafish	Phospholipase D family member 6 (PLD6)	Uncertainty	ZIWI、ZILI	[23,24]
Drosophila	Zuc	Trimmer	PIWI、Argonaute 3 (Ago3)、Aubergin（Aub）	[25,26]
Mice	PLD6	Poly(A)-specific ribonuclease-like domain containing 1 (PNLDC1)	MIWI、MIWI2、MILI	[27–29]

2.2. Secondary Amplification

Secondary amplification, also known as the "ping-pong cycle," represents a crucial stage in piRNA biogenesis, resulting in an increased production of piRNAs through synergistic interactions with diverse PIWI proteins [25,30]. In Drosophila, for instance, primary antisense piRNAs bind to the Aub protein to form the piRNA-Aub complex, which exhibits endonuclease activity. This complex subsequently targets the sense pre-piRNA through a process of base complementary pairing, then trimmed by exonucleases and modified by hua enhancer 1 (HEN1) methyltransferase, resulting in the formation of the secondary piRNA. The 5' end of the secondary piRNA begins at the 10th nt from the cleavage site of the target RNA, which typically features an adenine residue (10A bias) [30–32]. The secondary piRNA then associates with the Ago3 protein, forming a complex that targets antisense pre-piRNA through base-pairing. The Ago3 complex cleaves the target RNA, thereby generating a new antisense pre-piRNA. The 5' end of this new antisense pre-piRNA begins at the 10th nt from the cleavage site—typically featuring a uracil residue—and, after exonuclease trimming and modification by HEN1 methyltransferase, matures into a new primary antisense piRNA [33,34]. The newly formed primary antisense piRNA then re-associates with the Aub protein, initiating another round of the ping-pong cycle (Figure 1).

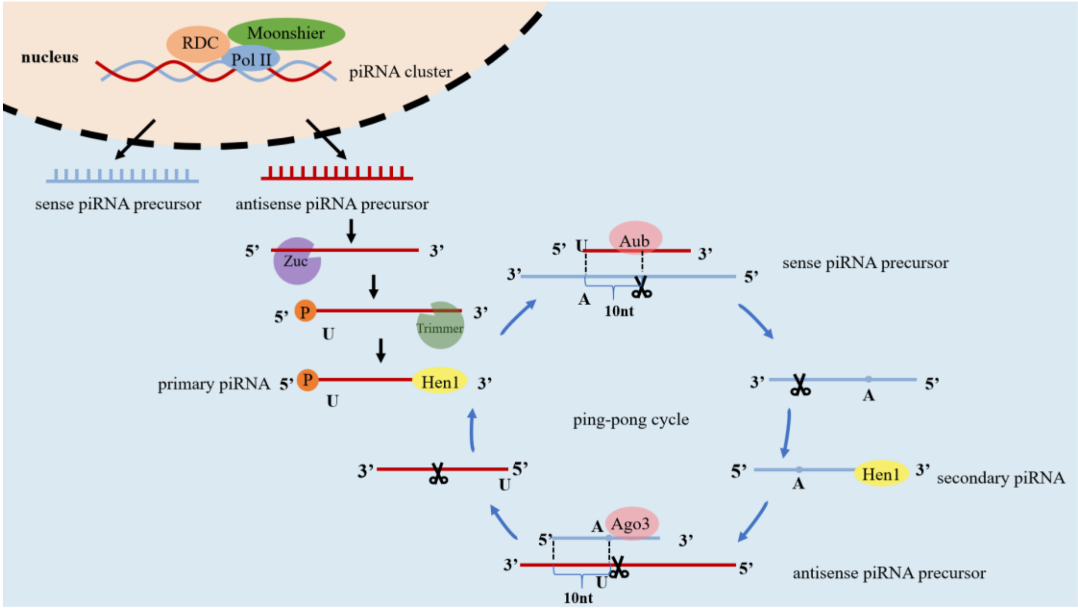


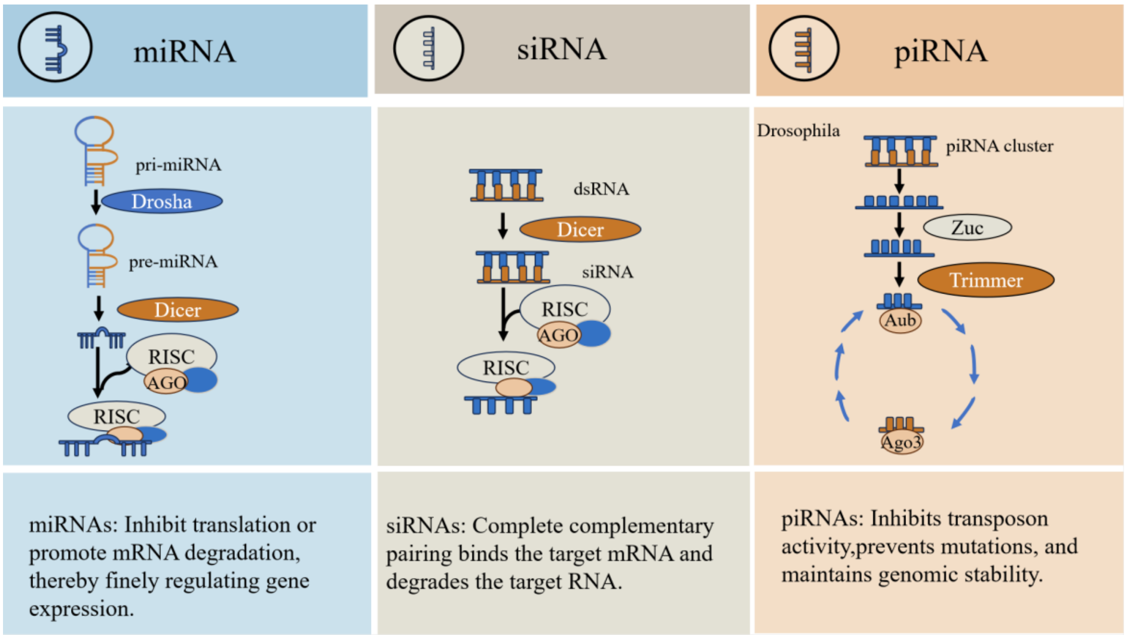
Figure 1. The primary and ping-pong mechanism for piRNAs biogenesis.

3. Characteristics and Functions of piRNAs

The principal function of piRNAs is believed to involve their binding to PIWI proteins, thereby forming functional piRNA–PIWI complexes. Their main functions include silencing transposons to ensure genomic stability, preserving fertility, and regulating disease development. In early studies, research focused on how piRNAs silence transposable elements at both the transcriptional and post-transcriptional levels in animal germ cells, in addition to regulating reproductive development [14,35]. Subsequent evidence has shown that piRNAs can also mediate the regulation of protein-coding genes [36,37]. More recent studies have focused on the regulatory roles of piRNAs in disease, with their expression being modulated by environmental, nutritional, stress-related, pathological, and genetic factors, as well as by maternal transgenerational effects [38,39].

3.1. The Characteristics of piRNAs

NcRNAs encompass a diverse array of categories, including but not limited to miRNAs, siRNAs, and piRNAs. Among these, piRNAs form a distinct class that plays a pivotal role in regulating transposons activity and ensuring genome stability, particularly in germ cells. piRNAs are generated through both diverse and highly conserved biogenesis pathways, underscoring their critical roles in cellular processes. While miRNAs primarily regulate gene expression by modulating mRNA translation and degradation, and siRNAs serve in defending against exogenous nucleic acids, piRNAs maintain genome stability chiefly by repressing transposons via a unique biosynthetic pathway. Distinct from miRNAs and siRNAs, piRNAs are generated via a Dicer-independent process from both dedicated piRNA clusters and non-cluster templates, yielding mature piRNAs typically 26–31 nt in length through primary processing and secondary amplification [14]; In contrast, miRNA biogenesis initiates with the transcription of primary miRNA transcripts (pri-miRNAs) by Pol II. These pri-miRNAs fold into characteristic hairpin structures within the nucleus, where they are recognized and cleaved by Drosha. This processing generates precursor miRNAs (pre-miRNAs), which are subsequently processed by the cytoplasmic RNase III enzyme Dicer into miRNA duplexes with length of 21-24 nt. One strand of the duplex is selectively loaded onto AGO subfamily proteins to form the functional RNA-induced silencing complex (RISC).[40]. Similar to miRNAs, siRNAs originate from exogenous viral genomes or endogenous repetitive sequences, which are transcribed into long double-stranded RNAs (dsRNAs). These dsRNAs undergo precise cleavage by the ribonuclease Dicer, generating short RNA duplexes, which are 20-24 nt long. Following processing, one strand of the duplex (the guide strand) is selectively incorporated into AGO subfamily proteins within the RISC.[41,42]. Secondly, it is important to note that the binding proteins of piRNAs are distinct from those of miRNAs and siRNAs. The silencing of small molecule RNAs has been observed to interact with Argonaute family proteins, which constitute the core part of RNA-induced silencing [43]. Argonaute proteins have been identified as multidomain proteins, primarily comprising the PAZ and PIWI domains, and encompassing both AGO subfamily and PIWI subfamily proteins. [44,45]. It has been demonstrated that, in contrast to the interaction of miRNAs and siRNAs with AGO subfamily proteins, piRNAs interact with the PIWI subfamily proteins [46].In summary, the core functions of these ncRNAs are distinct. piRNAs primarily silence transposons—thus maintaining genome stability via epigenetic modifications and direct cleavage of transposon RNAs [47–49]—whereas miRNAs regulate gene expression by modulating translation and mRNA degradation [50], and siRNAs mediate gene silencing by degrading complementary mRNAs[51]. A comparative analysis reveals significant functional disparities among these classes (Figure 2). Notably, the investigation of piRNAs in livestock is a relatively recent development compared to research on other ncRNAs.



miRNAs: Inhibit translation or promote mRNA degradation, thereby finely regulating gene expression.

siRNAs: Complete complementary pairing binds the target mRNA and degrades the target RNA.

piRNAs: Inhibits transposon activity,prevents mutations, and maintains genomic stability.

Figure 2. Differences between miRNA, siRNA, and piRNA.

3.2. The Role of piRNAs in Silencing Transposons and Stabilizing Genomes

The piRNA pathway is critical for maintaining transposons silencing and sustaining gene stability, and is centrally dependent on the integrity of PIWI proteins. It has been demonstrated that piRNA binds to PIWI proteins to form transposons capable of recognizing the transposons in Tc3, GypsyDR1, long interspersed nuclear element-1 (L1), etc. and able to silence them. This process is dependent on the RNA-dependent RNA polymerase (RdRPase) for the synthesis of 22-nucleotide guanine-starting RNA (22G-RNA), which in turn maintains long-term silencing through the WAGO pathway [52]. The absence of PIWI proteins (e.g., PRG-1, ZIWI, or PIWI) has been demonstrated to result in transposon activation, reduced germ cell numbers, and gonadal hypoplasia [24,31,53]. It has been established that certain proteins - defective P granules and sterile (DEPS-1), four ankyrin repeats, a sterile alpha motif, and leucine zipper 1 protein (Asz1), Maelstrom (Mael), etc. are indispensable for the maintenance of complex structure or function, and their absence directly disrupts transposons repression [54–56]. The repression of transposons by piRNAs can achieved through epigenetic mechanisms. It has been established that MILI and MIWI2, in conjunction with DNA methyltransferase 3-like (DNMT3L), are indispensable for the initiation of methylation processes. Furthermore, it has been determined that L1 and intracisternal A particle (IAP) play a pivotal role in this process. In the absence of MILI/MIWI2, there is a concomitant reduction in the level of transposon CpG methylation, resulting in the activation of the transposons [57,58]. 22G-RNA, in conjunction with the WAGO family protein heritable RNA interference (RNAi) defective-1 (HRDE-1) and the RNAi pathway protein nuclear RNAi defective-2 (NRDE-2), have been shown to collaborate in the recruitment of histone methyltransferase SET-25/32, thereby facilitating the catalysis of histone H3 lysine 9 trimethylation (H3K9me3) modification and, consequently, resulting in transcriptional repression through the establishment of heterochromatin structures by the heterochromatin protein like-2 (HPL-2). Concurrently, 22G-RNA has been demonstrated to directly mediate the degradation of target mRNAs and enhance the silencing effect [59]. Abnormal PIWI function has been demonstrated to reduce the level of H3K9me modification and heterochromatin protein 1 (HP1) enrichment, whilst concomitantly increasing the level of histone H3 lysine 4 methylation (H3K4me2/3) modification. This has been shown to inhibit chromatin formation in the transposons and its surrounding regions, thereby ultimately activating the transposons [60–62]. It has been established that piRNAs are capable of obstructing the process of translation of transposon mRNAs through their capacity to bind to the 3'untranslated region (3'UTR) region of the transposon mRNAs. Furthermore, it has been demonstrated that chromatin assembly factor 1 (CAF1) interacts with the MIWI/piRNA complex, thereby inducing the decay of the target mRNA [37].

3.3. Physiological Functions of piRNAs

The piRNA pathway is paramount for reproductive development, playing a critical role in safeguarding gametogenesis and maintaining fertility. At the level of chromosome dynamics, piRNAs play a pivotal role in assembling telomere protection complexes thereby ensuring genomic stability [63]. Furthermore, the piRNA pathway directly influences chromosome condensation and segregation. In Drosophila germ cells, mitotic bodies composed of piRNA pathway proteins have been observed to bind to pericentromeric, piRNA-producing loci, thereby regulating condensin loading. Mutations in the piRNA pathway lead to aberrant condensin loading, causing delays in chromosome condensation and segregation defects [64]. The role of PIWI proteins in maintaining germline stem cells (GSCs) is underscored by observations showing that deletion of PIWI results in reduced GSC numbers, whereas its overexpression enhances stem cell division [65]. Notably, mice deficient in the piRNA pathway protein Miwi2 exhibit a significant reduction in germ cell numbers with age. [58]. The importance of fertility maintenance is further evidenced by studies in mice showing that mitochondrial phospholipase D (MitoPLD) gene dysfunction disrupts the piRNA biogenesis pathway, resulting in spermatogonial meiotic arrest [66]. In golden hamsters, piRNAs are essential for the development of spermatogonia and the formation of fertile oocytes [67]. Thus, aberrant expression of genes associated with the piRNA pathway leads to unscheduled activation of transposons in germ cells, meiotic arrest, impaired spermatogenesis and oogenesis, disrupted early embryonic development, and ultimately infertility. Consequently, the piRNA pathway plays a pivotal role in preserving developmental potential and reproductive health.

3.4. Factors Regulating piRNAs

The expression of piRNAs is regulated by a multitude of factors. Recent studies have demonstrated that elevated temperatures convert Drosophila piRNA clusters from an inactive to an active state, leading to the generation of novel piRNAs. These novel piRNAs are characterized by enhanced stability and can be maternally inherited by subsequent generations [68]. Conversely, elevated temperatures have also been shown to decrease piRNA levels and reduce offspring fitness in C. elegans. However, following bacterial infection, the restoration of piRNA levels correlates with improved offspring fitness, suggesting that the piRNA pathway can dynamically respond to environmental signals and exert a lasting influence on progeny [69]. Altered nutritional levels modulate piRNA expression; for instance, differential piRNA profiles have been observed in the sperm of obese versus lean males [70]. Similarly, a Western-style diet has been found to alter piRNA expression in the testes of male mice [71], while a high-fat diet influences sperm piRNA profiles in both males and their offspring, subsequently impacting offspring metabolic function [72]. Furthermore, the present study demonstrates that short-term endurance training induces reversible changes in sperm piRNA expression [73]. Early traumatic stress has been shown to significantly downregulate piRNA cluster 110 in male mouse sperm [74]. Furthermore, microcystin-leucine-arginine (MC-LR) exposure has been reported to alter piRNA expression in the testes and prostate of offspring, leading to decreased testicular indices and prostate hyperplasia in male mice [38,75]. In the context of livestock production, environmental stressors—including nutritional imbalances and heat stress—often have detrimental effects on animal health and performance. Given the role of piRNAs in responding to environmental and nutritional cues, it is hypothesized that they may hold considerable potential for enhancing livestock productivity and resilience.

4. Characteristics of Mammary Gland Development

In dairy production, a cow's milk yield is the primary determinant of its economic viability. The dynamics of mammary gland development and the physiological homeostasis underlying lactation are the fundamental biological mechanisms that govern milk production performance. The mammary gland, a distinctive mammalian organ, displays diverse morphologies across different developmental stages—from embryonic morphogenesis and pubertal ductal expansion to gestational alveolar differentiation. Within livestock production systems, the extent of mammary gland development directly impacts the economic viability of the industry. This is substantiated by the strong positive correlations observed between core production indicators—such as the milk yield of dairy cows and the suckling capacity of sows, as reflected in piglet weaning weights [76–78]. Notably, reduced lactation performance and the incidence of mastitis cause significant economic losses in the livestock breeding industry. Therefore, research aimed at promoting mammary epithelial cell proliferation and differentiation, maintaining lactation homeostasis, and reducing mastitis through molecular regulation offers a promising avenue for addressing current challenges in the industry [79,80]. Within the ncRNA regulatory network, although the precise functional mechanisms of piRNAs in mammary gland development remain to be fully elucidated, ample evidence demonstrates that miRNAs and lncRNAs play significant roles in mammary gland development and milk synthesis. This underscores the multifaceted regulatory functions of the ncRNA family in mammary gland development.

4.1. The Role of ncRNAs in Mammary Gland Development

As a prominent member of the short ncRNA family, miRNAs regulate gene expression through sequence-specific recognition of the 3′ untranslated region (3′UTR) or other regulatory elements of target mRNAs. This regulatory mechanism exhibits unique network characteristics: a single miRNA can modulate the stability or translational efficiency of multiple target mRNAs, while a single mRNA may be concurrently regulated by several miRNAs, thereby orchestrating protein synthesis and signaling pathways [81]. Recent studies indicate that miRNAs serve as key regulators in the dynamic process of mammary gland development. Furthermore, the expression patterns of miRNAs undergo characteristic changes throughout mammary gland development—including the formation of the embryonic mammary gland primordium, pubertal ductal morphogenesis, gestational alveolar proliferation, and functional maturation during lactation (Table 2). Collectively, these dynamic changes play a crucial regulatory role in every stage of mammary gland development.

Table 2. The role of miRNAs in mammary gland development.

Particular year	miRNA	Research target	Outcomes	References
			Mammary gland development	
2007	let-7	Comma-Dβ	Inhibited self-renewal capacity of progenitor cells and promoted differentiation.	[82]
2014	miR-21	HC11、mice	Regulated mammary gland development and lactation.	[83]
2012	miR-30b	Mice	Inhibited normal mammary gland development and lipid droplet accumulation.	[84]
2020	miR-31	HC11、mice	Promoted mammary stem cells (MaSCS) self-renewal, alveogenesis, and lipid droplet accumulation.	[85]
2020	miR-34a	Comma-Dβ、SUM159pt、mice	Inhibited MaSCs self-renewal, terminal end bud (TEB) development.	[86]
2009	miR-101a	HC11、mice	Inhibited HC11 proliferation and β-casein expression, affected mammary gland development and degeneration.	[87]
2010	miR-132、miR-212	Mice	Promoted ducts growth and modulated epithelial-stromal interactions.	[88]
2015	miR-137	MDA-MB-231、293T、mice	Promoted thickening of the mammary substrate.	[89]
2006	miR-138	Mouse mammary epithelial cells、mice	Regulated mammary epithelial cell proliferation and mammary gland development, promoted β-casein expression.	[90]
2017	miR-139	BMEC、Holstein cows	Inhibited β-casein synthesis and BMEC proliferation.	[91]

2022	miR-142-5p、miR-148C、miR-152、miR-218、	Goats	Regulated mammary gland regenerative degeneration.	[92]
2014	miR-193b	MEC、mice	Inhibited mammary stem/progenitor cell activity and alveolar differentiation.	[93]
2009	miR-200c	293T、Tera-2 、mice	Inhibited mammary ducts formation.	[94]
2018	miR-205	MEC、mice	Impacted mammary regenerative capacity and mammary homeostasis.	[95]
2019	miR-489	Mouse mammary epithelial cells、mice	Inhibited ducts growth and TEB formation.	[96]
		Milk component synthesis		
2018	miR-15b	MCF-10A、mice、goats	Inhibited lipid synthesis and metabolism.	[97]
2017	miR-17-5p miR-148a	GMEC、goats	Promoted triacylglycerol (TAG) synthesis and milk fat droplet accumulation.	[98]
2015	miR-24	GMEC、goats	Increased unsaturated fatty acid concentrations, TAG levels and milk fat droplet accumulation.	[99]
2018	miR-25	GMEC、goats	Inhibited TAG synthesis and lipid droplet accumulation.	[100]
2013	miR-27a	GMEC、goats	Inhibited TAG synthesis and reduced the ratio of unsaturated/saturated fatty acids.	[101]
2015	miR-29s	DCMEC、293T、Chinese Holstein cows	Inhibited triglyceride, protein and lactose secretion.	[102]
2013	miR-103	GMEC、goats	Promoted lipid droplet accumulation and TAG accumulation.	[103]
2011/2017	miR-126-3p	MCF-10A、mice	Inhibited β-casein secretion and lipid synthesis.	[104,105]
2019	miR-142-3p	MMGEC、mice	Inhibited secretion of β-casein and TAG.	[106]
2017	miR-145	GMEC、goats	Promoted lipid droplet enlargement and TAG accumulation, increased the relative content of unsaturated fatty acids.	[107]
2016	miR-150-5p	Mice	Inhibited of the de novo synthesis of lipids and fatty acids.	[108]
2016	miR-181b	GMEC、goats	Increased TAG levels and cream droplet accumulation.	[109]
2020	miR-204	HC11、mice	Promoted β-casein and milk fat synthesis.	[110]
2019	miR-206	HC11、mice	Promoted lipid accumulation.	[111]
2018	miR-221	MEC、MCF-10A、mice	Promoted lipid synthesis.	[112]
2015	miR-486	BMEC、Holstein cows	Promoted beta-casein, lactose and lipid secretion.	[113]

LncRNAs, another significant class of ncRNAs, though not as extensively studied as miRNAs, have garnered increasing attention in recent years due to their critical roles in mammary gland development. It has been demonstrated that the lncRNA SOX2OT harbors the transcription factor SOX2—one of the Yamanaka factors—which plays a pivotal role in embryonic development and is essential for maintaining the pluripotency of various stem cells. SOX2 has also been shown to be a key determinant [114]. Steroid receptor RNA activator (SRA) is induced by steroid hormones and is implicated in post-pubertal mammary gland development. Studies have demonstrated that SRA significantly promotes the proliferation and differentiation of mammary epithelial cells through the synergistic activation of estrogen (ER) and progesterone (PR) receptors, while also triggering apoptosis. Furthermore, SRA enhances the progression of lobule-alveolar structures during pregnancy relative to control mice [115]. Zfas1 is expressed in the developing mammary gland after puberty, particularly in the epithelial cells of the ducts and alveoli during pregnancy. Knockdown of Zfas1 in HC11 cells has been shown to increase cellular proliferation, induce β-casein expression, and promote epithelial dome formation, suggesting that high Zfas1 expression in late gestational mammary glands may regulate proliferation and inhibit the terminal differentiation of alveolar cells [116].The H19 locus is regulated by estradiol and corticosterone, with high expression levels observed in alveolar cells during pregnancy and degeneration. H19 is developmentally regulated, exhibiting elevated transcript levels during both puberty and pregnancy [117]. Pregnancy-induced noncoding RNA (PINC) is a developmentally regulated lncRNA that is highly expressed in alveolar cells during pregnancy and in degenerating terminal ductal lobule-like structures during transplacental labor. PINC may inhibit the terminal differentiation of alveolar cells during pregnancy, thereby preventing the premature secretion of large quantities of milk [118,119]. Overall, both miRNAs and lncRNAs have been shown to exert direct influences on mammary gland development. Similarly, piRNAs—a distinct class of ncRNAs—have been shown to play unique roles in reproductive development. It is hypothesized that piRNAs may also be key factors influencing mammary gland development in domestic animals, potentially mediating transgenerational epigenetic inheritance and affecting offspring phenotypes. However, research on piRNAs in livestock remains scarce.

4.2. Prospects for piRNAs in Livestock Animals

As members of ncRNAs, piRNAs play critical roles in numerous biological processes, including maintaining genomic stability, silencing transposons, and facilitating germ cell development. Recent research has revealed the potential significance of piRNAs in livestock species, with ongoing studies focusing on pigs, cattle, and sheep. Numerous investigations have characterized the expression profiles of piRNAs in various gonadal tissues across different developmental stages in livestock, offering novel insights into their roles in germ cell, embryonic, and overall gonadal development. In pigs, a "ping-pong" amplification loop of piRNAs has been proposed, suggesting that these small ncRNAs not only repress transposons expression but also regulate the post-transcriptional expression of multiple protein-coding genes critical for normal spermatogenesis, thereby enhancing our understanding of porcine spermatozoa development [120,121]. In cattle, hybrid male sterility (HMS) has been linked to promoter hypermethylation-induced silencing of PIWI/piRNA pathway genes. DNA methylation influences this pathway by affecting gene expression and the production of robust piRNAs during spermatogenesis, underscoring its central role in bovine HMS [122]. Studies have shown that piRNA expression in bovine frozen semen differs significantly between high-motility (HM) and low-motility (LM) sperm, suggesting that piRNAs may be involved in sperm development and overall fertility [123]. Additionally, the expression profiles of piRNAs in sheep ovaries during the luteal (LP) and follicular (FP) phases have been examined to provide a reference for understanding the role of ovarian piRNAs throughout the estrous cycle [124]. The results of the present study suggest that piRNAs play an integral role in domestic animals (Table 3). Moreover, analysis of milk exosomal ncRNAs has revealed the presence of 88 piRNAs of unknown function within milk, indicating a potential association between piRNAs and immune function [125]. Additionally, the antiviral defense function of piRNAs has been demonstrated in mosquito cells—knocking down piRNA pathway proteins leads to enhanced replication of Semliki Forest virus, thereby underscoring the antiviral properties of the piRNA pathway. Consequently, the potential of piRNAs to enhance reproductive efficiency, optimize production performance, and augment disease resistance has been recognized. Compared with research on model organisms or humans, studies on piRNAs in animal husbandry are still in their infancy and warrant further investigation.

Table 3. The role of piRNAs in domestic animals.

Particular year	Detection Methods	Species	Outcomes	References
2021	Small RNA-seq	Porcine	Characterization of the composition of piRNAs in spermatozoa suggests that piRNAs may be potential negative regulatory markers of sperm quality.	[126]
2012	Small RNA-seq、qRT-PCR	Porcine	It was demonstrated that piRNAs were predominantly enriched in the mature gonads and were expressed more in the testis than in the ovary.	[127]
2023	Small RNA-seq	Porcine	Expression of piRNAs is regulated by Senecavirus A (SVA) and promotes apoptosis.	[128]
2015	Small RNA-seq	Porcine	Characterization of the composition of piRNAs in testis suggests that mammalian piRNAs exist in the ping-pong cycle and have a role in the post-transcriptional regulation of protein-coding genes.	[121]
2022	Small RNA-seq	Xiang pigs	Identification of the composition of piRNAs in testicular tissues at different stages demonstrates that piRNAs regulate spermatogenesis.	[120]

2017	Small RNA-seq 、qRT-PCR	Porcine	Characterization of the expression profiles of testicular piRNAs at different stages of sexual maturation demonstrated that piRNAs regulate testicular development and spermatogenesis. spermatogenesis.	[129]
2012	Small RNA-seq、qRT-PCR	Porcine	Evidence for a potential role of piRNAs in female germ cell development.	[130]
2020	Small RNA-seq 、qRT-PCR	Porcine	Characterization of the expression profile of sperm plasma extracellular vesicles (SP-EV) piRNAs suggests that piRNAs play a role in the physiological function of spermatozoa.	[131]
2017	Small RNA-seq、qRT-PCR	Bovids	The piRNAs in the testis were identified as longer than the piIRNAs in oocytes and embryos.	[132]
2020	Small RNA-seq	Yattle、cattle、yaks、	Promoter hypermethylation of PIWI/piRNA pathway genes leading to gene silencing and reduction of testis-thick piRNAs is a driver of bovine HMS.	[122]
2015	Small RNA-seq	Calves	Expression of piRNAs in bovine blood and plasma was revealed, suggesting that they may originate from tissues other than blood cells and thus enter the circulation.	[133]
2018	Small RNA-seq	Cattle、yaks、dzo	Comparison of the expression characteristics of three ruminant piRNAs provides theoretical references for exploring their regulatory mechanisms in spermatogenesis and dzo reproductive therapy.	[134]
2020	Small RNA-seq、qRT-PCR	Bulls	Expression of piRNAs in spermatozoa was detected, suggesting that they may play a role in embryonic development and may serve as biomarkers of semen fertility.	[135]
2017	Small RNA-seq	Bulls	Characterization of the composition of piRNAs in frozen spermatozoa suggests a role in sperm development and fertility.	[123]
2015	Small RNA-seq	Bovine	Detection of the composition of mature testicular and ovarian piRNAs revealed that ovarian piRNAs were very similar to spermatogenesis thick-walled stage piRNAs.	[136]
2018	Small RNA-seq	Bovids	Detection of milk exosomal piRNAs expression suggests that they may be related to immune and developmental functions.	[125]
2021	Small RNA-seq	Cattle	The presence of piRNAs in ejaculated sperm was confirmed, suggesting that they may regulate sperm maturation, fertilization process, and embryonic genome activation.	[137]
2021	Small RNA-seq	Bovids	Expression of piRNAs was detected separately in both milks, suggesting a possible regulatory role in calf immunity and development.	[138]
2023	Small RNA-seq	Murrah buffalo	Characterization of the composition of piRNAs at different stages of lactation implies that piRNAs can serve as potential targets for the regulation of lactation.	[139]
2019	Small RNA-seq	Mongolian horse	Characterization of piRNAs composition in sexually mature and immature testes suggests that piRNAs may regulate testicular development and spermatogenesis.	[140]
2022	Small RNA-seq	Sheep	Expression profiles of piRNAs in LP and FP ovaries were characterized to facilitate understanding of the role of piRNAs in the estrous cycle.	[124]
2022	Small RNA-seq	Sheep	Characterization of the composition of testicular piRNAs demonstrates that piRNAs may mediate blood-testis barrier stability and spermatogonial stem cell differentiation.	[141]
2021	Small RNA-seq	Sunite (SN)、Small-tailed Han (STH）	Identification of differential expression of testicular piRNAs in different breeds suggests that piRNAs may be associated with male fecundity.	[142]
2023	Small RNA-seq、qRT-PCR	Tibetan sheep	Characterization of piRNAs expression profiles in different stages of testis suggests that piRNAs regulate male fertility and spermatogenesis.	[143]

5. The Role of piRNAs in Breast Cancer

Although the precise mechanisms of piRNA action in mammary gland development remain ambiguous, mounting evidence suggests a strong correlation between aberrant piRNA expression—and consequent PIWI dysfunction—and the onset, progression, and metastasis of breast diseases, particularly breast cancer. Moreover, epigenetic regulation of the piRNA–PIWI axis indicates that piRNAs may serve as both prognostic markers and therapeutic targets for breast diseases. For instance, Huang et al. used small RNA sequencing to identify differentially expressed piRNAs in tumor versus non-tumor breast tissues; they reported that piR-4987, piR-20365, piR-20485, and piR-20582 are up-regulated in tumors, with elevated piR-4987 expression correlating with positive lymph node status. [144]. In addition, Hashim et al. found that in breast cancer cells, piR-34377, piR-35407, and piR-36743 are up-regulated, whereas piR-36026, piR-36249, piR-36318, and piR-36712 are down-regulated—suggesting roles in regulating the cell cycle, apoptosis, cell–cell interactions, and DNA replication and repair [145]. Further studies have identified differentially expressed piRNAs in various breast cancer types and following different treatments [146,147]. Recent evidence indicates that piRNAs function via multiple pathways. For example, piR-651 promotes cell proliferation and invasion by elevating levels of MDM2, CDK4, and Cyclin D1, and it inhibits apoptosis by forming a complex with PIWIL2 that enhances DNMT1-mediated methylation of the phosphatase and tensin homolog (PTEN) promoter; disruption of piR-651 results in the opposite effect [148]. Similarly, piR-823 has been shown to enhance the expression of stem cell regulators (OCT4, SOX2, KLF4, NANOG, h-TERT) and methyltransferases (DNMT1, DNMT3A, DNMT3B), thereby promoting hypermethylation of the adenomatous polyposis coli (APC) promoter, activating Wnt signaling, and driving tumor growth—whereas piR-823 knockdown, which also increases ERα and decreases h-TERT expression via inhibition of the PI3K/Akt/mTOR pathway, suppresses cancer cell proliferation [149,150]. In addition, piR-2158 inhibits IL11 expression by competing with FOS-related antigen 1 (FOSL1), inactivates the JAK/STAT pathway, and suppresses cell proliferation, migration, epithelial-mesenchymal transition (EMT), stemness, and angiogenesis [151]. Collectively, these findings underscore the pivotal role of piRNAs in breast cancer (Table 4).

Table 4. The role of piRNAs in breast cancer.

Particular year	piRNA	Expression	Research target	Finding	References
2021	piR-651	Upregulation	MDA-MB-231、MCF-7、AU565、HCC38	Bound to PIWIL2, promoted cell proliferation and migration through DNMT1-mediated methylation of the PTEN promoter.	[148]
2021	piR-823	Upregulation	MCF-7、T-47D、nude mice	Increased the expression of DNMT1, DNMT3A, and DNMT3B genes to promote DNA methylation of APC genes to activate the Wnt signaling pathway.	[149]
2022	piR-823	Upregulation	MDA-MB-231	Inhibited piR-823 expression inhibited cell proliferation, PI3K/Akt /mTOR gene expression, and increased gene and protein expression of ERα.	[150]
2013	piR-932	Upregulation	Cancer stem cells、SCID mice	Bound to PIWIL2, promoted methylation of promoter CpG islands to repress Latexin expression.	[9]
2023	piR-2158	Downregulation	ALDH+ breast cancer stem cells、MDA-MB-231、4T1、HUVEC、293T、nude mice、BALB/c mice	Competed with FOSL1 to inhibit IL-11 expression and secretion, inactivated JAK/STAT signaling and thereby inhibiting breast cancer.	[151]
2022	piR-17560	Upregulation	MDA-MB-231、MCF-7	Targeted FTO-mediated m6A demethylation enhances ZEB1	[152]

				expression, thereby promoting chemotherapy resistance and EMT.	
2013	piR-4987 piR-20365、piR-20485、piR-20582	Upregulation	breast tissue samples	Influenced cancer development and lymph node metastasis.	[144]
2017	piR-1282、piR-21131、piR-23672、piR-26526、piR-26527、piR-26528、piR-30293、piR-32745 piR-23662	Upregulation Downregulation	breast tissue samples	Can be used as a biomarker for breast cancer and provided a therapeutic target.	[146]
2014	piR-31106	Upregulation	MCF-7、SKBR3 、ZR-75.1、MCF10A	Responded to cell growth, cell cycle progression, and hormonal signaling.	[145]
2021	piR-31106、piR-34998、piR-40067	Upregulation	breast tissue samples	Can be used as a prognostic and therapeutic marker for breast cancer	[147]
2023	piR-31106	Upregulation	MDA-MB-231、MCF-7	Promoted cell proliferation and migration as well as oncogene expression and METTL3-mediated m6A methylation.	[153]
2025	piR-31115	Upregulation	MDA-MB-231、MCF-7、SK-BR-3、MCF-10A	Bound to PIWIL4 and inhibits the degradation of HSP90AA1 protein, thereby promoting cell migration.	[154]
2020	piR-31143	Upregulation	HCC1806、MDA-MB-468、Hs 578T	Can modulation of TNBC behavior through ERβ.	[155]
2014	piR-34377、piR-35407、piR-36743 piR-36026、piR-36249、piR-36318、piR-36712	Upregulation Downregulation	MCF-7、SKBR3 、ZR-75.1、MCF10A	Responded to cell growth, cell cycle progression, and hormonal signals.	[145]
2019	piR-36712	Downregulation	MCF-7、ZR75-1、293T、BALB/c nude mice	Knockdown of piR-36712 inhibits p53 activity through SEPW1, upregulates Slug/p21 and decreases E-calmodulin levels, ultimately inhibiting cell proliferation, migration and invasion.	[156]
2020	piR-016658 piR-016975	Upregulation Downregulation	MCF-7、SUM-159、Hs-578t、MDA-MB-231、MDA-MB-453、T-47D、BT474、SKBR3	Regulated by cell Cyclin D1, affects stem cell function.	[157]
2015	piR-021285	Upregulation	MCF-7、MDA-MB-231	Increases the methylation level of ARHGAP11A gene, which promotes cell invasion and inhibits cell apoptosis	[158]
2015	piR-sno75	Upregulation	MCF-7、HEK293T、NOD/SCID mice	Binding to WDR5 recruits the MLL3/UTX complex to the TRAIL promoter	[159]

				region thereby inducing H3K4 methylation and H3K27 demethylation.	
2022	piR-MW557525	Upregulation	FBs、Piwil2-iCSCs	Promotes the proliferation, migration and invasion of Piwil2-iCSCs, promotes the expression of CD24, CD133, KLF4 and SOX2, and inhibits apoptosis.	[160]
2018	piR-FTH1	Downregulation	MDA-MB-231、MCF-7、HEK293、A549、A2008、PC3、RF3、Hela	Binds to HILI/HIWI2 and down-regulates FTH1, increasing sensitivity to chemotherapy.	[161]
2024	piR-YBX1	Downregulation	MDA-MB-231、BT 549、BALB/c nude mice	Inhibition of YBX1 expression leads to inhibition of MEK and ERK1/2 MAPK signaling pathways ultimately inhibiting cell proliferation and migration.	[162]

Beyond oncogenesis, numerous studies have highlighted critical roles for piRNAs in maintaining genome stability, regulating stem cell function, and modulating molecular signaling pathways—findings that offer important clues to their physiological roles in normal mammary gland development. Mammary gland development is a complex, multi-stage process encompassing embryonic formation of the mammary anlage, pubertal ductal branching, and gestational differentiation of glandular structures [163]. The central roles of stem cells, hormonal stimulation, and signaling pathways underpin both normal development and breast cancer pathogenesis. Indeed, mammary stem cells MaSCs maintain tissue homeostasis and regeneration through self-renewal, whereas breast cancer stem cells (BCSCs) drive tumor growth and recurrence [163–165]. Moreover, both mammary development and breast cancer have been associated with the activation of key pathways—including Wnt, Notch, and Hedgehog—and with the pivotal influence of estrogen receptor ERα in mammary morphogenesis and tumorigenesis [166–168]. Clearly, the physiological development of breast tissue shares common molecular characteristics with the pathological progression of breast cancer; accordingly, it is plausible that piRNAs contribute to the regulation of cell proliferation, differentiation, and homeostasis in normal mammary gland development.

6. The Role of piRNAs in Inflammation

In addition to its well-established role in breast cancer, recent studies have shown that piRNAs also play a significant role in inflammation. The inflammatory response is characterized by the activation of immune cells and the release of pro-inflammatory factors [169]. It is hypothesized that piRNAs may influence the expression or secretion of critical inflammatory mediators, thereby modulating downstream signaling pathways and ultimately shaping the inflammatory response. For example, Saha et al. identified 19 differentially expressed piRNAs in the plasma of patients with chronic pancreatitis (CP) compared to healthy individuals. [170]. In endothelial cells, rno-piR-017330 is up-regulated in response to TNF-α stimulation, suggesting that piRNAs contribute to the regulation of inflammation in these cells [171]. Moreover, piRNA-6426 has been shown to inhibit inflammation by increasing methylation at the SOAT1 promoter via recruitment of DNMT3B, which reduces secretion of IL-1β and TNF-α and ameliorates the inflammatory microenvironment in heart failure [172]. Research further demonstrates that pir-has-216911 binds to TLR4 mRNA and suppresses the TLR4/NF-κB/NLRP3 inflammatory signaling pathway, thereby inhibiting caspase-1–induced activation of GSDMD and reducing the pro-inflammatory effects of pyroptosis [173].In addition, piR-112710 directly binds to the 3′UTR of thioredoxin interacting protein (Txnip), suppressing its expression and inactivating the Txnip/NLRP3 pathway; this leads to reduced levels of pro-inflammatory factors (IL-18 and IL-1β) and lower expression of proteins related to inflammasome activation (NLRP3, caspase-1, and GSDMD-N) [174,175]. Furthermore, hsa_piR_019949 is significantly down-regulated in response to IL-1β, and it may modulate the inflammatory response by repressing the expression of the lncRNA NEAT1—which in turn lowers NLRP3 levels and regulates the NOD-like receptor signaling pathway [176]. Additionally, IL-1β has been shown to promote expression of piRNA mmu_piR_037459 in chondrocytes, suggesting that inflammatory factors may drive osteoarthritis (OA) pathology by regulating piRNA levels [177]. It can thus be concluded that piRNAs also have an important role in the inflammatory response (Table 5).

Table 5. The role of piRNAs in inflammation.

Particular year	piRNA	Expression	Research target	Finding	References
2024	hsa-piR-3411、hsa-piR-24541、hsa-piR-27080、hsa-piR-28104、hsa-piR-32157 and 10 others	Upregulation	Human peripheral venous blood	Identification of piRNAs in the plasma of CP patients demonstrated that piRNAs are associated with inflammation.	[170]
	hsa-piR-32835、hsa-piR-32836、hsa-piR-32986、hsa-piR-33168	Downregulation			
2022	piRNA-6426	Downregulation	Rat cardiomyocytes、rats	Inhibits secretion of inflammatory factors IL-1β and TNF-α, cardiomyocyte apoptosis, oxidative stress, and improves the inflammatory microenvironment in heart failure.	[172]
2023	piR-has-27620、piR-has-27124	Upregulation	Blood samples	Identification of peripheral leukocyte piRNAs expression and their enrichment in Rap1, PI3K-Akt, and MAPK pathways as RA biomarkers.	[178]
2021	rno-piR-017330	Upregulation	Endothelial cells、rats	Identification of piRNAs expression in endothelial cells under inflammatory conditions suggests that piRNAs may regulate inflammatory processes.	[171]
2024	hsa_piR_019949	Downregulation	C28/I2、SW1353	Inhibition of NEAT1 and NLRP3 expression regulates the NOD-like receptor signaling pathway and modulates OA progression.	[176]
2024	mmu_piR_037459	Upregulation	Mice cardiomyocytes、mice	Inhibition of collagenase II expression, promotion of chondrocyte apoptosis and inhibition of proliferation, inhibition of USP7 expression, and regulation of OA progression.	[177]
2024	piR-112710	Downregulation	Mice cardiomyocytes、mice	Inhibits the Txnip/NLRP3 signaling pathway, reduces the levels of IL-18, IL-1β, and NLRP3, inhibits cardiomyocyte injury, and regulates inflammation progression.	[174]
2025	pir-has-216911	Upregulation	HL7702、Huh7、HepG2、Hep3B、nude mice	Inhibition of the TLR4/NFκB/NLRP3 inflammatory signaling pathway suppressed the inflammatory response.	[173]
2020	piRNAs	Differential expression	Sudani duck	The composition of piRNAs in brain and lung was characterized,	[179]

	suggesting that they may be associated with lung inflammation.

Although the mechanisms by which piRNAs regulate inflammatory responses are gradually being elucidated, their specific role in mastitis—a common inflammatory condition of the mammary gland—remains unreported. Mastitis exhibits pathological similarities to other systemic or organ-specific inflammatory diseases (e.g., arthritis, pneumonia), providing a useful theoretical foundation for investigating shared mechanisms. First, mastitis and other inflammatory diseases share a similar immune microenvironment, characterized by the infiltration of inflammatory cells such as monocytes, dendritic cells, and macrophages that release reactive oxygen species (ROS) and proteases, thereby exacerbating tissue damage [180]. Second, key pro-inflammatory cytokines—namely, TNF-α, IL-1β, and IL-6—are elevated in mastitis, driving the inflammatory response and contributing to tissue destruction [65,181]. Third, mastitis shares essential inflammatory pathways such as NF-κB, the NLRP3 inflammasome, and MAPK with other inflammatory conditions [182–184]. Therefore, although the role of piRNAs in mastitis remains ambiguous, their established functions in regulating cytokine secretion, NLRP3 inflammasome expression, and NF-κB signaling in other inflammatory disorders suggest that piRNAs may similarly influence mastitis progression.

In light of the functions of piRNAs in breast cancer progression and inflammatory responses, a comprehensive analysis was conducted regarding the potential roles of piRNAs in mammary gland development and mastitis (Figure 3). This investigation offers new insights into the advancement of animal husbandry.

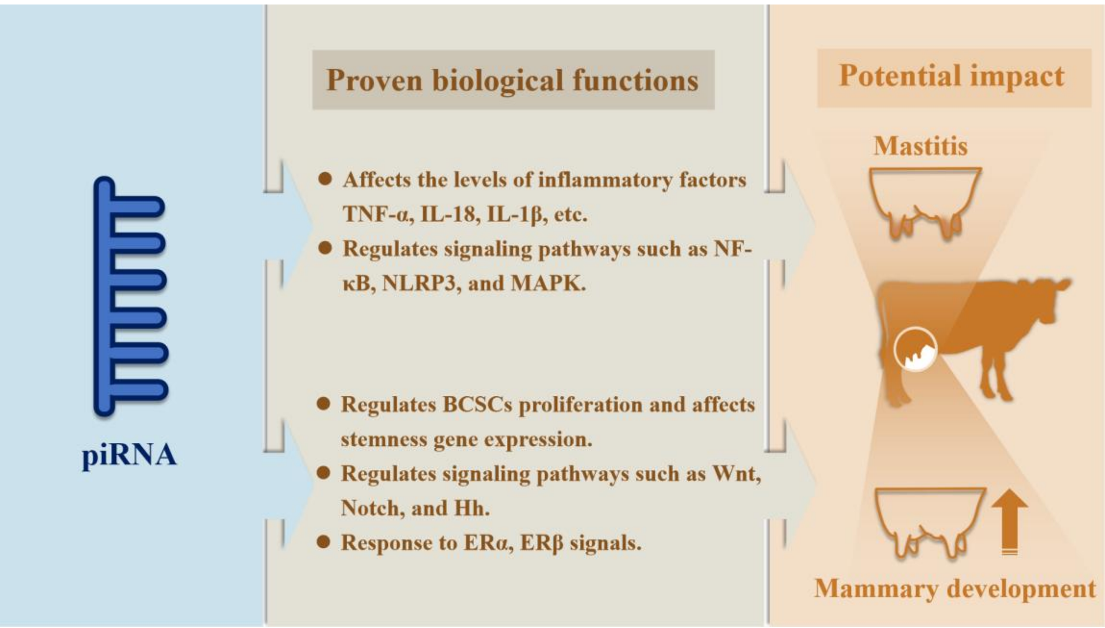


Figure 3. Possibility of piRNA in mammary development and mastitis.

7. Conclusions

Livestock animals are economically vital to the global industry, providing a stable, high-quality supply of meat, eggs, and milk, while also playing critical roles in environmental safety, disease prevention, and biosecurity. Despite their importance, research into the roles of piRNAs in livestock remains in its infancy, and systematic investigations are scarce. In this paper, we demonstrate that piRNAs are involved in numerous biological processes—including gamete formation, embryonic development, and disease regulation. Moreover, we propose that these small ncRNAs may influence livestock growth performance, disease resistance, and environmental adaptation through epigenetic mechanisms. A comprehensive analysis of the molecular mechanisms underlying piRNA function in livestock is imperative to optimize breeding strategies, mitigate disease risks, and cultivate high-quality breeds—ultimately contributing to the industrial upgrading and high-quality development of animal husbandry. Notably, this study also proposes a novel mechanism by which piRNAs might regulate the mammary developmental cycle and the pathological process of mastitis, offering new perspectives on enhancing lactation performance and developing innovative disease prevention and control strategies.

Author Contributions: W.Y. and Z.Z.: writing—original draft. W.Y., Z.Z., X.D.: writing—review and editing and literature search. X.D., and Q.H.: proofreading and supervision: X.D., Q.H., AND Z.W.: resources, and funding. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Cattle Agro-industry Technology Research System of Shandong Province (SDAIT-09-20), China Postdoctoral Science Foundation（2024M761856）, and Shandong Postdoctoral Science Foundation（SDBX202302020）.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this review, and there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the content of this review.

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