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MicroRNAs as potential biomarkers in ruminant, avian and porcine

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Review Article
Volume: 8, Issue: 1
April 2024
Pages: 54-63

ABSTRACT

In recent years, studies on microRNAs have increased considerably. miRNAs are small RNA molecules, ranging from 19 to 25 nucleotides in length, that control the suppression of target genes after transcription. MiRNAs serve as fine-tuning factors that influence the expression of up to 60% of all mammalian protein-coding genes. Unlike proteins, miRNA sequences are widely conserved across species. This conservation strongly suggests that miRNAs emerged early in evolution and maintain their functional importance. It has been revealed that these small structures containing a small number of nucleotides can act as critical points in the organism. While traditional cancer biomarkers are mainly produced by tumor tissues or normal embryo tissues, they are absent or present in small amounts in tissue organs and the blood of healthy adults. MiRNAs can be easily detected in the blood, making them selectable candidates as biomarkers for disease. The ruminant family, one of the most diverse subspecies of terrestrial mammals, lives in a wide variety of environments worldwide and is known to have a major impact on various ecosystems and industries, including agriculture, daily activities, and cultures. MiRNAs have a significant impact on the physiology of farm animals, biological development, and cell differentiation. In this review, we will examine miRNAs that have been identified as candidates or potential candidates for the diagnosis and treatment of diseases seen in ruminants, pigs, and avians in recent years. In this way, we will provide a perspective to prevent diseases that can cause great economic losses in veterinary medicine and the production industry.

Article History
Received: 27.01.2024
Accepted: 16.04.2024
Available online:
23.04.2024

Keywords: biomarkers, micro RNA, veterinary medicine

DOI: https://doi.org/10.30704/http-www-jivs-net.1426005
To cite this article: Kalaycılar, İ. B., & Yardibi, H. (2024). Importance MicroRNAs as potential biomarkers in ruminant, avian and porcine. ***Journal of Istanbul Veterinary Sciences, 8*(1), 54-63.** **Abbreviated Title*: J. İstanbul vet. sci.***

Introduction

Family numbers of miRNAs have exhibited significant growth in recent years. MiRNAs, which are RNA molecules with a length of 19 to 25 nucleotides, regulate the inhibition of specific genes following transcription (Lu and Rothenberg, 2018). MiRNAs are short, naturally occurring RNA molecules that are involved in regulating gene expression after the process of transcription. They are around 22 nucleotides long and function by binding to certain areas on mRNA molecules that are complementary to them. This binding leads to the repression of translation, preventing the target mRNA from being translated into protein. Each miRNA has several messenger RNAs (mRNAs) as its targets, and each mRNA has multiple miRNAs that bind to it. MiRNAs function as precise regulators for approximately 60% of all protein-coding genes in mammals. miRNA sequences exhibit a high degree of conservation across several species, in contrast to proteins. The evidence strongly indicates that miRNAs appeared early in their evolutionary history and have retained their functional significance (Antunes et al., 2020). These tiny

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structures, composed of a limited number of nucleotides, have been found to function as crucial focal areas inside the organism. Although tumor tissues or normal embryo tissues primarily generate traditional cancer biomarkers, these biomarkers are either absent or found in minimal quantities in the tissue organs and blood of healthy adults. MiRNAs may be readily identified in the bloodstream, rendering them viable contenders as biomarkers for illnesses (Wang et al., 2018). Our approach involves examining the miRNAs of ruminant pigs and avian species, which have been identified as diagnostic or treatment biomarkers, or have the potential to be biomarkers, in recent years based on veterinary medicine guidelines.

Ruminant

The ruminant family, one of the most diverse subspecies of terrestrial mammals, lives in a wide variety of environments worldwide and is known to have a major impact on various ecosystems and industries, including agriculture, daily activities, and cultures (Mendes, 2012). The ability of this group of animals to survive and reproduce on low-quality, low-protein, and high-fiber plant sources is an important factor in their success. Many of the various stakeholders believe that animal health is crucial to livestock production. However, there is disagreement among consumers, farmers, and veterinarians about what constitutes an acceptable health status (Ojo & Kreuzer-Redmer, 2023). Cell differentiation, biological development, and the physiology of farm animals are significantly affected by miRNAs (Wang et al., 2013). These processes include controlling muscle growth and hypertrophy, adipose tissue expansion, oocyte maturation, and early embryonic development. Recent studies have revealed the critical roles of miRNAs in sheep (Hou et al., 2018), goat (Zhong et al., 2020), and cattle rumen development as well as maintenance of intestinal homeostasis (Liang et al., 2014; Do et al., 2019).

Cattle

In a study, it was stated that mastitis, which is inflammation of the udder, is a challenging problem that causes high economic losses in dairy animals, and the complexity of the disease, the degree of economic losses, and the increasing importance of the dairy industry, together with public health concerns, necessitate the design of appropriate mastitis diagnostics that can provide rapid, accurate, and confirmatory diagnosis. It has been suggested that miRNAs may be potential biomarkers in the diagnosis of bovine mastitis (Li et al., 2015; Chakraborty et al., 2019). Again, in line with the results obtained in his study (Srikok et al., 2020), when MIR29B-2 is used together with the California mastitis test (CMT) and the number of days in milk (DIM) data, it is possible to screen and classify milk samples taken from cows as healthy, subclinical mastitis, or mastitis. This is applicable. It has been demonstrated that it appears to have sufficient discriminatory power to enable it to be used as a biomarker in cases where the condition of the milk sample cannot be determined based on CMT results.

In another study conducted by Lai et al., 2021) to detect bovine mastitis, although the sensitivity and specificity of miR-21 in serum were lower than miRNA biomarkers in milk, the significant increase of miR-21 in serum could reflect the effect of local inflammation on the systemic reaction.

Subclinical mastitis, that is, inflammation of the mammary gland without clinical symptoms, is known as one of the most common and costly diseases in dairy farming worldwide. Milk miRNAs encapsulated in extracellular vesicles (EVs) are proposed as potential biomarkers of diseases, including subclinical mastitis. In a study by Saenz-de-Juano et al. (2022), they looked at naturally infected and healthy mammary glands for subclinical mastitis. They found that bta-miR-223-3p was the miRNA that was most highly controlled in all of the individuals.

Given the importance of early disease detection to reduce the major financial and animal welfare impact of bovine mastitis worldwide, improved tools that can accurately detect early mastitis are urgently needed. The value of miRNAs as disease biomarkers has been demonstrated, but their potential to accurately detect early mammary inflammation has not been studied in detail. The study said that the rise in btamiR-223 and bta-miR-142-5p levels in the early stages shows that miRNAs can be useful as early diagnostic biomarkers of mastitis in cattle (Tzelos et al., 2022).

Studies examining the underlying molecular mechanisms associated with mastitis in high milk-producing dairy cattle by analysis of milk-released exosomal miRNA show that key target genes, including significantly downregulated miR-375, CTLA4, IHH, IRF1, and IL7R, can be upregulated. These genes are negative regulators of immune response pathways that may be associated with impaired inflammatory mechanisms in breast cells. In light of this information, a study conducted in dairy cattle stated that bta-miR-375 may be a promising biomarker for the development of mastitis in dairy cattle (Mahala et al., 2024).

In the review on the potential use of miRNAs in tissue and/or circulation as biomarkers in the assessment of health and welfare status in animal species, it was revealed which miRNAs control stress, immunity, milk, genetic infrastructure, management, and environment, which are some of the most important factors in animal welfare (Miretti et al., 2020).

Bovine milk and colostrum provide essential nutrients and immunologically active factors that are beneficial to a newborn calf. Exosomes derived from milk and colostrum are known to be the most important for cellular communication. Researchers looked for and described exosomal miRNA in the milk and colostrum of Holstein and East Anatolian Red (DAK) cows. They found 795 miRNAs that were expressed differently in the two groups of cows. A total of 545 of these were determined to be known miRNAs and 260 were new miRNAs, and it was stated that the data obtained from this study will make useful contributions to potential miRNA biomarker studies (Özdemir, 2020).

In a research project investigating embryonic development in cattle, significant downregulation of several DNA damage response (DDR) genes tested after increasing miR-30c or decreasing CDK12 expression suggested a possible role of miR-30c in regulating embryonic development through DDR pathways (Lin et al., 2019).

Extracellular vesicles (EVs), found in various biological fluids and especially reproductive fluids, have attracted considerable attention due to their possible role in cell-to-cell communication. Among the different bioactive molecule cargos of EVs, miRNAs are emerging as promising diagnostic biomarkers with high clinical potential. In line with this information, the presence of large amounts of miRNA in the fluids of the bovine reproductive system was revealed. 310 of these were characterized in oviduct fluids and 351 in uterine fluids. In this study, which was characterized and also looked at the level of differentiation, it was revealed that miRNAs play an important role in cell signalling, intercellular junctions, and related pathways such as reproductive functions that may play a role in the modulation of the oviduct and uterus throughout the cycle, as well as in the processes of embryonic existence and development (Hamdi et al., 2021).

MiRNAs modulate male fertility by regulating gene expression. Menezes et al. (2020) used RT-qPCR to look at the changes in sperm miR-15a, miR-29b, and miR-34a from bulls that were high fertile (HF) and low fertile (LF). MiRNA levels of miR-15a and miR-29 were found to be higher in LF sires compared to those present in HF sires. Finding miRNAs in the spermatozoa of fathers with different levels of in vivo fertility will help us understand how the ability to fertilize changes in cattle and other mammals. These possible biomarkers can be used as fertility markers in reproductive biotechnology to check the quality of semen and guess if a male will be fertile.

They looked at how miR-224 might help control adipogenic differentiation in bovine preadipocytes in their study (Zhang et al., 2019). Comparative transcriptome analysis between castrated male cattle with increased intramuscular fat (IMF) and intact male cattle revealed that miR-224 and lipoprotein lipase (LPL) were aberrantly expressed and negatively correlated, and LPL was the predicted protein of miR-224. They revealed that this was their goal. When miR-224 was overexpressed or silenced, qRT-PCR showed a negative regulatory effect on LPL. The mRNA expression levels of fat formation-associated biomarkers C/EBPα, C/EBPβ, PPARγ, FASN, and PLIN1 decreased when miR-224 was overexpressed, while the opposite effect occurred when miR-224 was inhibited, which was followed by adipogenic differentiation. All together, the results show that miR-224 controls the adipogenic differentiation of pre-adipocytes in cattle by focusing on LPL. It was concluded that this provides insight into the molecular basis of IMF accumulation in cattle.

The presence and biodegradable potential of miRNAs in other biofluids have been less studied, especially in the veterinary field. External miRNAs are an example of this; they are largely identified in rats and humans but have not been in cattle. As a result of research (Shaughnessy et al., 2020) in cattle, they have discovered that hsa-miR-548d-5p, hsa-miR-2113, and hsa-miR-1244 could be a potential bioeffect for diagnosing chronic granulomatous inflammation of the colon caused by Mycobacterium avium subspecies paratuberculosis (MAP).

Endometritis, seen as inflammation of the endometrium, can affect fertility and is known to cause serious economic losses in the dairy industry. Exosomes and exosomal miRNAs, which are widely found in various tissues and body fluids, have been shown to play an important regulatory role in immune responses. However, with the study on the mechanisms by which miR-218 regulates the release of cytokines and chemokines in endometritis, MiR-218 has been stated to be a potential biomarker for the detection of endometritis (Wang et al., 2020).

During a research study involving Piedmontese, which have double muscle groups in beef cattle breeding, miRNAs expressed in skeletal muscle were analyzed. miR-10b, miR-126-5p, miR-143, and miR-146b were found to be significantly up-regulated, while miR-21-5p, miR-221, miR-223, and miR-30b-5p were found to be significantly down-regulated. It has been reported that miR-23a is expressed at high levels in all groups created from these miRNAs in animals (Tewari et al., 2021).

As reported in the research by Huang et al. (2022), two small RNA libraries from fatty (S01) and normal livers (S02) from Holstein DairyCow (HDC) were analyzed by deep sequencing. A total of 12,964,411 and 15,426,289 clean reads were obtained, representing 370 known and 182 novel miRNAs, respectively. As a result of the analysis, 66 upregulated and seven downregulated differentially expressed miRNAs (DIE-miRNAs) were identified. DIE-miRNAs showed that ethyl lipid metabolism and cocaine addiction are closely related to liver metabolism. It has been reported that these findings will provide valuable information for further functional validation of miRNAs between normal and fatty livers, as they may benefit new attractive miRNA biomarkers for disease detection in HDC.

A study in Japanese black cows compared the circulating miRNAs of non-pregnant and pregnant animals, investigated miRNAs as biomarkers for early pregnancy diagnosis, and established a measurement system that included selecting an appropriate reference miRNA and determining the effect of hemolysis on miRNA measurement. By using microarray analysis, both pregnant and non-pregnant cows' plasma contained a total of 124 miRNAs. Levels of five circulating miRNAs were detected to be significantly higher in pregnant cows than in non-pregnant cows. 24 miRNAs were detected only in the pregnant group. showed that miR-2455 was a suitable reference miRNA in the plasma of non-pregnant and pregnant Japanese black cows, and miR-19b, miR-25, miR-29a, and miR-148a were significant reference miRNAs. In this study, four miRNAs, miR-19b, miR-25, miR-29a, and miR-148a, were detected at high levels in the plasma of pregnant Japanese black cows. Since these miRNAs are less affected by hemolysis, it has been demonstrated that they can potentially be used as biomarkers for early pregnancy diagnosis in cattle (Ono et al., 2022).

Senecio spp. It is known to be one of the most common plant-related poisonings in cattle. In a study that performed miRNA profile analysis to determine potential diagnostic biomarkers for Senecio brasiliensis poisoning, it was reported that miR-122, miR-885, and especially miR-21 could be potential biomarkers with high specificity and sensitivity in the detection of poisoning (Winter et al., 2022).

An investigation was carried out on the circulating expression levels of miRNAs that might serve as potential candidates for early pregnancy diagnosis in pregnant and near-pregnant heifers. The study showed that the Let-7d-5p miRNA could be used as a sign of early pregnancy and give information about how the fetus and cow's cells and molecules interact in the early stages of pregnancy. It has been demonstrated that it is of critical importance (De Los Santos Funes et al., 2023).

Subacute ruminal acidosis (SARA) is known to be a metabolic disease frequently seen in dairy cows fed with high-yield, concentrated feeds. A pilot study was conducted by Ojo et al. (2023) to determine that miRNAs circulating in the blood of cows could be used as potential biomarkers to detect animals with metabolic disorders such as SARA. According to the results of the study, bta-miR-30b-3p and bta-miR-2285 in the bloodstream were found to be good candidates for biomarkers that could help diagnose SARA.

It is known that endometritis is one of the most important diseases causing infertility in mammals. A study that looked at the endometrial transcriptome and miRNA profiles in cattle with clinical and subclinical endometritis disease found that the levels of miR-146a and miR-223 expression profiles went up significantly. As a result of this development, it has been stated that the relevant miRNAs can be safe biomarkers to distinguish between healthy and endometritis cattle (Shokri et al., 2023).

Sheep

Breeding stress-tolerant animals and mitigating the negative effects of environmental and pathogenic stress factors is a potential strategy to aid the health of ruminants. In the study conducted by Naylor et al. (2020), stress was administered to 15 female lambs with an intravenous bolus of lipopolysaccharide (LPS; 400 ng/kg) to evaluate a comprehensive set of circulatory mediators released in response to acute immune stress to identify candidate biomarkers that can be used in the selection of stress-resistant animals. Stress was applied, and blood was collected from the carotid artery at 0, 2, 4, and 6 hours after the LPS challenge to identify and monitor candidate stress biomarkers. The temperature was also recorded over time. Biomarker responses were evaluated with a repeated measures model to compare time points with baseline values, and miR-145, miR-233, and miR-1246 expression levels increased and remained high during the study.

In the study that analyzed potential miRNAs participating in metabolism with extracellular vesicles in the presence of inflammation in sheep, it was shown that miR-26a-5p, among miRNA regulators in the biological process of inflammation, may have an important role in the resolution of inflammation (Ciliberti et al., 2023).

In a study to detect miRNA expression in the proteom of the early antral follicles in sheep, based on in silico analyses, specific miRNAs associated with genes corresponding to the most abundant proteins in the sheep follicle (VIM, LMNA, ACTB, and HSPA5) were studied, and five key mIRNS potentially linked to the oocyte mayosis (hsa-let-7b-5p; hsa-mir-221-3p; Hsamir -17-5p; HS-mir-24-3p and Hsa-Mir-107) were identified (Otávio et al., 2023).

Avian

Stress-induced immunosuppression is one of the serious threats to the poultry industry, especially evident for young chickens. In a study conducted to investigate the molecular mechanism of stress-related immunosuppression in chickens, dexamethasone (Dex) was injected into the chicken thymus to suppress the immune system of chickens. Then, miRNA expression analysis was applied to the thymuses. As a result of the analysis, it was stated that the differentially expressed miRNAs ggamiR-2954, gga-miR-146b-3p, gga-miR-106-3p, and gga-miR-214 would provide a basis for revealing the molecular regulation mechanism of immunosuppression in poultry (Zhou et al., 2019).

Marek's disease virus (MDV) is known to be the causative agent of Marek's disease (MD), a complex pathology characterized by paralysis, immunosuppression, and T-cell lymphogenesis in chickens. MD is controlled in poultry production through in-egg or incubation-administered vaccines, and these practices have been shown to protect against lymphoma formation but not superinfections caused by MDV field strains. In the study, where full transcriptomic and proteomic analyses of chicken serum exosomes obtained from commercial leghorn and broiler Marek's disease virus vaccine studies were performed, it was stated that cellular cargo miRNAs showed different patterns in vaccinated chickens, suggesting that miRNAs target the MAP kinase, cellular proliferation pathway. It has been reported that the miRNA set in unvaccinated tumor chickens showed significant targeting of phosphoinositol signaling. As a result of these comprehensive analyses, bioinformatic analyses of miRNAs and predicted miRNA targets showed that there were more tumor suppressor miRNAs in VEX compared to TEX (Neerukonda et al., 2019).

Coccidiosis caused by Eimeria spp. infection in broiler chickens continues to be one of the most important diseases in terms of production and economy today. Considering that the development of a genetic biomarker panel for subclinical infection will be an important biological tool for the management of broiler flocks, the study by Giles et al. (2020) analyzed the expression of miRNAs to determine their potential in diagnosing coccidiosis in broiler flocks. As a result of analyses conducted clinically or among chickens infected with Eimeria maxima and Eimeria acervulina, Gallusgallus (gga)-miR-122-5p, gga-miR-205b, and gga-miR-144-3p showed that they can be used in the diagnosis of subclinical coccidiosis.

In the study conducted by Chen et al. (2022), the expression levels of circRNAs, miRNAs, and mRNAs in the spleen obtained from commercial dual-purpose Sasso T445 breed chickens infected and uninfected with Eimeria tenella were analyzed. In conclusion, among these, it has been shown that circMGAT5 can inhibit the activation of macrophages through the circMGAT5-miR-132c-5p-MMD (macrophage differentiation-associated) axis to participate in the immune response caused by Eimeria infection.

Circular RNAs (circRNA) are molecules that result from backsplicing events that connect a downstream 5' splice site to an upstream 3' splice site. Little is known about the potential pathogenesis-inducing function of circRNAs in chickens. circDNAJB6, found in chickens, is a stable and newly conserved circular RNA that is mainly expressed in the stomach, lung, spleen, and thymus. In light of this information, the study conducted by Tan et al. (2024) investigated the function of circDNAJB6, which can serve as potential biomarkers and act as potential targets for the treatment of bacterial infection. It has been found to interact with five miRNAs: 3p, gga-miR-1306–5p, gga-miR-6549–5p, and gga-miR-1684a–5p.

Young peregrine falcons (Falco peregrinus), which are wild birds, were studied to see how the endocrine system and immune system are connected. The study found that exposure to perfluoroalkyl acids (PFAAs) has big effects on both systems, and the researchers wanted to look at these effects in terms of miRNAs. As a result of the analyses, plasma miRNA-155 counts were identified to have significant negative relationships with PFAAs concentrations (Sun et al., 2021).

Porcine

Zearalenone (ZEN), a mycotoxin with estrogenic effects, is known to pose a risk to animal health. It is not easy to detect and diagnose the disorders caused by this effect. For this reason, in a study conducted to investigate the effects of ZEN on pigs, miRNA expression profiles in pig jejunum and serum were examined. As a result of the investigations, it was stated that ssc-miR-135a-5p, ssc-miR-432-5p, ssc-miR-542-3p, and ssc-miR-493-3p profiles may be effective when comparing pigs exposed to ZEN and pigs not exposed to ZEN (Grenier et al., 2019).

Research on miRNAs and porcine oocyte maturation and the molecular mechanisms behind this complex process has not been fully elucidated. To elucidate these mechanisms, in the study conducted by Hu et al. (2020), exosomal miRNA was obtained from porcine follicular fluid (PFF), and the differentiation levels of miRNAs were examined. As a result of the analyses, it was reported that miR-125b, let-7d-5p, miR-200b, miR -26a, and miR-92a may be potential biomarkers for the molecular identification of high-quality oocytes.

Early pregnancy diagnosis in sows, which can greatly increase the efficiency of the swine industry, is an important issue that needs to be examined and researched. Regarding this, (Zhou et al., 2020) examined the expression levels of miRNAs released into the bloodstream through exosomes in their study. They identified a large number of miRNAs in twenty-eight different ways between the pregnant and control groups. As a result, they successfully determined the exosomal miRNA profiles circulating in the serum of pigs in the early period of pregnancy and showed that miR-92b-3p and miR-17-5p can be used as potential circulating biomarkers for early pregnancy diagnosis.

It is known that impairment in fetal skeletal muscle growth developing in the uterus can lead to decreased birth weight and decreased carcass quality in pigs. Recently, their research showed the part that miRNAs and the genes they target play in the development of skeletal muscles during pregnancy and in the cause of intrauterine growth restriction (IUGR) (Ali et al., 2021b). In this study, they performed miRNA analysis in the longissimusdorsi muscle (LDM) of the pig. As a result, miR-140, miR-186, miR-101, miR-15, miR-24, miR-29, miR-449, miR-27, miR-142, miR-99, miR-181, They identified 13 miRNAs significantly associated with fetal weight, including miR-199 and miR-210. They reported that the miRNA profile of skeletal muscle can be used to predict fetal weight, and fetal weight-related miRNAs can serve as potential biomarkers of prenatal fetal health and growth (Ali et al., 2021a).

In a study to measure the sperm quality of sexually mature pigs, samples of seminal plasma surrounding the sperm were studied. miRNA expression profiles were examined in extracellular vesicles (SP-EV) obtained from seminal plasma. ssc-miR-205, ssc-miR-493-5p, and ssc-miR-378b-3p, which are expressed differently in high-quality and low-quality semen, are related to cellular localization (nuclear and cytosol) and molecular functions (acetylation, Ubl conjugation, and protein kinase). It has been revealed that it potentially impairs sperm quality by allowing gene targeting (Dlamini et al., 2023).

Porcine reproductive and respiratory syndrome (PRRS) is a serious infectious disease in the swine industry and is known to cause serious economic losses in current swine production worldwide. It is clear that there is no effective anti-viral strategy to prevent this disease. A study that tried to find a solution to this problem found that PRRSV infection lowers the expression of ssc-miR-124a and that ssc-miR-124a has a strong antiviral effect by decreasing the expression of CD163. Overall, these results suggest that ssc-miR-124a may be an important therapeutic target for the development of new anti-PRRSV therapies (Li et al., 2021).

Among the miRNAs examined in the porcine cardiopulmonary bypass model, which was created to investigate the protective effects of polarized cardioplegia (STH-Pol-B) and hyperkalemic cardioplegia (STH2-B) after cardiac surgery, miRNA-708-5p was significantly lower in STH-Pol-B hearts (P =.019) and miRNA-122 expression was found to be significantly higher (P =.046). In line with these results, it is stated that polarized and depolarized arrests are not superior to each other and confirm their potential (Santer et al., 2019). In a similar study, regarding the relationship between cardioplegia and temperature, only ssc-miR-451 was expressed differently between STH2-hot and STH2-cold, and these data were reported for the first time that cardiopulmonary bypass and the temperature of the cardioplegic solution affected the expression of miRNAs in the left ventricular tissue. shows. Consequently, specific miRNAs have been reported as potential therapeutic targets to limit ischemia-reperfusion injury in patients undergoing cardiac surgery (Kiss et al., 2020).

Clinical signs are typically how veterinarians diagnose inflammation in pigs. However, it is known that this method is not very reliable due to subjective clinical interpretations. In a study conducted to diagnose this inflammation response with miRNAs at the molecular level, inflammation was induced in pigs with Escherichia coli lipopolysaccharide (LPS). For inflammation responses, whole blood samples were taken from pigs after 0, 1, 3, and 8 hours, and miRNA expression levels in plasma were measured. As a result of the investigations, they revealed that the expression of ssc-let-7e-5p, ssc-mir-22-3p, and ssc-miR-146a-5p are the miRNAs that change most significantly over time (Swain et al., 2021).

In the study conducted by Bilinska et al. (2023), eight miRNAs (miR-21a, miR-26b, miR-30a, miR-92a, and miR-146a) were detected in pork in vitro in order to understand the importance of extracellular miRNAs in cell differentiation processes. MiR-148a, miR-199, and miR-383a were determined. Adipogenesis analysis revealed that miRNAs (miR-21a, miR-92a), especially those associated with the inflammatory process, were expressed at high levels in differentiated adipocytes and were also secreted by the cells. These findings may provide an important basis for understanding the role of extracellular miRNAs in cellular differentiation processes, and they have been reported to provide important guidance for the discovery of potential therapeutic and diagnostic targets.

African swine fever (ASF) is known to be a serious viral disease in domestic and wild pigs with a high mortality rate, for which no effective vaccine or antiviral drug is available (Dixon et al., 2019). MiR-451 is one of the most representative miRNAs described to present upregulation in ASF-infected pigs 3–7 days after infection (Núñez-Hernández et al., 2017; Pang et al., 2023). In the study for the diagnosis of this disease, a colorimetric detection strategy was aimed at the detection of an ASF-related miRNA based on isothermal rolling circle amplification (RCA) and salt-induced gold nanoparticle aggregation developed by Chi et al. (2024). As a result of the experiments, it was stated that its repeatability was satisfactory. This newly developed method has been reported to be successfully applied to the detection of ssc-miR-451 in pig serum samples.

Metabolites and miRNAs in plasma were looked at as possible biomarker candidates in a pilot study to learn more about molecular pathophysiology in a minipig model with the goal of finding lethal radiation early. The pilot study analyzed changes in miRNA and metabolites immediately after total body irradiation (TBI). As a result, it has been shown that 92 miRNAs can be potential biomarkers in the early diagnosis of radiation (Chakraborty et al., 2023).

The molecular mechanisms of deoxynivalenol (DON), which is a common mycotoxin worldwide and affects human and animal health, in living organisms have not been fully investigated. In the study conducted by Segura-Wang et al. (2021), the effects of this toxin on piglets were found to be associated with miRNA expression responses. As a result of the data obtained, only slight changes in miRNA expression were detected in the liver tissue of pigs, and ssc-miR-10b was down-regulated in the liver of piglets exposed to DON.

In the research undertaken by Lecchi et al. (2020), it was investigated whether the acute pain associated with castration and tail docking of male piglets could modulate the expression of salivary miRNAs, and the results obtained showed that miR- in the saliva of piglets castrated and tail docked without the application of painkillers. They showed that 19b, miR-27b, and miR-365 increased significantly. They stated that research on this subject should be expanded so that the detected miRNAs can be used as potential biomarkers.

In the study conducted by Li (2021), the place and importance of miRNAs in this production mechanism in the development of back fat in Landrace and Neijiang pigs were investigated. Eleven miRNAs that are unique to Landrance pigs and thirty-five miRNAs that are unique to Neijang pigs were identified among the miRNAs that were screened. It was reported that miR-1-3p can regulate lipid accumulation and synthesis and may serve as a potential marker for pig breeding as a consequence of the study.

It is known that intramuscular fat (IMF) content is a very important parameter in evaluating pork quality. Studies on this subject show that miRNAs play a role in IMF accumulation. In this research, a new miRNA that is involved in IMF adipogenesis in pigs was looked at in terms of the growth and differentiation of pre-adipocytes inside the muscle. As a result of the study, miR-146a-5p was determined to target SMAD4 and TRAF6 to inhibit porcine intramuscular adipogenesis by attenuating TGF-β and AKT/mTORC1 signaling, respectively. These findings imply a new miRNA biomarker that regulates intramuscular adipogenesis to improve pork quality (Zhang et al., 2021).

Conclusion

In overall, the rapidly expanding domain of miRNA investigation has unveiled the crucial function that these diminutive RNA molecules perform in controlling the expression of genes in numerous species. The extensive preservation of miRNA sequences indicates that they originated early in the course of evolution, highlighting their fundamental significance. Due to their capacity to regulate the expression of a considerable number of protein-coding genes in mammals, miRNAs are indispensable participants in cellular processes. MiRNAs are exceptionally promising candidates for disease biomarkers due to their distinctive properties, especially in the context of avians, swine, and ruminants. In contrast to conventional cancer biomarkers, miRNAs are readily detectable in the bloodstream, providing a non-invasive and conveniently accessible diagnostic approach. This has the capacity to prevent diseases that present substantial economic risks to the veterinary medicine and diverse manufacturing sectors. The review explores the effects of miRNAs on the cell differentiation, homeostasis, and biological development of farm animals belonging to the diverse ruminant family. Through the identification of particular miRNAs that are linked to diseases in these animals, scientists have the potential to establish diagnostic and therapeutic interventions. The ramifications transcend the domain of veterinary medicine, exerting an impact on sectors including agriculture and advancing knowledge regarding the complex interconnections between miRNAs and the well-being of various species. This overview provides an extensive examination of the latest developments in the identification of miRNAs for the purpose of diagnosing and treating diseases that impact important livestock species. The results of this study not only provide insights into the possible uses of miRNAs in the field of veterinary medicine, but also underscore their wider importance in protecting the well-being and financial sustainability of sectors that depend on these creatures.

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