Blood microRNAomes profiling reveals signatures of lameness phenotypes in feedlot cattle

by

Wentao Li

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Animal Science

Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

Maintaining the health of feedlot beef cattle is essential because diseases are directly associated with economic losses and animal welfare. Lameness is an important health issue in feedlot cattle. However, the molecular mechanisms behind cattle lameness have not been well studied. Moreover, there are different types of lameness with various causes, and visual diagnosis methods may not differentiate them, leading to misdiagnosis and ineffective treatment. Recent evidence has demonstrated that circulating microRNAs (miRNAs) in biological fluids can reflect the changes in the physiological status and biological processes in the tissues/organs, which can be used as biomarkers for disease diagnosis. Therefore, this thesis research aimed to characterize miRNA profiles in the whole blood of beef cattle using RNA-sequencing and to determine whether they were different between healthy (HC) and lame beef cattle with different lameness phenotypes, including digital dermatitis (DD), foot rot (FR), toe tip necrosis (TTN) and foot rot & digital dermatitis combined (FRDD). In the first study (Chapter 2), although profiles of blood total miRNAs were not significantly different between healthy and lame cattle, 4 and 12 miRNAs were exclusively expressed in healthy and lameness phenotypes, respectively. In addition, 3, 7, 6, and 14 miRNAs were differentially expressed (FDR < 0.05, and $\log_2$ fold change < -1 or > 1)) in the blood of DD, FR, TTN, and FRDD cattle when compared to that of healthy cattle. Further RT-qPCR validation analysis of 6 selected miRNAs, including three lameness phenotype-specific miRNAs (DD-specific: bta-miR-2904; FR-specific: bta-miR-200a; and TTN-specific: bta-miR-483) and three differentially expressed miRNAs (bta-miR-6119-3p, down-regulated in all

lameness phenotypes; bta-miR-133a, up-regulated in the FR and TTN group; and bta-miR-1, up-regulated in the TTN group) confirmed that bta-miR-133a was highly expressed in the TTN group compared with HC and bta-miR-483 was highly expressed in the TTN group compared with FR as detected based on RNA-seq. Moreover, predicted functions of all differentially expressed miRNAs revealed that they were involved in functions of inflammation response and muscle cell development. The second study compared the temporal expression of miRNAs in the whole blood collected for three weeks after lameness diagnosis and treatment (DD, TTN, and FRDD) to identify the relationship between miRNA temporal expression changes and lameness recovery patterns. The comparison of blood miRNA profiles between recovered and unrecovered lame cattle revealed that some miRNAs (bta-miR-1 and bta-miR-206 in the DD and FRDD groups, respectively) were only expressed in the blood of unrecovered cattle and vice versa. The predicted functions of those uniquely and differentially expressed miRNAs in unrecovered cattle were mainly related to bacterial infection and muscle cell self-repair. Further time-series analysis revealed miRNAs with specific expression-changing patterns over three weeks following the first assessment for each lameness phenotype. The miRNAs identified in the patterns may involve functions related to infection and inflammatory response during the treatment period. In summary, the comparative miRNAome analysis revealed that the blood miRNAs might affect the functions related to muscle and immune functions of cattle as a result of varied pathogenesis of different lameness phenotypes. The two miRNAs: bta-miR-133a and bta-miR-483, are potential markers for TTN, which warrant future research on their functions and validation using large populations.

Preface

This thesis is an original work by Wentao Li with the collaborations led by Dr. Leluo Guan at the University of Alberta and Dr. Karen Schwartzkopf-Genswein at Agriculture and Agri-Food Canada.

This thesis work includes two animal studies, both received ethical approval from the Lethbridge RDC Animal care committee (Animal Use protocol Review 1817), and the procedures were conducted following the guidelines of the Canadian Council on Animal Care (2020).

Dedication

This thesis is dedicated to my beloved family.

Acknowledgements

First, I would like to show my sincere appreciation to my supervisor Dr. Leluo Guan for providing me with the opportunity to join her research team in September 2019. Innumerable guidance, advice, support, and encouragement from Dr. Guan inspired me in my research. I sincerely appreciate all her help, I learned how to be a strong person in both research and my own life.

I would like to thank Dr. Karen Schwartzkopf-Genswein for being my supervisory committee member and giving me many suggestions in each stage of my study. I am also thankful to Dr. Richard Uwiera for devoting his time to be the chair and arm's length examiner in my thesis defense. I greatly appreciate your time and patience.

I would like to express my thanks to all the members of Dr. Guan's group, it's my honor to study and work with Dr. Mi Zhou, Dr. Eóin O'Hara, Dr. Huizeng Sun, Yanhong Chen, Dr. Nilusha Malmuthuge, Dr. Tao Ma, Dr. Wei Guo, Yajing Ban, Jian Wang, Yixin Wang, Zhe Pan, Sonja Bialobzyski, Dr. Xinfeng Wang, and Dr. Hangshu Xin. I wish to extend my special thanks to my dear friends, Junhong Liu and Dr. Xuechun Bai for sharing your life.

In addition, I would like to say thanks to the researchers from Dr. Karen S. Schwartzkopf-Genswein's group in the Lethbridge research and development center, thanks for the help of Dr. Holtshausen, Nicholaus Johnson, and Dawn Gray.

I gratefully acknowledge the funding support from the Ministry of Alberta Agriculture and Forestry and John Prentice Graduate Scholarship in Beef Genomics that help me finish my studies in Canada.

My deepest appreciation goes to my dear parents Mr. Qingping Li and Mrs. Xiaohui Li. Without their inspirational encouragement and financial support, I could not complete my research work. Finally, I would also like to thank my girlfriend Miss. Zihan Wang for her spiritual support throughout my two years of study.

Table of Contents

List of figures

List of tables

List of abbreviations

ADG: average daily gain

ANOVA: analysis of variance

BRD: bovine respiratory disease

D: downregulated

DAVID: Database for Annotation, Visualization and Integrated Discovery

DAZL: DAZ-like

DD: digital dermatitis

DE: differential expressed

E. coli: Escherichia coli

F. necrophorum: Fusobacterium necrophorum

FDR: false discovery rate

FR: foot rot

FRDD: foot rot & digital dermatitis combined lameness

HC: health control

miRNAs: microRNAs

N: no significant difference

NGS: next-generation sequencing

PCA: principal component analysis

pre-miRNA: precursor miRNA

RE: recovered

RPM: reads per million mapped reads

RT-qPCR: quantitative real-time PCR

TTN: toe tip necrosis

U: up regulated

UNR: unrecovered

W0~W2: week 0~ week 2

Chapter 1. Literature review

1.0 Introduction

Canada is one of the largest beef exporting countries globally (Pogue et al., 2018). Alberta owns the most cattle inventories among all provinces and territories, with 40.4 percent of all Canadian beef cattle and significantly contributes to the Canadian beef industry (Statistic Canada, 2020). Canada's traditional beef cattle operation consists of three parts: cow-calf, backgrounding, and finishing periods (Alemu et al., 2016). Beef cattle spend from sixteen to two hundred days in the feedlot during the finishing period, which is also the main weight-gaining period during their life (Greenwood, 2021). In feedlots, cattle are confined to a narrow place to restrict their movement and fed with a high-grain ration to quickly increase muscle and body fat (Greenwood, 2021). The advantages of this feeding operation are apparent to allow farmers to achieve maximum production efficiency compared with pasture grazing. Moreover, cattle can be checked more frequently and closely monitored for their behaviors and health conditions under this operational setting. However, the long-distance transportation of the herd from pasture to the feedlot (Holman et al., 2017), limited space in one pen that confines numerous cattle, and the rapid dietary change from grass to high grain have been reported to cause health and welfare issues in the feedlot cattle (Scheuer et al., 2010).

Feet health problems in cattle, which are defined as lameness, are usually caused by multiple factors such as long-standing in their feces and urine; the muddy environment makes the cattle hoof moist and weaken the protective function of the epidermis (Davis-Unger et al., 2017a). Lameness is one of the leading health and animal welfare issues that usually happen in the feedlot. Cattle with lameness behave significantly differently compared to healthy ones, such as the arched

back, resistance movement (Whay, 2002a), and reduced food and water intake (Norring et al., 2014a). Consequently, lameness in the feedlot cattle can cause an economic loss because of the poor performance of cattle and additional costs for labor/treatment (Shearer et al., 2013a). Moreover, cattle may suffer from pain for a long time because lameness is a chronic health problem (Babatunde et al., 2019). Currently, the detection of cattle lameness is mainly based on the visual observation of their back and pace, scoring their gaits to assess the severity of lameness, and foot exam to determine the severity of lameness (Qiao et al., 2021a). However, the foot exams are always time-consuming and laborious, some similar lesions even lead to misdiagnosis, which makes these methods defective and inaccurate. Therefore, a novel detection approach is needed to diagnose lameness accurately and effectively.

In recent years, disease detection and prediction at the molecular level have become popular, particularly regarding the regulatory molecules that can affect cellular and tissue functions involved in animal physiology and metabolism. MicroRNAs (miRNAs) are small RNA molecules in eukaryotes that regulate gene expression at the post-transcription level (Wilczynska and Bushell, 2014a). In the last decade, miRNA-based diagnosis has been proposed to detect complicated diseases in humans. There is accumulating evidence suggesting that miRNAs in animal body fluid can be identified as potential biomarkers for specific disease diagnosis, not only in humans but also in livestock animals, including dairy cows (Q. Li et al., 2018a), pigs (Huang et al., 2011) and horses (Unger et al., 2019). However, only a few studies focused on the application of miRNAs as diagnostic tools in beef cattle (McDaneld, 2009). Therefore, the following literature review aims to 1) review the current knowledge of beef cattle production focusing on feedlot production in Alberta, Canada; 2) how lameness affects production; and 3) background information on circulating miRNAs and their applications in disease detection.

1.1 Beef cattle production system

1.1.1 Canadian beef cattle production system

According to Statistics Canada, 1.55 million tonnes of beef had been produced in Canada, and approximately 45 percent of beef products were exported to other countries in 2020 (Greenwood, 2021). Among all provinces and territories in Canada, the beef production industry of Alberta makes a significant contribution to the country, providing over 40 percent of all beef cattle yield (Lee et al., 2017). Moreover, Alberta has the highest portion of beef cattle in Canada accounting for 59.6 % of the breeding stock and 42.3% of the replacement cattle, also producing the highest number of slaughtered cattle in the country (Statistics Canada, 2017).

As described above, the beef cattle production in Canada is typically separated into three different sectors: Cow-calf, backgrounding and finishing. Although these phases are interconnected, most farmers only focus on one process to maximize production efficiency (Pogue et al., 2018). During the cow-calf operation, calves spend their time with the dams on the pasture. The cows then consume the new grass growing in spring and provide sufficient milk for calves (Feuz and Umberger, 2003). During this period, the calves are grown with dams and their diet gradually transformed from milk into forage. Since the cows also need to enter the next breeding cycle (Damiran et al., 2018), they are separated for the backgrounding stage.

During the backgrounding stage, calves typically remain on the pasture from weaning until they are bought for the finishing period to maximize the usage of pasture (Greenwood, 2021), while some of them are backgrounded in the feedlot (Kumar et al., 2012). Traditionally, when the heifer and steer calves grow between 400 to 600 pounds, they are weaned, vaccinated, dehorned, and castrated (Peterson et al., 1989) to reduce the risk of high morbidity and mortality. As the most

critical phase for heifers and steers to build up the frame size, backgrounding aims for the beef cattle to gain weight primarily by muscle development. In other words, growth during the backgrounding stage decides how much weight the cattle can gain in the next stage and can significantly affect the cost when the heifers and steers are in the feedlot (McMillan et al., 2018). The calves then consume fertile forage until reaching 850 to 1000 pounds in the backgrounding period.

Although Alberta produces the largest amount of feedlot cattle in Canada, there are still a few small farms finishing their backgrounding beef cattle on the pasture to produce grass-fed beef. Instead, most backgrounding cattle are bought or owned by the large commercial feedlot for the last weight-gaining period (Sheppard et al., 2015). When the cattle are transported to the feedlot, individual information for each animal is recorded, including ID, weight, age, and the vaccine injection record; this process aims to ensure the cattle are healthy when entering the feedlot to prevent potential diseases. The diet of finishing beef cattle changes from a main-forage ration during the backgrounding period to a high grain diet after two weeks of transition feeding (Wagner et al., 2014). A typical diet for the finishing cattle consists of approximately 80% of grain (corn, barley, or wheat), 10% forage, and 10 % supplements (Wagner et al., 2014). These diets are high in proteins and carbohydrates with higher energy density, making the cattle grow quickly to reach the slaughter weight (Hannon et al., 2020). The feedlot is a highly intensive system where a large number of cattle are housed and are fed high-energy diets for rapid and efficient weight gain thereby reducing the cost of production for each pound of beef (Pogue et al., 2018). Moreover, beef cattle in the feedlot cannot move as freely as on the pasture, thus reducing energy expenditure. However, high stocking densities and the feeding of high-grain diets are known to increase cattle morbidity and mortality. For example, ruminal acidosis is one of the most common digestive

disorders resulting from feeding high-grain diets (Nagaraja and Lechtenberg, 2007). In addition to ruminal acidosis, the most prevalent infectious disease in the feedlot is bovine respiratory disease (BRD) which is also widely known as shipping fever, which can significantly affect cattle growth performance by causing depression, and reduced appetite, and increased temperature and respiration (Griffin, 2014). As the main cause of feedlot cattle disease, BRD is highly related to the change in cattle behaviour and immune functions (White et al., 2012). Lameness, which is defined as a foot health problem, has also been widely identified in both dairy and beef cattle herds, and the prevalence of lameness is only second to BRD in Alberta feedlots (Davis-Unger et al., 2019a). When cattle were affected by lameness, a higher likelihood of BRD prevalence was also detected (Davis-Unger et al., 2019b), suggesting the potential relationship between diseases happens in the feedlot.

In summary, diseases that happen in the feedlot can affect beef cattle production and industry income. Thus, monitoring and diagnosing those diseases in time are essential for keeping the herd healthy. Furthermore, lameness is a severe animal health problem that affects animals' health and productivity in feedlots, which will be the main focus of the rest of the literature review chapter.

1.2 Lameness in feedlot

1.2.1 Prevalence of lameness

Lameness is a significant economic and welfare concern for beef cattle raised in the feedlot. Griffin et al. (1993a) reported that 13.1% of feedlot cattle in the US are affected by various health issues. Among them, lameness accounted for 16% (Griffin et al., 1993a), suggesting over 2% of beef cattle in America are diagnosed with lameness. A western Canadian study assessed the prevalence of lameness in 28 feedlots over a 10-year period and found that 32% of the cattle were diagnosed

with lameness, which is second to the BRD (40%) among all diseases detected in feedlots (Davis-Unger et al., 2019c). Lameness was not only detected in the feedlot, but its high prevalence was also reported at slaughter (Bras et al., 2017), suggesting this disease deserves more attention. However, from the perspective of many feedlots' owners, mortality rates caused by lameness are low in comparison to BRD or other acute illnesses and therefore are not viewed with the same urgency as long as the cattle can reach the slaughter weight.

1.2.2 Symptoms and disadvantages of lameness

Lameness is a foot disease in livestock whose clinical signs are hard to detect, usually with leg or foot pain that affects their movement. One significant lameness symptom is the arched back that the whole back of the cattle arches up, while the back of a normal cattle is straight (Poursaberi et al., 2011). Abnormal behaviour caused by lameness includes refusing to move and reducing food and water intake because of foot or leg pain that can affect their daily life significantly (Norring et al., 2014b). Usually, lameness occurs after the damage occurs to leg or foot due to physical injury or pathogenic infections (Stokka et al., 2001a). These open wounds or ulcer-like injured tissue are the source of pain, which can indirectly cause a decrease in production performance (Shearer et al., 2013b). The average daily gain (ADG) of the lame cattle is significantly lower than that of healthy cattle, which decreased from 1.34 kg to 0.79 kg, and they require two more weeks to achieve the slaughter weight compared to the steers without lameness (Rouillard, 2020). Apart from the longer feeding time, lameness in feedlot also causes significant economic loss. It is reported that the estimated economic value of lame cattle is 53% lower than their original price in a Nebraska feedlot (Griffin et al., 1993b), and it ranged from 18% to 58% in Alberta based on the severity of lameness (Davis-Unger et al., 2017b).

The occurrence of lameness in the feedlot is caused by various factors, including genetics, injury or infection, environment, and nutrition. Boettcher et al. (1998) reported that genetically associated lower foot angles (-0.76, with a threshold of -0.64) in cattle were associated with clinical lameness (Boettcher et al., 1998a). In addition, some nutrition factors can also lead to lameness. For example, there is substantial evidence indicating that glucose and fructans can increase the probability of laminitis in the feedlot, which can be represented as the symptoms of lameness due to the pain (Lean et al., 2013a). This disease may be caused by the lactic acid production in the rumen due to a high glucose diet, especially when cattle are held on feed too long. However, physical injury and pathogen infection are the leading cause of lameness. Many studies reported that the concrete or rough flooring could cause physical injury to cattle hoofs (Shearer, 1998), especially when the cattle are stressed because they are more likely to step or scramble (Shearer et al., 2012a; Endres, 2017). Hoof damage and tissue morphological changes caused by these abnormal behaviours provide an entry point for pathogen infection, which is becoming the most common cause of lameness (Zhao et al., 2015).

1.2.3 Different types of lameness and diagnostic strategies

1.2.3.1 Foot rot

Foot rot, also termed infectious pododermatitis, has been widely diagnosed in both beef and dairy cattle herds worldwide (van Metre, 2017). Foot rot was the most common type of lameness in beef cattle in 28 western Canada feedlots over ten years, accounting for 74.5 % of all diagnosed lameness (Davis-Unger et al., 2019d). The leading cause of foot rot is secondary infection after physical damage caused by hard rocks and sharp objects on the feedlot ground (Allenstein, 1981). Feedlot cattle are housed in pens for about 200 days where they are exposed to muddy pen

conditions (Kara et al., 2011), particularly around water troughs or feed bunks, making the skin between the hooves more likely to soften and get damaged (Green et al., 2006). During the winter, the ice and dried mud become sharp and slippery, which are other factors causing foot injuries (Davis-Unger et al., 2019d). The most common pathogen causing foot rot is *Fusobacterium necrophorum*, a gram-negative bacterium widely inhabiting healthy skin and the environment (Brazier, 2006). This pathogen needs a physical injury or weakened barrier on the skin to enter the tissue. Foot rot is a polymicrobial disease, and the most abundant pathogens associated with the lesions are *F. necrophorum, Escherichia coli* and *Porphyromonas levii* (Smith et al., 1989). Thus, cattle are more likely to suffer from lameness when exposed to these three bacteria.

When cattle get foot rot, the soft tissue between two claws on the hoof usually be infected. In this case, careful observation of the infected area by the veterinarian is needed to have an accurate diagnosis. Under the foot rot diseased condition, the erythema and swelling tissue can be easily spotted in this area (Cortes et al., 2021a). Usually, the claws are separated by the swelling, and they are parallel to the axial midline of the foot (van Metre, 2017), which is also a critical judgment factor for veterinary inspection. Thus, cleaning the infected area is a top priority to reduce the source of subsequent infections (Currin et al., 2014). Based on the severity of foot rot, different types and doses of wide-spectrum antibiotics injection is the most effective treatment for lameness (van Metre, 2017), sometimes accompanied by topical administration. From previous research and practical application in the industry, ceftiofur sodium (Kausche et al., 2003) and oxytetracycline (Kausche et al., 2003) have been widely applied to treat foot rot in cattle in North America, which are highly efficient, and cattle have shorter withdrawal times to clear the medicine from their body. Furthermore, the foot bath with heavy metal salt as a means of preventing foot disease (Zanolari et al., 2021), especially the copper sulphate solution, has been widely used as ovine foot rot

treatment with good results (Härdi-Landerer et al., 2019).

The expenditure on disease prevention is usually less than the medical and labour costs for curing ill cattle, which may only account for 4% to 7% of total lameness case costs (Dolecheck and Bewley, 2018). Environmental management of the feedlot can reduce the occurrence of foot rot, as well as other phenotypes of lameness significantly, including removing iced feces, sharp rocks, and having well-drained pens. In addition, feeding cattle with extra minerals is another solution, with the zinc additive in the cattle diet reducing the incidence of foot rot (2.45% vs 5.38%, $p < 0.06$) (Brazle, 1993). A *Fusobacterium necrophorum* vaccine is another economical and convenient preventative method, which has demonstrated promising results from a study conducted at the University of Saskatchewan in 2005. Half of 447 mixed breed beef steers were pre-injected with 2 ml *Fusobacterium necrophorum* vaccine when they entered the feedlot, whereas the rest of the animals were given the placebo injection. The results showed that vaccine injection reduced the occurrence of foot rot by 80% (Checkley et al., 2005a). Unfortunately, its use was not widely adopted by the industry due to a lack of effectiveness in commercial feedlots (Checkley et al., 2005b). In summary, as the most common lameness phenotype in the feedlot, foot rot is not difficult to cure during the early stages. Therefore, disease prevention and more accurate diagnosis techniques are needed to avoid additional labour, treatment costs and treatment delay.

1.2.3.2 Digital dermatitis

Digital dermatitis (DD) is a foot lesion that was first reported in dairy herds in Italy in 1974 (Cheli R and Mortellaro C, 1974) and has become another health problem in cattle worldwide (van Amstel et al., 1995). DD has become one of the most significant lameness problems in dairy herds, with

over 92% of herds being infected worldwide, and the prevalence in single herds is highest and attainable at 83% (Holzhauer et al., 2006). In Alberta, the prevalence of DD was reported to range between 19% and 84% in dairy cows (Jacobs et al., 2017). However, in the past 40 years, the majority of studies have only focused on dairy cows because this disease reduces milk yield (Argaez-Rodriguez et al., 1997) and reproductive performance (Garbarino et al., 2004). Due to DD being one of the main animal welfare concerns, researchers started to pay more attention to the DD incidence in feedlot beef cattle (Julián A, 2020).

The clinical sign of DD usually presents as lameness, which is similar to other lameness phenotypes (RW and MW, 1988), and cattle typically walk on their toe tips (Read and Walker, 2016). DD lesions appear as an ulcer (2 to 6 cm in diameter) with hyperkeratotic, and hair or wart-like projections (Plummer and Krull, 2017) surrounding the lesion. Histopathological studies have described DD as a highly proliferative epidermis, hyperplastic stratum corneum as well as lesions infiltrated by inflammatory cells (Döpfer et al., 1997). *Treponema spp* (Schrank et al., 1999; Trott et al., 2003) and other spirochete-like, filamentous organisms (RW and MW, 1988) have been widely identified in the DD tissue using bacterial culture-based methods; thus, they were once considered to be the pathogens of DD. However, the pathogenic result in cattle induced by the pure culture of *Treponema spp* is inconsistent with the naturally occurring DD (Gomez et al., 2012). In addition, vaccines against this bacterium did not reduce the incidence or alleviate the severity of DD (Plummer and Krull, 2017). Thus, the primary pathogen cause DD is still controversial and inconclusive. Unlike FR, treatment of DD is costly and lacks efficiency (RA and DN, 2006; Evans et al., 2016). In fact, the direct-applied antibiotic application to the lesion (Plummer and Krull, 2017) and foot bath (Cook, 2017a) are the common treatments. To be specific, oxytetracycline is the most-used antibiotic for DD treatment. A dose of 2.0 to 25 g oxytetracycline soluble powder

to the lesion has been reported by vets and hoof trimmers, whereas there was no evidence to support if the larger dose is helpful or not for disease recovery (Plummer and Krull, 2017). However, these treatment strategies still lack efficiency. For example, a study that assessed the treatment of DD Holsten dairy cattle for three years in Iowa demonstrated that 93% of them showed better lesion recovery after local antibiotic treatment (Krull et al., 2016), but only 9% of them recovered to normal healthy skin. Some of them needed secondary treatment in the following year (Krull et al., 2016). This indicates that antibiotic treatment can alleviate symptoms but cannot completely resolve the infection. Similar to FR, prevention is an excellent way to reduce the prevalence of DD. Footbath is the most common method to control lameness in dairy farms and feedlots (Cook, 2017b). A study comparing two different footbath methods when treating Holstein and Simmental cows using 5% copper sulphate and non-heavy metal-based proprietary dip for three months demonstrated that both methods reduced the incidence of DD and increased milk yield (Prastiwi et al., 2019). In addition, 5% copper sulphate footbath showed a better performance than other footbath methods when treating DD (Logue et al., 2012). The clinical symptom of DD in beef cattle is the same as in dairy cows with similar predisposing factors (Wilson-Welder et al., 2015). With the increasing attention to beef cattle welfare, DD has become one of the leading health and welfare concerns in beef cattle.

1.2.3.3 Toe-tip necrosis

Toe-tip necrosis (TTN) is not as frequent as other lameness phenotypes (FR and DD discussed above) in feedlot cattle, which has an average incidence rate of 3.8% (Jelinski et al., 2016a). However, this disease is much more devastating than other foot diseases. TTN typically affects the hind feet and is usually observed during the first three weeks when the cattle enter the feedlot. An important clinical sign of TTN is white line separation which means the area that connects the sole

and hoof wall allowing bacteria to easily invade and infect the internal tissues (Johnston et al., 2019a). It is proposed that TTN happens due to external physical damage to the hooves followed by bacterial infection, which is also accepted by the mainstream and called “abrasion theory” (Jelinski et al., 2016b). After the white line separation by external force damage, bacteria in the environment invade and infect the soft tissues of the toes and finally form the inflammation, which continues to spread upward in most cases causing other diseases, such as cellulitis, myositis, arthritis, tendonitis, tenosynovitis, and other systemic diseases (Jelinski et al., 2016b).

Compared to other lameness phenotypes, TTN is difficult to diagnose because the lesions occur inside the hoof. The disease definition of TTN is observing necrosis inside the toes on the third phalangeal bone when cutting the toe tip into two pieces (Jelinski et al., 2016c). In this case, TTN in the cattle is usually detected after euthanasia or slaughter. Although “white line separation” is defined as the clinical sign of this disease, this pathognomonic is not always evident in actual inspection (Jelinski et al., 2016d), which sometimes makes it misdiagnosed as a physical injury. In most cases, a single pathogen infection does not cause TTN. A study assessed over 200 beef steers who died of TTN in western Canadian feedlots over two years demonstrated that the heavy growth of *Escherichia coli* and *Trueperella pyogenes* were identified in the lesion of TTN in cattle based on the bacteria testing using anaerobic swabs (Paetsch et al., 2017). Moreover, other organic fragments, such as straw, were found inside the white line (Paetsch et al., 2017). The possible explanation is that the separation of the white line leads to the entry of foreign organic matter into the claw, then causes anaerobic bacterial infection and leads to soft tissue and systemic infections. Besides, some people believe that the enzymes produced by the bacteria can cause further degradation of the white line and hoof (Jelinski et al., 2016d; Johnston et al., 2019b). Therefore, the mainstream recognition of TTN caused by external injuries still needs further exploration.

Like other lameness phenotypes, prevention has a far better effect on TTN than post-treatment, especially when TTN is extremely difficult to cure. A previous study compared the effectiveness of antimicrobial treatment in TTN beef cattle from 1904 batches of beef cattle in 48 feedlots located in western Canada; it revealed that around 25% of affected cattle died 6-days after treatment, and over 70% of affected cattle died when not administered with an antimicrobial (Jelinski et al., 2016d). Once the infection affects the third phalangeal bone, the use of antimicrobials is not effective in curing the lesion. This suggests that more radical treatment is in demand, including amputation and euthanasia.

1.2.3.4 Traditional lameness detection methods

The most common method of diagnosing lameness employed by veterinarians, farmers and hoof trimmers is visual assessment. Clinical signs including an arched back, impaired movement, abnormal gait, and foot lesions are observed in an animal (Shearer et al., 2012b; Nuffel et al., 2015). Different gait assessment schemes for lameness detection have been identified by various modes, including gait patterns like a slower and shorter stride (Maertens et al., 2011; van Nuffel et al., 2013; van Nuffel et al., 2015), posture or body movement patterns like arched back (Distl and Mair, 1993; Nordlund et al., 2004), weight distribution patterns by measuring the "leg/weight ratio" (Scott, 1989; Phillips, 2007), and behavioural changes in the cattle (Blackie et al., 2011). Manson and Leaver (1988) proposed a 9-point system to describe different levels of lameness, with the higher the score indicating the more serious the lameness (Manson and Leaver, 1988). Some other locomotion indices have also been developed by different researchers, including the Five-point system (Winckler and Willen, 2010); Visual analogue scale (VAS) (Flower and Weary, 2006), and the most used visual scoring method being proposed by Sprecher (Sprecher et al., 1997a). Sprecher determines the lameness severity into five levels: 0~4, where 0 means no

lameness and 4 represents the most severe lameness. All the monitoring programs are based on the changes in the cattle locomotion itself, and it still needs professional observations. Although automated lameness detection such as automatic deep image analysis (Condotta et al., 2020) and accelerometers system (O'Leary et al., 2020a) has become a hot research topic in recent years, these detection methods are only effective after lameness occurs. In addition, compared with dairy cows, beef cattle herds are not subjected to daily uninterrupted inspections, especially in feedlots (Paetsch et al., 2017). Dairy cows are continuously monitored so that lameness and other diseases can be detected earlier. However, lameness detection in beef cattle only occurs when they show obvious lame conditions and are observed by the pen operators. At present, lameness detection is only performed after the occurrence of the disease, usually based on visual observation and expertise of the stock personals, and the corresponding lameness phenotypes cannot be accurately diagnosed.

1.3 MiRNAs as molecular biomarkers in lameness diagnosis

1.3.1 MiRNAs feature and functions

MiRNAs are a subset of non-coding RNAs, usually 21 to 25 nt single-strand RNA molecules, which represent and account for 1 to 2 % of identified transcripts in eukaryote species (John et al., 2004). In 1993, Rosalind C. Lee was the first to identify two genes: lin-4 and let-7 in nematode *C.elegans* that encode small RNAs rather than proteins (Lee et al., 1993), which were then defined as miRNA. To date, 38,589 miRNAs have been identified from eukaryotes and are recorded in the miRNA database (miRbase R 22.1) (Kozomara et al., 2019a). Interestingly, the sequences of miRNAs are highly conserved in different species, which play essential roles in regulating almost every cellular process (Wilczynska and Bushell, 2014b). The conservation of miRNAs has

also been found among different plants. From the first group of identified miRNAs in Arabidopsis, 8 out of 16 matched the orthologous locus in rice and the mature miRNA sequence is almost unchanged (Ha et al., 2008), validating the conservation of miRNAs to a large extent and its importance in biological functions.

In general, the mature miRNA is produced by a two-step cleavage derived from primary miRNA (pri-miRNA) (MacFarlane and Murphy, 2010a). Briefly, pri-miRNA is first transcribed from miRNA genes and then forms precursor miRNA (pre-miRNA) through the cleavage by enzymes Drosha and Pasha. Afterwards, pre-miRNA form miRNA duplexes in the cytoplasm through the second cleavage by protein Exportin-5. The miRNA duplexes usually have a stem-loop structure called miRNA/miRNA* duplex with two complementary strands. After Dicer enzyme-mediated hairpin excision, dsRNA containing mature miRNA bind to the Argonaute (Ago) protein (Meister et al., 2004), and this effector complex is called RNA-induced silencing complex (RISC) (Okamura et al., 2004). The antisense strand of miRNA can be complemented with the target mRNA and induce the endonucleolytic, therefore achieving the gene silencing. Most functions of miRNA rely on their binding to mRNA, which leads to post-transcriptional suppression (Bartel, 2018).

In animals, there are three main mechanisms to silence mRNAs by miRNAs: mRNA cleavage (Xu et al., 2016), reducing translation efficiency (MacFarlane and Murphy, 2010b)and poly (A) tail shortening (Fabian et al., 2010a). Among them, target mRNA cleavage is the most frequently reported post-transcriptional regulation mechanism, in which miRNAs can pair the 3’ untranslated regions (3′-UTRs), following the cleavage and destroying of target mRNAs by the catalysis of the Ago protein (Rhoades et al., 2002). This activity directly reduces mRNA levels and protein production, thereby regulating gene expression at the post-transcriptional level (Guo et al., 2010).

It is proposed that miRNA can inhibit the translation of mRNA by shortening the poly (A) tail based on the finding of the alleviation of the miR-340 suppression of *tdrd7* mRNA via shortening the poly (A) tail of mRNA with the function of germline-specific RNA-binding protein termed DAZ-like (DAZL) (Takeda et al., 2009). In summary, miRNA can inhibit protein synthesis by mRNA degradation or deadenylation (Eulalio et al., 2008; Filipowicz et al., 2008). However, the inhibition also happens at or after the beginning stage of translation by targeting the cap recognition on ribosomes or preventing the assembly of ribosome 80s complex (Fabian et al., 2010b). Many studies have started focusing on the gene regulation function of miRNAs at the post-transcription level to explore the roles of miRNAs in expression and processing in various biological processes (Ladomery et al., 2011).

1.3.2 Circulating miRNA in body fluids

There are two types of miRNAs presenting in animals: cellular miRNAs and circulating miRNAs, which play different roles in the body (Duttagupta et al., 2011a). The mature miRNAs were firstly formatted and integrated into the RISC in the cytoplasm to target mRNA (Yu et al., 2016). However, some researchers have found that miRNAs are not only functional in the cell where they are produced (Kumar et al., 2013a), but also are exported to target mRNAs in distant target cells (Valadi et al., 2007; Vickers et al., 2011). In addition, miRNAs were found in different bodily fluids including blood (Z.-M. Luoreng et al., 2018; Gasparello et al., 2020; Umezu et al., 2020), serum (Elias et al., 2017; Leão et al., 2018; Shiino et al., 2019), urine (Kutwin et al., 2018; Lekchnov et al., 2018; Jeon et al., 2020), milk (Golan-Gerstl et al., 2017; Quan et al., 2020; Kupsco et al., 2021), saliva (Rapado-González et al., 2019), and semen (Barceló et al., 2019; Mayes et al., 2019; Mercadal et al., 2020). Generally, most circulating miRNAs in body fluids are highly stable and bound to exosomes and other extracellular vesicles (Kosaka et al., 2010) to avoid RNase

degradation (Duttagupta et al., 2011a). This structural complexity is also beneficial for the transportation of miRNAs to remotely regulate their targets (Sun et al., 2018). In this case, body fluids are a good source to detect circulating miRNAs related to disease progress or specific physiological condition (Anindo and Yaqinuddin, 2012; Azzalini et al., 2019; Coenen-Stass et al., 2019; Isabel Gessner et al., 2021). In the meantime, body fluids such as saliva, urine and/or blood also have the advantage during detection, including easy access, low invasiveness, and time-saving.

1.3.3 MiRNAs as a novel diagnosing tool in human diseases

Accumulating evidence suggests that circulating miRNAs can be selected as critical biomarkers for human disease detection and diagnosis (Jiang and Zhu, 2020). The first application was published in 2006 by Dr. Slack's group at Yale University, who identified that miRNA's mutation was related to various human cancers and functioned as cancer-related gene expression repression (Esquela-Kerscher and Slack, 2006). This study was the first to demonstrate the potential of miRNA as a diagnostic molecule for human cancers. In the following decade, the role of miRNAs in human cancer has been widely studied (Tong and Nemunaitis, 2008; Mulrane et al., 2013a; Wang et al., 2013; McGuire et al., 2015a; Qadir and Faheem, 2017) with over half of the identified miRNA genes reported being located in the cancer-associated region or fragile sites (Reddy, 2015). To date, miRNAs have been proved to function in the formation of different types of cancers, including breast cancer (Iorio et al., 2005; Mulrane et al., 2013b; McGuire et al., 2015b), colon cancer (Akao et al., 2007; Schepeler et al., 2008; Zhang et al., 2013), gastric cancer (Shin and Chu, 2014; Wu et al., 2014; Hao et al., 2017), lung cancer (Wang et al., 2010; Võsa et al., 2013; Uddin and Chakraborty, 2018), prostate cancer (Brase et al., 2011; Filella and Foj, 2017; Cochetti et al., 2020) and thyroid cancer (Marini et al., 2011; Lodewijk et al., 2012; Qiu et al., 2018). Recent findings further indicate that molecular marker-based diagnostic tool has the potential for disease

diagnosis and prognosis (Yerukala Sathipati and Ho, 2018b), and the miRNA expression profile is more accurate than gene expression profiles for classifying human diseases (Lu et al., 2005).

With the development of new diagnostic methods, more miRNAs in different body fluids have been identified as biomarkers for specific disease diagnosis (Backes et al., 2016). To be more specific, miRNA profiles have been identified and applied in disease diagnosis in type II diabetes (Bunt et al., 2013; Chakraborty et al., 2014; Satake et al., 2018), Alzheimer's disease (Kumar et al., 2013b; Leidinger et al., 2013; Swarbrick et al., 2019), vascular disease (Zhang, 2009; Zhang, 2010; Jamaluddin et al., 2014) and other clinical diseases like Creutzfeldt-Jakob disease; bladder or liver diseases(Murakami et al., 2012; Norsworthy et al., 2020). These findings provide new ideas for early disease diagnosis because the miRNA level changes in bodily fluids before the onset stage (Zhou and Zhang, 2014). Based on these findings, miRNAs might have potential in the detection and characterization of various causes of lameness in livestock.

1.3.4 Current application of miRNA as a detection tool on livestock

In addition to the human diseases, application of specific miRNAs related to various livestock physiological conditions and diseases could be applied to diagnose the similar situations in animals (Wara et al., 2019; Gupta et al., 2018a; Z.M. Luoreng et al., 2018). The current studies on animal miRNAs are related to the assessment of the relationships between the miRNA profiles and/or expression with specific phenotypes including productivity (Strotbek et al., 2013; Ioannidis and Donadeu, 2016a), fertility (McIver et al., 2012; Hu et al., 2020; Turri et al., 2021) and other production indicators. As described previously, livestock diseases can lead to significant economic loss and welfare issues (Bishop and Woolliams, 2014; Hu et al., 2020). Therefore, a novel disease diagnosis method is needed to improve diagnostic accuracy, reduce economic losses, reduce the

use of antimicrobials and improve animal welfare (Bennett, 2003). Currently, most miRNA studies conducted on animal diseases have focused on the stage of disease detection without clinical or commercial application. Although the specific miRNAs and their functions have been identified in these studies, the regulatory mechanisms of miRNAs in these health-related traits have not been identified (Do et al., 2021). More in-depth study of the regulating roles of miRNA underlying the disease is required.

There is substantial evidence that miRNAs can serve as biomarkers for disease diagnosis in different livestock species. In cattle, miRNAs have been widely studied in dairy cows for their roles in affecting different biological processes under various physiological conditions (Ioannidis et al., 2018). The comparison of miRNAs expressed in the mammary gland identified seven differentially expressed miRNAs between four heat-stressed and four healthy lactating Holstein cows (Li et al., 2018b). Similarly, eleven differentially expressed miRNAs were identified in the whole blood of heat-stressed Holstein cows when compared to those without being heat stressed (Lee et al., 2020a). In addition, miR-1976, miR-873-3p, miR-520f-3p, and miR-126-3p in cattle serum were found to be differentially expressed in those diagnosed with Johne's disease when compared with healthy ones (Gupta et al., 2018). Similarly, Ssc-let-7d-3p has been identified as differentially expressed in serum between commercial specific pathogen-free swine herds and those affected by *Trichuris suis* (Hansen et al., 2016). Likewise, gga-miR-15b was significantly differentially expressed between specific pathogen free white leghorn chickens' spleen and the spleen tumor tissue with Marek's disease (Tian et al., 2012). These findings suggest that blood circulating miRNAs can be informative biomarkers to detect the different physiological conditions and diseases in livestock.

1.3.5 Methods to detect miRNAs

Currently, the most used methods for miRNA detection and comparation are miRNA hybridization microarray, small RNA sequencing (miRNA-seq), and miRNA real time-quantitative reverse transcription PCR (RT-qPCR) (Meyer et al., 2010). Microarray is based on the high sensitivity hybridization technology which targets the specific miRNAs using predesigned fluorescence probes and produces the desired expression data by different levels of fluorescence indicators (Nagl et al., 2005). The small RNA-seq is a sequencing method through small RNA ligation or poly-A tailing to extend the length of small RNAs, generating the overall expression profiles of small RNAs (Giraldez et al., 2018). RT-qPCR usually uses predesigned TaqMan miRNA probes to detect the expression level of selected miRNAs based on the reaction product of step-loop RT-qPCR (Johansen and Andreassen, 2014). Each method has its pros and cons. For example, the microarray is a commercially designed tool based on hybridization to detect over 1500 mature miRNAs from 474 human and 373 mice miRNA genes at the same time, which can generate data quickly and is cost-effective (Pradervand et al., 2018). However, the limitation of this method is that microarray can only detect known miRNAs with predesigned probes. Because it is based on hybridization, novel miRNAs cannot be detected. RNA-seq is a sequencing method based on next-generation sequencing (NGS), including cDNA library construction and sequencing, which allows detection, prediction, and quantification of all miRNAs within a biological sample based on the different sequencing depths (Meyer et al., 2010) making it the most comprehensive detection method. However, it is costly and time-consuming, and the results may be highly variable depending on the analytical methods used (Maria D Giraldez et al., 2018). MiRNA RT-qPCR can detect the expression level of particular miRNAs based on specially designed probes or primers (Forero et al., 2019) and it is highly sensitive and accurate, requires less time and is cost-effective

(Chen et al., 2005). However, it can only detect the targeted miRNAs and is not have a high throughput.

1.4 Knowledge gaps and hypothesis

To date, most published studies on lameness in cattle have been conducted on dairy cows because they are more accessible and observable, and lameness in dairy cows can cause obvious economic loss. Only a few studies have assessed lameness in feedlot beef cattle, even though lameness is considered the second most prevalent health issue in the feedlot (Davis-Unger et al., 2019b). Moreover, the visual detection methods of lameness are limited, including time-consuming, laborious, and inaccurate. Currently, the molecular regulatory mechanism behind lameness is still unclear and no study yet assesses the functions of miRNAs in regulating the biological process associated with lameness. We hypothesize that when cattle have different types of lameness, they have different physiological changes, and miRNAs and their expression shift can be one of the regulators behind those changes. And the specific miRNAs can be detected differentially expressed between lame and healthy cattle and between different lameness phenotypes. The objectives of the present thesis research were (1) to identify if there are differences between the miRNA profiles of healthy and different lameness phenotypes in cattle, and (2) to identify if the level of differentially expressed miRNAs changes during the recovery period. Specifically, we aimed to generate the miRNA profile from lame and healthy beef cattle blood to demonstrate how the miRNA profiles differ and predict their potential regulating roles associated with lameness. The long-term goal of this thesis research is to provide the basic knowledge of miRNAs in cattle blood under lameness conditions and potentially develop a novel and accurate miRNA-based diagnostic tool for different lameness phenotypes. The study of the miRNAs in beef cattle blood and their associations with the formation of different phenotypes of lameness will advance our knowledge about how the

miRNAs regulate the formation of lameness, which will help to develop a potential novel detection method for beef cattle lameness through comparing the miRNA profiles between lame and healthy cattle.

1.5 References

Akao, Y., Y. Nakagawa, and T. Naoe. 2007. MicroRNA-143 and -145 in Colon Cancer. https://home-liebertpub-com.login.ezproxy.library.ualberta.ca/dna. 26:311–320. doi:10.1089/DNA.2006.0550. Available from: https://www-liebertpub-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1089/dna.2006.0550

Alberta has the most beef cattle in Canada and the second largest total farm area. Available from: https://www150.statcan.gc.ca/n1/pub/95-640-x/2016001/article/14808-eng.htm

Alemu, A. W., B. D. Amiro, S. Bittman, D. MacDonald, and K. H. Ominski. 2016. A typological characterization of Canadian beef cattle farms based on a producer survey. Canadian Journal of Animal Science. 96:187–202. doi:10.1139/CJAS-2015-0060/ASSET/IMAGES/CJAS-2015-0060TAB6.GIF. Available from: https://cdnsciencepub-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1139/cjas-2015-0060

Allenstein, L. C. 1981. Lamenesses of cattle. The Canadian Veterinary Journal. 22:65. Available from: https://www-ncbi-nlm-nih-gov.login.ezproxy.library.ualberta.ca/pmc/articles/PMC1789870/

van Amstel, S. R., S. van Vuuren, and L. C. Tutt. 1995. DIGITAL DERMATITIS REPORT OF AN OUTBREAK. Journal of the South African ’ ~terinary Association. 66:177–181. doi:10.10520/AJA00382809_1548.

Anindo, M. I. K., and A. Yaqinuddin. 2012. Insights into the potential use of microRNAs as biomarker in cancer. International Journal of Surgery. 10:443–449. doi:10.1016/J.IJSU.2012.08.006.

Azzalini, E., E. de Martino, P. Fattorini, V. Canzonieri, G. Stanta, and S. Bonin. 2019. Reliability of miRNA Analysis from Fixed and Paraffin-Embedded Tissues. International Journal of Molecular Sciences 2019, Vol. 20, Page 4819. 20:4819. doi:10.3390/IJMS20194819. Available from: https://www.mdpi.com/1422-0067/20/19/4819/htm

Babatunde, S. M., S. Z. Ramanoon, W. M. S. Mossadeq, R. Mansor, and S. S. S. Hussain. 2019. Dairy Farmers’ Perceptions of and Actions in Relation to Lameness Management. Animals 2019, Vol. 9, Page 270. 9:270. doi:10.3390/ANI9050270. Available from: https://www.mdpi.com/2076-

2615/9/5/270/htm

Backes, C., E. Meese, and A. Keller. 2016. Specific miRNA Disease Biomarkers in Blood, Serum and Plasma: Challenges and Prospects. Molecular Diagnosis & Therapy 2016 20:6. 20:509–518. doi:10.1007/S40291-016-0221-4. Available from: https://link.springer.com/article/10.1007/s40291-016-0221-4

Barceló, M., M. Castells, L. Bassas, F. Vigués, and S. Larriba. 2019. Semen miRNAs Contained in Exosomes as Non-Invasive Biomarkers for Prostate Cancer Diagnosis. Scientific Reports 2019 9:1. 9:1–16. doi:10.1038/S41598-019-50172-6. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-019-50172-6

Bartel, D. P. 2018. Metazoan MicroRNAs. Cell. 173:20. doi:10.1016/J.CELL.2018.03.006. Available from: /pmc/articles/PMC6091663/

Bennett, R. 2003. The 'Direct Costs'of Livestock Disease: The Development of a System of Models for the Analysis of 30 Endemic Livestock Diseases in Great Britain. Journal of Agricultural Economics. 54:55–71. doi:10.1111/J.1477-9552.2003.TB00048.X. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/j.1477-9552.2003.tb00048.x

Blackie, N., E. Bleach, J. Amory, and J. Scaife. 2011. Impact of lameness on gait characteristics and lying behaviour of zero grazed dairy cattle in early lactation. Applied Animal Behaviour Science. 129:67–73. doi:10.1016/J.APPLANIM.2010.10.006.

Boettcher, P. J., J. C. M. Dekkers, L. D. Warnick, and S. J. Wells. 1998. Genetic Analysis of Clinical Lameness in Dairy Cattle. Journal of Dairy Science. 81:1148–1156. doi:10.3168/JDS.S0022-0302(98)75677-2.

Bras, A. L., H. W. Barkema, M. R. Woodbury, C. S. Ribble, J. Perez-Casal, and M. C. Windeyer. 2017. Clinical presentation, prevalence, and risk factors associated with Mycoplasma bovis-associated disease in farmed bison (Bison bison) herds in western Canada. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.2460/javma.250.10.1167. 250:1167–1175. doi:10.2460/JAVMA.250.10.1167.

Brase, J. C., M. Johannes, T. Schlomm, M. Fälth, A. Haese, T. Steuber, T. Beissbarth, R. Kuner,

and H. Sültmann. 2011. Circulating miRNAs are correlated with tumor progression in prostate cancer. International Journal of Cancer. 128:608–616. doi:10.1002/IJC.25376. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/ijc.25376

Brazier, J. S. 2006. Human infections with Fusobacterium necrophorum. Anaerobe. 12:165–172. doi:10.1016/J.ANAEROBE.2005.11.003.

Brazle, F. K. 1993. Effect of Zinpro 100® in a mineral mixture on gain and incidence of footrot in steers grazing native grass pastures. Kansas Agricultural Experiment Station Research Reports. 745:145–146. doi:10.4148/2378-5977.2148. Available from: https://newprairiepress.org/kaesrr/vol0/iss1/745

Bunt, M. van de, K. J. Gaulton, L. Parts, I. Moran, P. R. Johnson, C. M. Lindgren, J. Ferrer, A. L. Gloyn, and M. I. McCarthy. 2013. The miRNA Profile of Human Pancreatic Islets and Beta-Cells and Relationship to Type 2 Diabetes Pathogenesis. PLOS ONE. 8:e55272. doi:10.1371/JOURNAL.PONE.0055272. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0055272

Chakraborty, C., C. G. P. Doss, S. Bandyopadhyay, and G. Agoramoorthy. 2014. Influence of miRNA in insulin signaling pathway and insulin resistance: micro-molecules with a major role in type-2 diabetes. Wiley Interdisciplinary Reviews: RNA. 5:697–712. doi:10.1002/WRNA.1240. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/wrna.1240

Checkley, S. L., E. D. Janzen, J. R. Campbell, and J. J. McKinnon. 2005a. Efficacy of vaccination against Fusobacterium necrophorum infection for control of liver abscesses and footrot in feedlot cattle in western Canada. The Canadian Veterinary Journal. 46:1002. Available from: /pmc/articles/PMC1259145/

Checkley, S. L., E. D. Janzen, J. R. Campbell, and J. J. McKinnon. 2005b. Efficacy of vaccination against Fusobacterium necrophorum infection for control of liver abscesses and footrot in feedlot cattle in western Canada. The Canadian Veterinary Journal. 46:1002. Available from: /pmc/articles/PMC1259145/

Chen, C., D. A. Ridzon, A. J. Broomer, Z. Zhou, D. H. Lee, J. T. Nguyen, M. Barbisin, N. L. Xu,

V. R. Mahuvakar, M. R. Andersen, K. Q. Lao, K. J. Livak, and K. J. Guegler. 2005. Real-time quantification of microRNAs by stem–loop RT–PCR. Nucleic Acids Research. 33:e179–e179. doi:10.1093/NAR/GNI178. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/nar/article/33/20/e179/1082564

Cochetti, G., J. A. Rossi de Vermandois, V. Maulà, M. Giulietti, M. Cecati, M. del Zingaro, R. Cagnani, C. Suvieri, A. Paladini, and E. Mearini. 2020. Role of miRNAs in prostate cancer: Do we really know everything? Urologic Oncology: Seminars and Original Investigations. 38:623–635. doi:10.1016/J.UROLONC.2020.03.007.

Coenen-Stass, A. M. L., M. J. Pauwels, B. Hanson, C. M. Perez, M. Conceição, M. J. A. Wood, I. Mäger, and T. C. Roberts. 2019. Extracellular microRNAs exhibit sequence-dependent stability and cellular release kinetics. https://doi-org.login.ezproxy.library.ualberta.ca/10.1080/15476286.2019.1582956. 16:696–706. doi:10.1080/15476286.2019.1582956. Available from: https://www-tandfonline-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1080/15476286.2019.1582956

Condotta, I. C. F. S., T. M. Brown-Brandl, G. A. Rohrer, and K. O. Silva-Miranda. 2020. Development of method for lameness detection based on depth image analysis. ASABE 2020 Annual International Meeting. 1-. doi:10.13031/AIM.202001082. Available from: https://elibrary-asabe-org.login.ezproxy.library.ualberta.ca/azdez.asp?JID=&AID=51535&CID=virt2020&v=&i=&T=1

Cook, N. B. 2017a. A Review of the Design and Management of Footbaths for Dairy Cattle. Veterinary Clinics of North America: Food Animal Practice. 33:195–225. doi:10.1016/J.CVFA.2017.02.004.

Cook, N. B. 2017b. A Review of the Design and Management of Footbaths for Dairy Cattle. Veterinary Clinics: Food Animal Practice. 33:195–225. doi:10.1016/J.CVFA.2017.02.004. Available from: http://www.vetfood.theclinics.com/article/S0749072017300129/fulltext

Cortes, J. A., S. Hendrick, E. Janzen, E. A. Pajor, and K. Orsel. 2021. Economic impact of digital dermatitis, foot rot, and bovine respiratory disease in feedlot cattle. Translational Animal Science. 5. doi:10.1093/TAS/TXAB076. Available from: /pmc/articles/PMC8244990/

Currin, J. F., W. D. Whittier, and N. Currin. 2014. Foot Rot in Beef Cattle. Available from: https://vtechworks.lib.vt.edu/handle/10919/50702

Damiran, D., K. A. Larson, L. T. Pearce, N. E. Erickson, and B. H. A. Lardner. 2018. Effect of calving period on beef cow longevity and lifetime productivity in western Canada. Translational Animal Science. 2:S61–S65. doi:10.1093/TAS/TXY020. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/2/suppl_1/S61/5108326

Davis-Unger, J., E. A. Pajor, K. Schwartzkopf-Genswein, S. Marti, C. Dorin, E. Spackman, and K. Orsel. 2017a. Economic impacts of lameness in feedlot cattle. Translational Animal Science. 1:467–479. doi:10.2527/TAS2017.0052. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/1/4/467/4780403

Davis-Unger, J., E. A. Pajor, K. Schwartzkopf-Genswein, S. Marti, C. Dorin, E. Spackman, and K. Orsel. 2017b. Economic impacts of lameness in feedlot cattle. Translational Animal Science. 1:467–479. doi:10.2527/TAS2017.0052. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/1/4/467/4780403

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019a. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Translational Animal Science. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/3/2/595/5377510

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019b. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Transl Anim Sci. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://pubmed.ncbi.nlm.nih.gov/32704830/

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019c. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Translational Animal Science. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/3/2/595/5377510

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019d. Prevalence and lameness-associated risk factors in Alberta feedlot cattle.

Translational Animal Science. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://academic.oup.com/tas/article/3/2/595/5377510

Distl, O., and A. Mair. 1993. Computerized analysis of pedobarometric forces in cattle at the ground surface/floor interface. Computers and Electronics in Agriculture. 8:237–250. doi:10.1016/0168-1699(93)90036-Z.

Do, D. N., P.-L. Dudemaine, M. Mathur, P. Suravajhala, X. Zhao, and E. M. Ibeagha-Awemu. 2021. miRNA Regulatory Functions in Farm Animal Diseases, and Biomarker Potentials for Effective Therapies. International Journal of Molecular Sciences 2021, Vol. 22, Page 3080. 22:3080. doi:10.3390/IJMS22063080. Available from: https://www.mdpi.com/1422-0067/22/6/3080/htm

Dolecheck, K., and J. Bewley. 2018. Animal board invited review: Dairy cow lameness expenditures, losses and total cost. animal. 12:1462–1474. doi:10.1017/S1751731118000575. Available from: https://www-cambridge-org.login.ezproxy.library.ualberta.ca/core/journals/animal/article/animal-board-invited-review-dairy-cow-lameness-expenditures-losses-and-total-cost/1DD4186D5AC3943FC5DB8E6536C0277A

Döpfer, D., A. A. H. M. ter Huurne, J. L. Cornelisse, A. J. A. M. van Asten, A. Koopmans, F. A. Meijer, Y. H. Schukken, I. Szakáll, W. Klee, and R. B. Bosma. 1997. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and Campylobacter faecalis. Veterinary Record. 140:620–623. doi:10.1136/VR.140.24.620. Available from: https://bvajournals.onlinelibrary.wiley.com/doi/full/10.1136/vr.140.24.620

Duttagupta, R., R. Jiang, J. Gollub, R. C. Getts, and K. W. Jones. 2011. Impact of Cellular miRNAs on Circulating miRNA Biomarker Signatures. PLOS ONE. 6:e20769. doi:10.1371/JOURNAL.PONE.0020769. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0020769

Elias, K. M., W. Fendler, K. Stawiski, S. J. Fiascone, A. F. Vitonis, R. S. Berkowitz, G. Frendl, P. Konstantinopoulos, C. P. Crum, M. Kedzierska, D. W. Cramer, and D. Chowdhury. 2017. Diagnostic potential for a serum miRNA neural network for detection of ovarian cancer. Elife. 6. doi:10.7554/ELIFE.28932.

Endres, M. I. 2017. The Relationship of Cow Comfort and Flooring to Lameness Disorders in Dairy Cattle. Veterinary Clinics: Food Animal Practice. 33:227–233. doi:10.1016/J.CVFA.2017.02.007. Available from: http://www.vetfood.theclinics.com/article/S0749072017300154/fulltext

Esquela-Kerscher, A., and F. J. Slack. 2006. Oncomirs — microRNAs with a role in cancer. Nature Reviews Cancer 2006 6:4. 6:259–269. doi:10.1038/NRC1840. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/nrc1840

Eulalio, A., E. Huntzinger, and E. Izaurralde. 2008. Getting to the Root of miRNA-Mediated Gene Silencing. Cell. 132:9–14. doi:10.1016/J.CELL.2007.12.024.

Evans, N. J., R. D. Murray, and S. D. Carter. 2016. Bovine digital dermatitis: Current concepts from laboratory to farm. The Veterinary Journal. 211:3–13. doi:10.1016/J.TVJL.2015.10.028.

Fabian, M. R., N. Sonenberg, and W. Filipowicz. 2010a. Regulation of mRNA Translation and Stability by microRNAs. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.1146/annurev-biochem-060308-103103. 79:351–379. doi:10.1146/ANNUREV-BIOCHEM-060308-103103. Available from: https://www-annualreviews-org.login.ezproxy.library.ualberta.ca/doi/abs/10.1146/annurev-biochem-060308-103103

Fabian, M. R., N. Sonenberg, and W. Filipowicz. 2010b. Regulation of mRNA Translation and Stability by microRNAs. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.1146/annurev-biochem-060308-103103. 79:351–379. doi:10.1146/ANNUREV-BIOCHEM-060308-103103. Available from: https://www-annualreviews-org.login.ezproxy.library.ualberta.ca/doi/abs/10.1146/annurev-biochem-060308-103103

Feuz, D. M., and W. J. Umberger. 2003. Beef cow-calf production. Veterinary Clinics: Food Animal Practice. 19:339–363. doi:10.1016/S0749-0720(03)00023-9. Available from: http://www.vetfood.theclinics.com/article/S0749072003000239/fulltext

Filella, X., and L. Foj. 2017. miRNAs as novel biomarkers in the management of prostate cancer. Clinical Chemistry and Laboratory Medicine (CCLM). 55:715–736. doi:10.1515/CCLM-2015-1073. Available from: https://www-degruyter-com.login.ezproxy.library.ualberta.ca/document/doi/10.1515/cclm-2015-1073/html

Filipowicz, W., S. N. Bhattacharyya, and N. Sonenberg. 2008. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nature Reviews Genetics 2008 9:2. 9:102–114. doi:10.1038/NRG2290. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/nrg2290

Flower, F. C., and D. M. Weary. 2006. Effect of Hoof Pathologies on Subjective Assessments of Dairy Cow Gait. Journal of Dairy Science. 89:139–146. doi:10.3168/JDS.S0022-0302(06)72077-X.

Focus on Herd Health: Hoof Health - Issuu. Available from: https://issuu.com/ontariobeefmagazine/docs/ontbeef_feb2020_final_lr/s/10255914

Forero, D. A., Y. González-Giraldo, L. J. Castro-Vega, and G. E. Barreto. 2019. qPCR-based methods for expression analysis of miRNAs. Biotechniques. 67:192–199. doi:10.2144/BTN-2019-0065/ASSET/IMAGES/LARGE/FIGURE4.JPEG. Available from: https://www.future-science.com/doi/abs/10.2144/btn-2019-0065

Garbarino, E. J., J. A. Hernandez, J. K. Shearer, C. A. Risco, and W. W. Thatcher. 2004. Effect of Lameness on Ovarian Activity in Postpartum Holstein Cows. Journal of Dairy Science. 87:4123–4131. doi:10.3168/JDS.S0022-0302(04)73555-9.

Gasparello, J., C. Papi, M. Allegretti, E. Giordani, F. Carboni, S. Zazza, E. Pescarmona, P. Romania, P. Giacomini, C. Scapoli, R. Gambari, and A. Finotti. 2020. A Distinctive microRNA (miRNA) Signature in the Blood of Colorectal Cancer (CRC) Patients at Surgery. Cancers 2020, Vol. 12, Page 2410. 12:2410. doi:10.3390/CANCERS12092410. Available from: https://www.mdpi.com/2072-6694/12/9/2410/htm

Giraldez, Maria D., R. M. Spengler, A. Etheridge, P. M. Godoy, A. J. Barczak, S. Srinivasan, P. L. de Hoff, K. Tanriverdi, A. Courtright, S. Lu, J. Khoory, R. Rubio, D. Baxter, T. A. P. Driedonks, H. P. J. Buermans, E. N. M. Nolte-'T Hoen, H. Jiang, K. Wang, I. Ghiran, Y. E. Wang, K. van Keuren-Jensen, J. E. Freedman, P. G. Woodruff, L. C. Laurent, D. J. Erle, D. J. Galas, and M. Tewari. 2018. Comprehensive multi-center assessment of accuracy, reproducibility and bias of small RNA-seq methods for quantitative miRNA profiling. Nat Biotechnol. 36:746. doi:10.1038/NBT.4183. Available from: /pmc/articles/PMC6078798/

Giraldez, Maria D, R. M. Spengler, A. Etheridge, P. M. Godoy, A. J. Barczak, S. Srinivasan, P. L. de Hoff, K. Tanriverdi, A. Courtright, S. Lu, J. Khoory, R. Rubio, D. Baxter, T. A. P. Driedonks, H. P. J. Buermans, E. N. M. Nolte-'t Hoen, H. Jiang, K. Wang, I. Ghiran, Y. E. Wang, K. van Keuren-Jensen, J. E. Freedman, P. G. Woodruff, L. C. Laurent, D. J. Erle, D. J. Galas, and M. Tewari. 2018. Comprehensive multi-center assessment of small RNA-seq methods for quantitative miRNA profiling. Nature Biotechnology 2018 36:8. 36:746–757. doi:10.1038/NBT.4183. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/nbt.4183

Golan-Gerstl, R., Y. E. Shiff, V. Moshayoff, D. Schecter, D. Leshkowitz, and S. Reif. 2017. Characterization and biological function of milk-derived miRNAs. Molecular Nutrition & Food Research. 61:1700009. doi:10.1002/MNFR.201700009. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/mnfr.201700009

Gomez, A., N. B. Cook, N. D. Bernardoni, J. Rieman, A. F. Dusick, R. Hartshorn, M. T. Socha, D. H. Read, and D. Döpfer. 2012. An experimental infection model to induce digital dermatitis infection in cattle. Journal of Dairy Science. 95:1821–1830. doi:10.3168/JDS.2011-4754.

Green, L., Z. E. Barker, J. Scaife, J. Amory, and M. Speijers. 2006. RISK FACTORS ASSOCIATED WITH FOOT LAMENESS IN DAIRY CATTLE AND A SUGGESTED APPROACH FOR LAMENESS REDUCTION. undefined.

Greenwood, P. L. 2021. Review: An overview of beef production from pasture and feedlot globally, as demand for beef and the need for sustainable practices increase. Animal. 15:100295. doi:10.1016/J.ANIMAL.2021.100295.

Griffin, D. 2014. The monster we don't see: subclinical BRD in beef cattle. Animal Health Research Reviews. 15:138–141. doi:10.1017/S1466252314000255. Available from: https://www-cambridge-org.login.ezproxy.library.ualberta.ca/core/journals/animal-health-research-reviews/article/monster-we-dont-see-subclinical-brd-in-beef-cattle/013BE1899D30E2807F7B4D1C9530BF7A

Griffin, D., L. Perino, and D. Hudson. 1993a. G93-1159 Feedlot Lameness. Available from: https://digitalcommons.unl.edu/extensionhist

Griffin, D., L. Perino, and D. Hudson. 1993b. G93-1159 Feedlot Lameness. Available from:

https://digitalcommons.unl.edu/extensionhist

Guo, H., N. T. Ingolia, J. S. Weissman, and D. P. Bartel. 2010. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 2010 466:7308. 466:835–840. doi:10.1038/nature09267. Available from: https://www.nature.com/articles/nature09267

Gupta, S. K., P. H. Maclean, S. Ganesh, D. Shu, B. M. Buddle, D. N. Wedlock, and A. Heiser. 2018. Detection of microRNA in cattle serum and their potential use to diagnose severity of Johne's disease. Journal of Dairy Science. 101:10259–10270. doi:10.3168/JDS.2018-14785.

Hannon, S. J., S. A. Brault, S. J. G. Otto, P. S. Morley, T. A. McAllister, C. W. Booker, and S. P. Gow. 2020. Feedlot Cattle Antimicrobial Use Surveillance Network: A Canadian Journey. Frontiers in Veterinary Science. 7:968. doi:10.3389/FVETS.2020.596042/BIBTEX.

Hansen, E. P., H. Kringel, S. M. Thamsborg, A. Jex, and P. Nejsum. 2016. Profiling circulating miRNAs in serum from pigs infected with the porcine whipworm, Trichuris suis. Veterinary Parasitology. 223:30–33. doi:10.1016/J.VETPAR.2016.03.025.

Hao, N.-B., Y.-F. He, X.-Q. Li, K. Wang, and R.-L. Wang. 2017. The role of miRNA and lncRNA in gastric cancer. Oncotarget. 8:81572. doi:10.18632/ONCOTARGET.19197. Available from: /pmc/articles/PMC5655310/

Härdi-Landerer, M. C., A. C. Stoffel, S. Dürr, and A. Steiner. 2019. [Footbath as treatment of footrot in sheep. Current -situation on Swiss sheep farms]. Schweizer Archiv fur Tierheilkunde. 161:377–386. doi:10.17236/SAT00210. Available from: https://europepmc-org.login.ezproxy.library.ualberta.ca/article/med/31145680

Holman, D. B., E. Timsit, S. Amat, D. W. Abbott, A. G. Buret, and T. W. Alexander. 2017. The nasopharyngeal microbiota of beef cattle before and after transport to a feedlot. BMC Microbiology. 17:1–12. doi:10.1186/S12866-017-0978-6/TABLES/1. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12866-017-0978-6

Holzhauer, M., C. Hardenberg, C. J. M. Bartels, and K. Frankena. 2006. Herd- and Cow-Level Prevalence of Digital Dermatitis in The Netherlands and Associated Risk Factors. Journal of Dairy Science. 89:580–588. doi:10.3168/JDS.S0022-0302(06)72121-X.

Hu, H., Q. Jia, J. Xi, B. Zhou, and Z. Li. 2020. Integrated analysis of lncRNA, miRNA and mRNA

reveals novel insights into the fertility regulation of large white sows. BMC Genomics 2020 21:1. 21:1–15. doi:10.1186/S12864-020-07055-2. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-020-07055-2

Huang, T. H., J. J. Uthe, S. M. D. Bearson, C. Y. Demirkale, D. Nettleton, S. Knetter, C. Christian, A. E. Ramer-Tait, M. J. Wannemuehler, and C. K. Tuggle. 2011. Distinct Peripheral Blood RNA Responses to Salmonella in Pigs Differing in Salmonella Shedding Levels: Intersection of IFNG, TLR and miRNA Pathways. PLOS ONE. 6:e28768. doi:10.1371/JOURNAL.PONE.0028768. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0028768

Ioannidis, J., and F. X. Donadeu. 2016. Circulating miRNA signatures of early pregnancy in cattle. BMC Genomics 2016 17:1. 17:1–12. doi:10.1186/S12864-016-2529-1. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-016-2529-1

Ioannidis, J., E. Sánchez-Molano, A. Psifidi, F. X. Donadeu, and G. Banos. 2018. Association of plasma microRNA expression with age, genetic background and functional traits in dairy cattle. Scientific Reports 2018 8:1. 8:1–10. doi:10.1038/S41598-018-31099-W. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-31099-w

Iorio, M. v., M. Ferracin, C.-G. Liu, A. Veronese, R. Spizzo, S. Sabbioni, E. Magri, M. Pedriali, M. Fabbri, M. Campiglio, S. Ménard, J. P. Palazzo, A. Rosenberg, P. Musiani, S. Volinia, I. Nenci, G. A. Calin, P. Querzoli, M. Negrini, and C. M. Croce. 2005. MicroRNA Gene Expression Deregulation in Human Breast Cancer. Cancer Research. 65:7065–7070. doi:10.1158/0008-5472.CAN-05-1783. Available from: https://cancerres-aacrjournals-org.login.ezproxy.library.ualberta.ca/content/65/16/7065

Isabel Gessner, J. W. U. Fries, Veronika Brune, and Sanjay Mathur. 2021. Magnetic nanoparticle-based amplification of microRNA detection in body fluids for early disease diagnosis. Journal of Materials Chemistry B. 9:9–22. doi:10.1039/D0TB02165B. Available from: https://pubs-rsc-org.login.ezproxy.library.ualberta.ca/en/content/articlehtml/2021/tb/d0tb02165b

Jacobs, C., K. Orsel, and H. W. Barkema. 2017. Prevalence of digital dermatitis in young stock in Alberta, Canada, using pen walks. Journal of Dairy Science. 100:9234–9244.

doi:10.3168/JDS.2017-13044.

Jamaluddin, M. S., S. M. Weakley, L. Zhang, P. Kougias, P. H. Lin, Q. Yao, and C. Chen. 2014. miRNAs: roles and clinical applications in vascular disease. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.1586/erm.10.103. 11:79–89. doi:10.1586/ERM.10.103. Available from: https://www-tandfonline-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1586/erm.10.103

Jelinski, M., K. Fenton, T. Perrett, and C. Paetsch. 2016a. Epidemiology of toe tip necrosis syndrome (TTNS) of North American feedlot cattle. The Canadian Veterinary Journal. 57:829. Available from: /pmc/articles/PMC4944558/

Jelinski, M., K. Fenton, T. Perrett, and C. Paetsch. 2016b. Epidemiology of toe tip necrosis syndrome (TTNS) of North American feedlot cattle. The Canadian Veterinary Journal. 57:829. Available from: /pmc/articles/PMC4944558/

Jelinski, M., K. Fenton, T. Perrett, and C. Paetsch. 2016c. Epidemiology of toe tip necrosis syndrome (TTNS) of North American feedlot cattle. The Canadian Veterinary Journal. 57:829. Available from: /pmc/articles/PMC4944558/

Jelinski, M., K. Fenton, T. Perrett, and C. Paetsch. 2016d. Epidemiology of toe tip necrosis syndrome (TTNS) of North American feedlot cattle. The Canadian Veterinary Journal. 57:829. Available from: /pmc/articles/PMC4944558/

Jeon, J., E. Olkhov-Mitsel, H. Xie, C. Q. Yao, F. Zhao, S. Jahangiri, C. Cuizon, S. Scarcello, R. Jeyapala, J. D. Watson, M. Fraser, J. Ray, K. Commisso, A. Loblaw, N. E. Fleshner, R. G. Bristow, M. Downes, D. Vesprini, S. Liu, B. Bapat, and P. C. Boutros. 2020. Temporal Stability and Prognostic Biomarker Potential of the Prostate Cancer Urine miRNA Transcriptome. JNCI: Journal of the National Cancer Institute. 112:247–255. doi:10.1093/JNCI/DJZ112. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/jnci/article/112/3/247/5510559

Jiang, L., and J. Zhu. 2020. Review of MiRNA-Disease Association Prediction. Current Protein & Peptide Science. 21:1044–1053. doi:10.2174/1389203721666200210102751.

Johansen, I., and R. Andreassen. 2014. Validation of miRNA genes suitable as reference genes in qPCR analyses of miRNA gene expression in Atlantic salmon (Salmo salar). BMC Research Notes.

7:1–9. doi:10.1186/1756-0500-7-945/FIGURES/1. Available from: https://bmcresnotes-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/1756-0500-7-945

John, B., A. J. Enright, A. Aravin, T. Tuschl, C. Sander, and D. S. Marks. 2004. Human MicroRNA Targets. PLOS Biology. 2:e363. doi:10.1371/JOURNAL.PBIO.0020363. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosbiology/article?id=10.1371/journal.pbio.0020363

Johnston, J. D., D. J. R. Eichhorn, S. A. Kontulainen, S. D. Noble, and M. D. Jelinski. 2019a. Investigation of white line separation under load in bovine claws with and without toe-tip necrosis. American Journal of Veterinary Research. 80:736–742. doi:10.2460/AJVR.80.8.736. Available from: https://avmajournals-avma-org.login.ezproxy.library.ualberta.ca/view/journals/ajvr/80/8/ajvr.80.8.736.xml

Johnston, J. D., D. J. R. Eichhorn, S. A. Kontulainen, S. D. Noble, and M. D. Jelinski. 2019b. Investigation of white line separation under load in bovine claws with and without toe-tip necrosis. https://doi-org.login.ezproxy.library.ualberta.ca/10.2460/ajvr.80.8.736. 80:736–742. doi:10.2460/AJVR.80.8.736.

Kara, N. K., A. Galic, and M. Koyuncu. 2011. Effects of stall type and bedding materials on lameness and hygiene score and effect of lameness on some reproductive problems in dairy cattle. http://www-tandfonline-com.login.ezproxy.library.ualberta.ca/action/journalInformation?show=aimsScope&journalCode=taar20#.VsXoziCLRhE. 39:334–338. doi:10.1080/09712119.2011.607890. Available from: https://www-tandfonline-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1080/09712119.2011.607890

Kausche, F., E. Robb, R. Bergman, and M. Eysker. 2003. A comprehensive review of ceftiofur sodium and hydrochloride formulations for treatment of acute bovine foot rot. Veterinary Therapeutics : Research in Applied Veterinary Medicine. 4:83–93. Available from: https://europepmc-org.login.ezproxy.library.ualberta.ca/article/med/12756639

Kosaka, N., H. Iguchi, and T. Ochiya. 2010. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Science. 101:2087–2092. doi:10.1111/J.1349-7006.2010.01650.X. Available from: https://onlinelibrary-wiley-

com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/j.1349-7006.2010.01650.x

Kozomara, A., M. Birgaoanu, and S. Griffiths-Jones. 2019. miRBase: from microRNA sequences to function. Nucleic Acids Research. 47:D155–D162. doi:10.1093/NAR/GKY1141. Available from: https://academic.oup.com/nar/article/47/D1/D155/5179337

Kumar, P., Z. Dezso, C. MacKenzie, J. Oestreicher, S. Agoulnik, M. Byrne, F. Bernier, M. Yanagimachi, K. Aoshima, and Y. Oda. 2013a. Circulating miRNA Biomarkers for Alzheimer's Disease. PLOS ONE. 8:e69807. doi:10.1371/JOURNAL.PONE.0069807. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0069807

Kumar, P., Z. Dezso, C. MacKenzie, J. Oestreicher, S. Agoulnik, M. Byrne, F. Bernier, M. Yanagimachi, K. Aoshima, and Y. Oda. 2013b. Circulating miRNA Biomarkers for Alzheimer's Disease. PLOS ONE. 8:e69807. doi:10.1371/JOURNAL.PONE.0069807. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0069807

Kumar, R., H. A. Lardner, J. J. McKinnon, D. A. Christensen, D. Damiran, and K. Larson. 2012. Comparison of alternative backgrounding systems on beef calf performance, feedlot finishing performance, carcass traits, and system cost of gain. The Professional Animal Scientist. 28:541–551. doi:10.15232/S1080-7446(15)30403-4.

Kupsco, A., D. Prada, D. Valvi, L. Hu, M. S. Petersen, B. Coull, P. Grandjean, P. Weihe, and A. A. Baccarelli. 2021. Human milk extracellular vesicle miRNA expression and associations with maternal characteristics in a population-based cohort from the Faroe Islands. Scientific Reports 2021 11:1. 11:1–17. doi:10.1038/S41598-021-84809-2. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-021-84809-2

Kutwin, P., T. Konecki, E. M. Borkowska, M. Traczyk-Borszyńska, and Z. Jabłonowski. 2018. Urine miRNA as a potential biomarker for bladder cancer detection – a meta-analysis. Central European Journal of Urology. 71:177. doi:10.5173/CEJU.2018.1605. Available from: /pmc/articles/PMC6051360/

Ladomery, M. R., D. G. Maddocks, and I. D. Wilson. 2011. MicroRNAs: their discovery,

biogenesis, function and potential use as biomarkers in non-invasive prenatal diagnostics. International Journal of Molecular Epidemiology and Genetics. 2:253. Available from: /pmc/articles/PMC3166153/

Lean, I. J., C. T. Westwood, H. M. Golder, and J. J. Vermunt. 2013. Impact of nutrition on lameness and claw health in cattle. Livestock Science. 156:71–87. doi:10.1016/J.LIVSCI.2013.06.006.

Leão, R., T. van Agthoven, A. Figueiredo, M. A. S. Jewett, K. Fadaak, J. Sweet, A. E. Ahmad, L. Anson-Cartwright, P. Chung, A. Hansen, P. Warde, P. Castelo-Branco, M. O'Malley, P. L. Bedard, L. H. J. Looijenga, and R. J. Hamilton. 2018. Serum miRNA Predicts Viable Disease after Chemotherapy in Patients with Testicular Nonseminoma Germ Cell Tumor. Journal of Urology. 200:126–135. doi:10.1016/J.JURO.2018.02.068.

Lee, J., Soohyun Lee, J. Son, H. Lim, E. Kim, D. Kim, S. Ha, T. Hur, Seunghwan Lee, and I. Choi. 2020. Analysis of circulating-microRNA expression in lactating Holstein cows under summer heat stress. PLOS ONE. 15:e0231125. doi:10.1371/JOURNAL.PONE.0231125. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0231125

Lee, R. C., R. L. Feinbaum, and V. Ambros. 1993. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 75:843–854. doi:10.1016/0092-8674(93)90529-Y.

Lee, T., K. Good, W. Jamieson, M. Quinn, and A. Krishnamurthy. 2017. Cattle and Carnivore Coexistence in Alberta: The Role of Compensation Programs. Rangelands. 39:10–16. doi:10.1016/J.RALA.2016.11.002.

Leidinger, P., C. Backes, S. Deutscher, K. Schmitt, S. C. Mueller, K. Frese, J. Haas, K. Ruprecht, F. Paul, C. Stähler, C. J. Lang, B. Meder, T. Bartfai, E. Meese, and A. Keller. 2013. A blood based 12-miRNA signature of Alzheimer disease patients. Genome Biology 2013 14:7. 14:1–16. doi:10.1186/GB-2013-14-7-R78. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/articles/10.1186/gb-2013-14-7-r78

Lekchnov, E. A., E. v. Amelina, O. E. Bryzgunova, I. A. Zaporozhchenko, M. Yu. Konoshenko,

S. v. Yarmoschuk, I. S. Murashov, O. A. Pashkovskaya, A. M. Gorizkii, A. A. Zheravin, and P. P. Laktionov. 2018. Searching for the Novel Specific Predictors of Prostate Cancer in Urine: The Analysis of 84 miRNA Expression. International Journal of Molecular Sciences 2018, Vol. 19, Page 4088. 19:4088. doi:10.3390/IJMS19124088. Available from: https://www.mdpi.com/1422-0067/19/12/4088/htm

Li, Q., C. Yang, J. Du, B. Zhang, Y. He, Q. Hu, M. Li, Y. Zhang, C. Wang, and J. Zhong. 2018a. Characterization of miRNA profiles in the mammary tissue of dairy cattle in response to heat stress. BMC Genomics. 19:1–11. doi:10.1186/S12864-018-5298-1/TABLES/5. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-018-5298-1

Li, Q., C. Yang, J. Du, B. Zhang, Y. He, Q. Hu, M. Li, Y. Zhang, C. Wang, and J. Zhong. 2018b. Characterization of miRNA profiles in the mammary tissue of dairy cattle in response to heat stress. BMC Genomics 2018 19:1. 19:1–11. doi:10.1186/S12864-018-5298-1. Available from: https://link.springer.com/articles/10.1186/s12864-018-5298-1

Lodewijk, L., A. M. Prins, J. W. Kist, G. D. Valk, O. Kranenburg, I. H. M. B. Rinkes, and M. R. Vriens. 2012. The value of miRNA in diagnosing thyroid cancer: A systematic review. Cancer Biomarkers. 11:229–238. doi:10.3233/CBM-2012-0273.

Logue, D. N., T. Gibert, T. Parkin, S. Thomson, and D. J. Taylor. 2012. A field evaluation of a footbathing solution for the control of digital dermatitis in cattle. The Veterinary Journal. 193:664–668. doi:10.1016/J.TVJL.2012.06.050.

Luoreng, Z.-M., X.-P. Wang, C.-G. Mei, and L.-S. Zan. 2018. Expression profiling of peripheral blood miRNA using RNAseq technology in dairy cows with Escherichia coli-induced mastitis. Scientific Reports 2018 8:1. 8:1–10. doi:10.1038/S41598-018-30518-2. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-30518-2

MacFarlane, L.-A., and P. R. Murphy. 2010a. MicroRNA: Biogenesis, Function and Role in Cancer. Current Genomics. 11:537. doi:10.2174/138920210793175895. Available from: /pmc/articles/PMC3048316/

MacFarlane, L.-A., and P. R. Murphy. 2010b. MicroRNA: Biogenesis, Function and Role in Cancer. Current Genomics. 11:537. doi:10.2174/138920210793175895. Available from:

/pmc/articles/PMC3048316/

Maertens, W., J. Vangeyte, J. Baert, A. Jantuan, K. C. Mertens, S. de Campeneere, A. Pluk, G. Opsomer, S. van Weyenberg, and A. van Nuffel. 2011. Development of a real time cow gait tracking and analysing tool to assess lameness using a pressure sensitive walkway: The GAITWISE system. Biosystems Engineering. 110:29–39. doi:10.1016/J.BIOSYSTEMSENG.2011.06.003.

Manson, F. J., and J. D. Leaver. 1988. The influence of concentrate amount on locomotion and clinical lameness in dairy cattle. Animal Science. 47:185–190. doi:10.1017/S0003356100003251. Available from: https://www.cambridge.org/core/journals/animal-science/article/abs/influence-of-concentrate-amount-on-locomotion-and-clinical-lameness-in-dairy-cattle/983225C537C45191FD0EDBA758972761

Marini, F., E. Luzi, and M. L. Brandi. 2011. MicroRNA role in thyroid cancer development. Journal of Thyroid Research. 2011. doi:10.4061/2011/407123.

Mayes, C., R. Houston, S. Seashols-Williams, B. LaRue, and S. Hughes-Stamm. 2019. The stability and persistence of blood and semen mRNA and miRNA targets for body fluid identification in environmentally challenged and laundered samples. Legal Medicine. 38:45–50. doi:10.1016/J.LEGALMED.2019.03.007.

McDaneld, T. G. 2009. MicroRNA: Mechanism of gene regulation and application to livestock. Journal of Animal Science. 87:E21–E28. doi:10.2527/JAS.2008-1303. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/jas/article/87/suppl_14/E21/4731144

McGuire, A., J. A. L. Brown, and M. J. Kerin. 2015a. Metastatic breast cancer: the potential of miRNA for diagnosis and treatment monitoring. Cancer and Metastasis Reviews 2015 34:1. 34:145–155. doi:10.1007/S10555-015-9551-7. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s10555-015-9551-7

McGuire, A., J. A. L. Brown, and M. J. Kerin. 2015b. Metastatic breast cancer: the potential of miRNA for diagnosis and treatment monitoring. Cancer and Metastasis Reviews 2015 34:1. 34:145–155. doi:10.1007/S10555-015-9551-7. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s10555-015-9551-7

McIver, S. C., S. D. Roman, B. Nixon, and E. A. McLaughlin. 2012. miRNA and mammalian male germ cells. Human Reproduction Update. 18:44–59. doi:10.1093/HUMUPD/DMR041. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/humupd/article/18/1/44/855033

McMillan, S., G. Penner, J. McKinnon, K. Larson, F. Añez-Osuna, D. Damiran, and H. Lardner. 2018. Use of extensive winter feeding systems for backgrounding beef calves and the effect on finishing. doi:10.15232/pas.2017-01614. Available from: https://doi.org/10.15232/pas.2017-01614

Meister, G., M. Landthaler, A. Patkaniowska, Y. Dorsett, G. Teng, and T. Tuschl. 2004. Human Argonaute2 Mediates RNA Cleavage Targeted by miRNAs and siRNAs. Molecular Cell. 15:185–197. doi:10.1016/J.MOLCEL.2004.07.007.

Mercadal, M., C. Herrero, O. López-Rodrigo, M. Castells, A. de la Fuente, F. Vigués, L. Bassas, and S. Larriba. 2020. Impact of Extracellular Vesicle Isolation Methods on Downstream miRNA Analysis in Semen: A Comparative Study. International Journal of Molecular Sciences 2020, Vol. 21, Page 5949. 21:5949. doi:10.3390/IJMS21175949. Available from: https://www.mdpi.com/1422-0067/21/17/5949/htm

van Metre, D. C. 2017. Pathogenesis and Treatment of Bovine Foot Rot. Veterinary Clinics of North America: Food Animal Practice. 33:183–194. doi:10.1016/J.CVFA.2017.02.003.

Meyer, S. U., M. W. Pfaffl, and S. E. Ulbrich. 2010. Normalization strategies for microRNA profiling experiments: a 'normal' way to a hidden layer of complexity? Biotechnology Letters 2010 32:12. 32:1777–1788. doi:10.1007/S10529-010-0380-Z. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s10529-010-0380-z

Mulrane, L., S. F. McGee, W. M. Gallagher, and D. P. O'Connor. 2013a. miRNA Dysregulation in Breast Cancer. Cancer Research. 73:6554–6562. doi:10.1158/0008-5472.CAN-13-1841. Available from: https://cancerres-aacrjournals-org.login.ezproxy.library.ualberta.ca/content/73/22/6554

Mulrane, L., S. F. McGee, W. M. Gallagher, and D. P. O'Connor. 2013b. miRNA Dysregulation in Breast Cancer. Cancer Research. 73:6554–6562. doi:10.1158/0008-5472.CAN-13-1841.

Available from: https://cancerres-aacrjournals-org.login.ezproxy.library.ualberta.ca/content/73/22/6554

Murakami, Y., H. Toyoda, T. Tanahashi, J. Tanaka, T. Kumada, Y. Yoshioka, N. Kosaka, T. Ochiya, and Y. Taguchi. 2012. Comprehensive miRNA Expression Analysis in Peripheral Blood Can Diagnose Liver Disease. PLOS ONE. 7:e48366. doi:10.1371/JOURNAL.PONE.0048366. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0048366

Nagaraja, T. G., and K. F. Lechtenberg. 2007. Acidosis in Feedlot Cattle. Veterinary Clinics of North America: Food Animal Practice. 23:333–350. doi:10.1016/J.CVFA.2007.04.002.

Nagl, S., M. Schaeferling, and O. S. Wolfbeis. 2005. Fluorescence Analysis in Microarray Technology. Microchimica Acta 2005 151:1. 151:1–21. doi:10.1007/S00604-005-0393-9. Available from: https://link.springer.com/article/10.1007/s00604-005-0393-9

Nordlund, K. v., N. B. Cook, and G. R. Oetzel. 2004. Investigation Strategies for Laminitis Problem Herds. Journal of Dairy Science. 87:E27–E35. doi:10.3168/JDS.S0022-0302(04)70058-2.

Norring, M., J. Häggman, H. Simojoki, P. Tamminen, C. Winckler, and M. Pastell. 2014a. Short communication: Lameness impairs feeding behavior of dairy cows. Journal of Dairy Science. 97:4317–4321. doi:10.3168/JDS.2013-7512.

Norring, M., J. Häggman, H. Simojoki, P. Tamminen, C. Winckler, and M. Pastell. 2014b. Short communication: Lameness impairs feeding behavior of dairy cows. Journal of Dairy Science. 97:4317–4321. doi:10.3168/JDS.2013-7512.

Norsworthy, P. J., A. G. B. Thompson, T. H. Mok, F. Guntoro, L. C. Dabin, A. Nihat, R. W. Paterson, J. M. Schott, J. Collinge, S. Mead, and E. A. Viré. 2020. A blood miRNA signature associates with sporadic Creutzfeldt-Jakob disease diagnosis. Nature Communications 2020 11:1. 11:1–11. doi:10.1038/S41467-020-17655-X. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41467-020-17655-x

van Nuffel, A., W. Saeys, B. Sonck, J. Vangeyte, K. C. Mertens, B. de Ketelaere, and S. van Weyenberg. 2015. Variables of gait inconsistency outperform basic gait variables in detecting

mildly lame cows. Livestock Science. 177:125–131. doi:10.1016/J.LIVSCI.2015.04.008.

van Nuffel, A., J. Vangeyte, K. C. Mertens, L. Pluym, S. de Campeneere, W. Saeys, G. Opsomer, and S. van Weyenberg. 2013. Exploration of measurement variation of gait variables for early lameness detection in cattle using the GAITWISE. Livestock Science. 156:88–95. doi:10.1016/J.LIVSCI.2013.06.013.

Nuffel, A. van, I. Zwertvaegher, L. Pluym, S. van Weyenberg, V. M. Thorup, M. Pastell, B. Sonck, and W. Saeys. 2015. Lameness Detection in Dairy Cows: Part 1. How to Distinguish between Non-Lame and Lame Cows Based on Differences in Locomotion or Behavior. Animals 2015, Vol. 5, Pages 838-860. 5:838–860. doi:10.3390/ANI5030387. Available from: https://www.mdpi.com/2076-2615/5/3/387/htm

of Alberta – Agriculture, G., F. Economics, and C. Branch. 2020. July 1 2020 livestock inventory estimates : Alberta/Canada highlights.

Okamura, K., A. Ishizuka, H. Siomi, and M. C. Siomi. 2004. Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways. Genes & Development. 18:1655–1666. doi:10.1101/GAD.1210204. Available from: http://genesdev.cshlp.org.login.ezproxy.library.ualberta.ca/content/18/14/1655.full

O'Leary, N. W., D. T. Byrne, A. H. O'Connor, and L. Shalloo. 2020. Invited review: Cattle lameness detection with accelerometers. Journal of Dairy Science. 103:3895–3911. doi:10.3168/JDS.2019-17123.

Paetsch, C., K. Fenton, T. Perrett, E. Janzen, T. Clark, J. Shearer, and M. Jelinski. 2017. Prospective case-control study of toe tip necrosis syndrome (TTNS) in western Canadian feedlot cattle. The Canadian Veterinary Journal. 58:247. Available from: /pmc/articles/PMC5302198/

Peterson, E. B., D. R. Strohbehn, G. W. Ladd, and R. L. Willham. 1989. Effects of Preconditioning on Performance of Beef Calves before and after Entering the Feedlot. Journal of Animal Science. 67:1678–1686. doi:10.2527/JAS1989.6771678X. Available from: https://academic.oup.com/jas/article/67/7/1678/4696962

Phillips, C. 2007. Cattle Behaviour and Welfare: Second Edition. Cattle Behaviour and Welfare: Second Edition. 1–264. doi:10.1002/9780470752418. Available from:

https://onlinelibrary.wiley.com/doi/book/10.1002/9780470752418

Plummer, P. J., and A. Krull. 2017. Clinical Perspectives of Digital Dermatitis in Dairy and Beef Cattle. Veterinary Clinics of North America: Food Animal Practice. 33:165–181. doi:10.1016/J.CVFA.2017.02.002.

Pogue, S. J., R. Kröbel, H. H. Janzen, K. A. Beauchemin, G. Legesse, D. M. de Souza, M. Iravani, C. Selin, J. Byrne, and T. A. McAllister. 2018. Beef production and ecosystem services in Canada's prairie provinces: A review. Agricultural Systems. 166:152–172. doi:10.1016/J.AGSY.2018.06.011.

Poursaberi, A., C. Bahr, A. Pluk, D. Berckmans, I. Veermäe, E. Kokin, and V. Pokalainen. 2011. Online lameness detection in dairy cattle using Body Movement Pattern (BMP). International Conference on Intelligent Systems Design and Applications, ISDA. 732–736. doi:10.1109/ISDA.2011.6121743.

Pradervand, S., J. Weber, F. Lemoine, F. Consales, A. Paillusson, M. Dupasquier, J. Thomas, H. Richter, H. Kaessmann, E. Beaudoing, O. Hagenbüchle, and K. Harshman. 2018. Concordance among digital gene expression, microarrays, and qPCR when measuring differential expression of microRNAs. https://doi.org/10.2144/000113367. 48:219–222. doi:10.2144/000113367. Available from: https://www.future-science.com/doi/abs/10.2144/000113367

Prastiwi, A., Z. Okumuş, A. Hayirli, D. Çelebi, L. E. Yanmaz, E. Doğan, M. G. Şenocak, and U. Ersoz. 2019. Effectiveness of Copper Sulfate for Footbath Solutions in Dairy Cattle. Atatürk University Journal of Veterinary Sciences. 14:238–245. doi:10.17094/ATAUNIVBD.538342. Available from: https://dergipark.org.tr/en/pub/ataunivbd/538342

Qadir, M. I., and A. Faheem. 2017. miRNA: A Diagnostic and Therapeutic Tool for Pancreatic Cancer. Critical Reviews™ in Eukaryotic Gene Expression. 27:197–204. doi:10.1615/CRITREVEUKARYOTGENEEXPR.2017019494. Available from: https://www-dl-begellhouse-com.login.ezproxy.library.ualberta.ca/journals/6dbf508d3b17c437,3e1db9ff199ae23c,4c2b35b40e0bd753.html

Qiao, Y., H. Kong, C. Clark, S. Lomax, D. Su, S. Eiffert, and S. Sukkarieh. 2021. Intelligent

Perception-Based Cattle Lameness Detection and Behaviour Recognition: A Review. Animals 2021, Vol. 11, Page 3033. 11:3033. doi:10.3390/ANI11113033. Available from: https://www.mdpi.com/2076-2615/11/11/3033/htm

Qiu, J., W. Zhang, C. Zang, X. Liu, F. Liu, R. Ge, Y. Sun, Q. Xia, J. Qiu, W. Zhang, C. Zang, X. Liu, F. Liu, R. Ge, Y. Sun, and Q. Xia. 2018. Identification of key genes and miRNAs markers of papillary thyroid cancer. Biological Research. 51:45. doi:10.1186/S40659-018-0188-1. Available from: http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0716-97602018000100237&lng=es&nrm=iso&tlng=en

Quan, S., X. Nan, K. Wang, L. Jiang, J. Yao, and B. Xiong. 2020. Characterization of Sheep Milk Extracellular Vesicle-miRNA by Sequencing and Comparison with Cow Milk. Animals 2020, Vol. 10, Page 331. 10:331. doi:10.3390/ANI10020331. Available from: https://www.mdpi.com/2076-2615/10/2/331/htm

RA, L., and L. DN. 2006. Treatment strategies for digital dermatitis for the UK. Vet J. 171:79–88. doi:10.1016/J.TVJL.2004.08.009. Available from: https://pubmed.ncbi.nlm.nih.gov/16427584/

Rapado-González, Ó., B. Majem, A. Álvarez-Castro, R. Díaz-Peña, A. Abalo, L. Suárez-Cabrera, A. Gil-Moreno, A. Santamaría, R. López-López, L. Muinelo-Romay, and M. M. Suarez-Cunqueiro. 2019. A Novel Saliva-Based miRNA Signature for Colorectal Cancer Diagnosis. Journal of Clinical Medicine 2019, Vol. 8, Page 2029. 8:2029. doi:10.3390/JCM8122029. Available from: https://www.mdpi.com/2077-0383/8/12/2029/htm

Read, D. H., and R. L. Walker. 2016. Papillomatous Digital Dermatitis (Footwarts) in California Dairy Cattle: Clinical and Gross Pathologic Findings: http://dx.doi.org/10.1177/104063879801000112. 10:67–76. doi:10.1177/104063879801000112. Available from: https://journals.sagepub.com/doi/abs/10.1177/104063879801000112

Reddy, K. B. 2015. MicroRNA (miRNA) in cancer. Cancer Cell International 2015 15:1. 15:1–6. doi:10.1186/S12935-015-0185-1. Available from: https://cancerci-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12935-015-0185-1

Rhoades, M. W., B. J. Reinhart, L. P. Lim, C. B. Burge, B. Bartel, and D. P. Bartel. 2002. Prediction of Plant MicroRNA Targets. Cell. 110:513–520. doi:10.1016/S0092-8674(02)00863-2.

RW, B., and S. MW. 1988a. Digital dermatitis in dairy cattle. The Veterinary Record. 122:505–508. doi:10.1136/VR.122.21.505. Available from: https://europepmc.org/article/med/3407109

RW, B., and S. MW. 1988b. Digital dermatitis in dairy cattle. The Veterinary Record. 122:505–508. doi:10.1136/VR.122.21.505. Available from: https://europepmc.org/article/med/3407109

SA, H., W. WR, and M. RD. 1993. Effects of lameness on the behaviour of cows during the summer. The Veterinary Record. 132:578–580. doi:10.1136/VR.132.23.578. Available from: https://europepmc.org/article/med/8337801

Satake, E., M. G. Pezzolesi, Z. I. M. Dom, A. M. Smiles, M. A. Niewczas, and A. S. Krolewski. 2018. Circulating miRNA Profiles Associated With Hyperglycemia in Patients With Type 1 Diabetes. Diabetes. 67:1013–1023. doi:10.2337/DB17-1207. Available from: https://diabetes-diabetesjournals-org.login.ezproxy.library.ualberta.ca/content/67/5/1013

Schepeler, T., J. T. Reinert, M. S. Ostenfeld, L. L. Christensen, A. N. Silahtaroglu, L. Dyrskjøt, C. Wiuf, F. J. Sørensen, M. Kruhøffer, S. Laurberg, S. Kauppinen, T. F. Ørntoft, and C. L. Andersen. 2008. Diagnostic and Prognostic MicroRNAs in Stage II Colon Cancer. Cancer Research. 68:6416–6424. doi:10.1158/0008-5472.CAN-07-6110. Available from: https://cancerres-aacrjournals-org.login.ezproxy.library.ualberta.ca/content/68/15/6416

Scheuer, C., E. Boot, N. Carse, A. Clardy, J. Gallagher, S. Heck, S. Marron, L. Martinez-Alvarez, D. Masarykova, P. Mcmillan, F. Murphy, E. Steel, H. van Ekdom, and H. Vecchione. 2010. The origins and consequences of the American feedlot system. G. Balint, B. Antala, C. Carty, J.-M. A. Mabieme, I. B. Amar, and A. Kaplanova, editors. Physical Education and Sport for Children and Youth with Special Needs Researches – Best Practices – Situation. 343–354. doi:10.2/JQUERY.MIN.JS. Available from: https://baylor-ir-tdl-org.login.ezproxy.library.ualberta.ca/handle/2104/8032

Schrank, K., B.-K. Choi, S. Grund, A. Moter, K. Heuner, H. Nattermann, and U. B. Göbel. 1999. Treponema brennaborense sp. nov., a novel spirochaete isolated from a dairy cow suffering from digital dermatitis. International Journal of Systematic and Evolutionary Microbiology. 49:43–50. doi:10.1099/00207713-49-1-43. Available from: https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/00207713-49-1-43

Scott, G. B. 1989. Changes in limb loading with lameness for a number of friesian cattle. British Veterinary Journal. 145:28–38. doi:10.1016/0007-1935(89)90005-5.

Shearer, J. K. 1998. Lameness of dairy cattle: The Bovine Practitioner. 79–85. doi:10.21423/BOVINE-VOL1998NO32.1P79-85. Available from: https://bovine-ojs-tamu-tdl-org.login.ezproxy.library.ualberta.ca/bovine/index.php/bovine/article/view/1563

Shearer, J. K., S. R. van Amstel, and B. W. Brodersen. 2012a. Clinical Diagnosis of Foot and Leg Lameness in Cattle. Veterinary Clinics: Food Animal Practice. 28:535–556. doi:10.1016/J.CVFA.2012.07.003. Available from: http://www.vetfood.theclinics.com/article/S0749072012000540/fulltext

Shearer, J. K., S. R. van Amstel, and B. W. Brodersen. 2012b. Clinical Diagnosis of Foot and Leg Lameness in Cattle. Veterinary Clinics of North America: Food Animal Practice. 28:535–556. doi:10.1016/J.CVFA.2012.07.003.

Shearer, J. K., M. L. Stock, S. R. van Amstel, and J. F. Coetzee. 2013a. Assessment and Management of Pain Associated with Lameness in Cattle. Veterinary Clinics: Food Animal Practice. 29:135–156. doi:10.1016/J.CVFA.2012.11.012. Available from: http://www.vetfood.theclinics.com/article/S0749072012001004/fulltext

Shearer, J. K., M. L. Stock, S. R. van Amstel, and J. F. Coetzee. 2013b. Assessment and Management of Pain Associated with Lameness in Cattle. Veterinary Clinics: Food Animal Practice. 29:135–156. doi:10.1016/J.CVFA.2012.11.012. Available from: http://www.vetfood.theclinics.com/article/S0749072012001004/fulltext

Sheppard, S. C., S. Bittman, G. Donohoe, D. Flaten, K. M. Wittenberg, J. A. Small, R. Berthiaume, T. A. McAllister, K. A. Beauchemin, J. McKinnon, B. D. Amiro, D. Macdonald, F. Mattos, and K. H. Ominski. 2015. Beef cattle husbandry practices across ecoregions of canada in 2011. Canadian Journal of Animal Science. 95:305–321. doi:10.4141/CJAS-2014-158/SUPPL_FILE/CJAS-2014-158-SUPPLTAB1.DOC. Available from: https://cdnsciencepub-com.login.ezproxy.library.ualberta.ca/doi/abs/10.4141/cjas-2014-158

Shiino, S., J. Matsuzaki, A. Shimomura, J. Kawauchi, S. Takizawa, H. Sakamoto, Y. Aoki, M. Yoshida, K. Tamura, K. Kato, T. Kinoshita, Y. Kitagawa, and T. Ochiya. 2019. Serum miRNA–

based Prediction of Axillary Lymph Node Metastasis in Breast Cancer. Clinical Cancer Research. 25:1817–1827. doi:10.1158/1078-0432.CCR-18-1414. Available from: https://clincancerres-aacrjournals-org.login.ezproxy.library.ualberta.ca/content/25/6/1817

Shin, V. Y., and K.-M. Chu. 2014. MiRNA as potential biomarkers and therapeutic targets for gastric cancer. World Journal of Gastroenterology : WJG. 20:10432. doi:10.3748/WJG.V20.I30.10432. Available from: /pmc/articles/PMC4130850/

Smith, G. R., D. Wallace, and D. E. Noakes. 1989. Enhancement of the infectivity of Fusobacterium necrophorum by other bacteria. Epidemiology & Infection. 102:447–458. doi:10.1017/S0950268800030168. Available from: https://www-cambridge-org.login.ezproxy.library.ualberta.ca/core/journals/epidemiology-and-infection/article/enhancement-of-the-infectivity-of-fusobacterium-necrophorum-by-other-bacteria/24DB4FC8DF9B6DCDFD516F2046FBABA9

Sprecher, D. J., D. E. Hostetler, and J. B. Kaneene. 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology. 47:1179–1187. doi:10.1016/S0093-691X(97)00098-8.

Stokka, G. L., K. Lechtenberg, T. Edwards, S. MacGregor, K. Voss, D. Griffin, D. M. Grotelueschen, R. A. Smith, and L. J. Perino. 2001. Lameness in Feedlot Cattle. Veterinary Clinics of North America: Food Animal Practice. 17:189–207. doi:10.1016/S0749-0720(15)30062-1.

Strotbek, M., L. Florin, J. Koenitzer, A. Tolstrup, H. Kaufmann, A. Hausser, and M. A. Olayioye. 2013. Stable microRNA expression enhances therapeutic antibody productivity of Chinese hamster ovary cells. Metabolic Engineering. 20:157–166. doi:10.1016/J.YMBEN.2013.10.005.

Sun, Z., K. Shi, S. Yang, J. Liu, Q. Zhou, G. Wang, J. Song, Z. Li, Z. Zhang, and W. Yuan. 2018. Effect of exosomal miRNA on cancer biology and clinical applications. Molecular Cancer 2018 17:1. 17:1–19. doi:10.1186/S12943-018-0897-7. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12943-018-0897-7

Swarbrick, S., N. Wragg, S. Ghosh, and A. Stolzing. 2019. Systematic Review of miRNA as Biomarkers in Alzheimer's Disease. Molecular Neurobiology 2019 56:9. 56:6156–6167. doi:10.1007/S12035-019-1500-Y. Available from: https://link-springer-

com.login.ezproxy.library.ualberta.ca/article/10.1007/s12035-019-1500-y

Takeda, Y., Y. Mishima, T. Fujiwara, H. Sakamoto, and K. Inoue. 2009. DAZL Relieves miRNA-Mediated Repression of Germline mRNAs by Controlling Poly(A) Tail Length in Zebrafish. PLOS ONE. 4:e7513. doi:10.1371/JOURNAL.PONE.0007513. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0007513

Tian, F., J. Luo, H. Zhang, S. Chang, and J. Song. 2012. MiRNA expression signatures induced by Marek's disease virus infection in chickens. Genomics. 99:152–159. doi:10.1016/J.YGENO.2011.11.004.

Tong, A. W., and J. Nemunaitis. 2008. Modulation of miRNA activity in human cancer: a new paradigm for cancer gene therapy? Cancer Gene Therapy 2008 15:6. 15:341–355. doi:10.1038/CGT.2008.8. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/cgt20088

Trott, D. J., M. R. Moeller, R. L. Zuerner, J. P. Goff, W. R. Waters, D. P. Alt, R. L. Walker, and M. J. Wannemuehler. 2003. Characterization of Treponema phagedenis-like spirochetes isolated from papillomatous digital dermatitis lesions in dairy cattle. Journal of Clinical Microbiology. 41:2522–2529. doi:10.1128/JCM.41.6.2522-2529.2003. Available from: https://journals.asm.org/journal/jcm

Turri, F., E. Capra, B. Lazzari, P. Cremonesi, A. Stella, and F. Pizzi. 2021. A Combined Flow Cytometric Semen Analysis and miRNA Profiling as a Tool to Discriminate Between High- and Low-Fertility Bulls. Frontiers in Veterinary Science. 8. doi:10.3389/FVETS.2021.703101. Available from: /pmc/articles/PMC8329915/

Uddin, A., and S. Chakraborty. 2018. Role of miRNAs in lung cancer. Journal of Cellular Physiology. doi:10.1002/JCP.26607. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/jcp.26607

Umezu, T., K. Tsuneyama, K. Kanekura, M. Hayakawa, T. Tanahashi, M. Kawano, Y. Taguchi, H. Toyoda, A. Tamori, M. Kuroda, and Y. Murakami. 2020. Comprehensive analysis of liver and blood miRNA in precancerous conditions. Scientific Reports 2020 10:1. 10:1–11. doi:10.1038/S41598-020-78500-1. Available from: https://www-nature-

com.login.ezproxy.library.ualberta.ca/articles/s41598-020-78500-1

Unger, L., V. Jagannathan, A. Pacholewska, T. Leeb, and V. Gerber. 2019. Differences in miRNA differential expression in whole blood between horses with sarcoid regression and progression. Journal of Veterinary Internal Medicine. 33:241–250. doi:10.1111/JVIM.15375. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/jvim.15375

Valadi, H., K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee, and J. O. Lötvall. 2007. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nature Cell Biology 2007 9:6. 9:654–659. doi:10.1038/NCB1596. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/ncb1596

Vickers, K. C., B. T. Palmisano, B. M. Shoucri, R. D. Shamburek, and A. T. Remaley. 2011. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nature Cell Biology 2011 13:4. 13:423–433. doi:10.1038/NCB2210. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/ncb2210

Võsa, U., T. Vooder, R. Kolde, J. Vilo, A. Metspalu, and T. Annilo. 2013. Meta-analysis of microRNA expression in lung cancer. International Journal of Cancer. 132:2884–2893. doi:10.1002/IJC.27981. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/ijc.27981

Wagner, J. J., S. L. Archibeque, and D. M. Feuz. 2014. The Modern Feedlot for Finishing Cattle. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.1146/annurev-animal-022513-114239. 2:535–554. doi:10.1146/ANNUREV-ANIMAL-022513-114239. Available from: https://www-annualreviews-org.login.ezproxy.library.ualberta.ca/doi/abs/10.1146/annurev-animal-022513-114239

Wang, X. C., L. L. Tian, H. L. Wu, X. Y. Jiang, L. Q. Du, H. Zhang, Y. Y. Wang, H. Y. Wu, D. G. Li, Y. She, Q. F. Liu, F. Y. Fan, and A. M. Meng. 2010. Expression of miRNA-130a in Nonsmall Cell Lung Cancer. The American Journal of the Medical Sciences. 340:385–388. doi:10.1097/MAJ.0B013E3181E892A0.

Wang, Y., X. Zhang, H. Li, J. Yu, and X. Ren. 2013. The role of miRNA-29 family in cancer. European Journal of Cell Biology. 92:123–128. doi:10.1016/J.EJCB.2012.11.004.

Wara, A. B., S. Chhotaray, B. U. D. Shafi, S. Panda, M. Tarang, S. Yosuf, N. A. Baba, and A. Kumar. 2019. Role of miRNA Signatures in Health and Productivity of Livestock. International Journal of Current Microbiology and Applied Sciences. 8:727–738. doi:10.20546/IJCMAS.2019.804.079.

Whay, H. 2002. Locomotion scoring and lameness detection in dairy cattle. In Practice. 24:444–449. doi:10.1136/INPRACT.24.8.444. Available from: https://bvajournals-onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1136/inpract.24.8.444

White, B. J., D. E. Anderson, D. G. Renter, R. L. Larson, D. A. Mosier, L. L. Kelly, M. E. Theurer, B. D. Robért, and M. L. Walz. 2012. Clinical, behavioral, and pulmonary changes in calves following inoculation with Mycoplasma bovis. American Journal of Veterinary Research. 73:490–497. doi:10.2460/AJVR.73.4.490. Available from: https://avmajournals-avma-org.login.ezproxy.library.ualberta.ca/view/journals/ajvr/73/4/ajvr.73.4.490.xml

Wilczynska, A., and M. Bushell. 2014a. The complexity of miRNA-mediated repression. Cell Death & Differentiation 2014 22:1. 22:22–33. doi:10.1038/CDD.2014.112. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/cdd2014112

Wilczynska, A., and M. Bushell. 2014b. The complexity of miRNA-mediated repression. Cell Death & Differentiation 2014 22:1. 22:22–33. doi:10.1038/CDD.2014.112. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/cdd2014112

Wilson-Welder, J. H., D. P. Alt, and J. E. Nally. 2015. The etiology of digital dermatitis in ruminants: recent perspectives. Veterinary Medicine : Research and Reports. 6:155. doi:10.2147/VMRR.S62072. Available from: /pmc/articles/PMC6070020/

Winckler, C., and S. Willen. 2010. The Reliability and Repeatability of a Lameness Scoring System for Use as an Indicator of Welfare in Dairy Cattle. http://dx.doi.org/10.1080/090647001316923162. 51:103–107. doi:10.1080/090647001316923162. Available from: https://www.tandfonline.com/doi/abs/10.1080/090647001316923162

Wu, H.-H., W. Lin, and K.-W. Tsai. 2014. Advances in molecular biomarkers for gastric cancer: miRNAs as emerging novel cancer markers. Expert Reviews in Molecular Medicine. 16.

doi:10.1017/ERM.2013.16. Available from: https://www-cambridge-org.login.ezproxy.library.ualberta.ca/core/journals/expert-reviews-in-molecular-medicine/article/advances-in-molecular-biomarkers-for-gastric-cancer-mirnas-as-emerging-novel-cancer-markers/8BC834935B87146C58D23AF7606E60D4

Xu, K., J. Lin, R. Zandi, J. A. Roth, and L. Ji. 2016. MicroRNA-mediated target mRNA cleavage and 3′-uridylation in human cells. Scientific Reports 2016 6:1. 6:1–14. doi:10.1038/srep30242. Available from: https://www.nature.com/articles/srep30242

Yerukala Sathipati, S., and S.-Y. Ho. 2018. Identifying a miRNA signature for predicting the stage of breast cancer. Scientific Reports 2018 8:1. 8:1–11. doi:10.1038/S41598-018-34604-3. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-34604-3

Yu, X., M. Odenthal, and J. W. U. Fries. 2016. Exosomes as miRNA Carriers: Formation–Function–Future. International Journal of Molecular Sciences 2016, Vol. 17, Page 2028. 17:2028. doi:10.3390/IJMS17122028. Available from: https://www.mdpi.com/1422-0067/17/12/2028/htm

Zanolari, P., S. Dürr, J. Jores, A. Steiner, and P. Kuhnert. 2021. Ovine footrot: A review of current knowledge. The Veterinary Journal. 271:105647. doi:10.1016/J.TVJL.2021.105647.

Zhang, C. 2009. MicroRNA-145 in vascular smooth muscle cell biology: A new therapeutic target for vascular disease. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.4161/cc.8.21.9837. 8:3469–3473. doi:10.4161/CC.8.21.9837. Available from: https://www-tandfonline-com.login.ezproxy.library.ualberta.ca/doi/abs/10.4161/cc.8.21.9837

Zhang, C. 2010. MicroRNAs in Vascular Biology and Vascular Disease. Journal of Cardiovascular Translational Research 2010 3:3. 3:235–240. doi:10.1007/S12265-010-9164-Z. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s12265-010-9164-z

Zhang, J. X., W. Song, Z. H. Chen, J. H. Wei, Y. J. Liao, J. Lei, M. Hu, G. Z. Chen, B. Liao, J. Lu, H. W. Zhao, W. Chen, Y. L. He, H. Y. Wang, D. Xie, and J. H. Luo. 2013. Prognostic and predictive value of a microRNA signature in stage II colon cancer: a microRNA expression analysis. The Lancet Oncology. 14:1295–1306. doi:10.1016/S1470-2045(13)70491-1.

Zhao, X. J., X. Y. Wang, J. H. Wang, Z. Y. Wang, L. Wang, and Z. H. Wang. 2015. Oxidative

Stress and Imbalance of Mineral Metabolism Contribute to Lameness in Dairy Cows. Biological Trace Element Research. 164:43–49. doi:10.1007/S12011-014-0207-1/FIGURES/1. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s12011-014-0207-1

Zhou, J., and J. Zhang. 2014. Identification of miRNA-21 and miRNA-24 in plasma as potential early stage markers of acute cerebral infarction. Molecular Medicine Reports. 10:971–976. doi:10.3892/MMR.2014.2245. Available from: http://www.spandidos-publications.com.login.ezproxy.library.ualberta.ca/10.3892/mmr.2014.2245/abstract

Chapter 2. Potential regulatory role of microRNAs in different types of beef cattle lameness

2.1 Introduction

The beef cattle industry makes significant economic and social contributions to Canadian agriculture (Chen, 2021). However, various health and welfare issues have a severe impact on cattle performance and become challenges to the beef cattle industry (Thomas et al., 1978). Lameness is a worldwide health problem for both dairy and beef cattle which affects animals' productivity and welfare (Tunstall et al., 2021). The most recent report indicates that the prevalence of lameness in Alberta dairy farms ranges from 2 to 56% (van Huyssteen et al., 2020), and it was estimated at 4.5% in feedlot beef cattle, accounting for more than 30% of the diagnosed diseases, found in feedlot cattle (Davis-Unger et al., 2019).

Lameness is a complex clinical symptom that can be caused by a multitude of lesions and injuries. The most common lesions include footrot (FR), digital dermatitis (DD), toe tip necrosis (TTN) and other types of lesions (Cortes et al., 2021b), among which FR is the most prevalent (74.5% of all cattle diagnosed with lameness) (Davis-Unger et al., 2019b). Lame cattle show a series of abnormal features such as reduced feed/water intake, reluctance to move, and an arched back (Thomsen et al., 2008; Norring et al., 2014c). A recent study based on beef cattle involving 28 Alberta feedlots over ten years revealed that cattle affected with FR had reduced average returns ranging between $690 and $568 per animal (Davis-Unger et al., 2017b). A similar study compared the cost of operation between lame and healthy cattle in another two feedlots in southern Alberta also proved that the partial budget of their farm operation decreased by $96 - $98 when the heifers and steers were affected by DD (Cortes et al., 2021c).

Different lameness phenotypes require specific diagnosis and treatment methods. Traditionally,

lameness detection relies on the visual assessments of beef herds. During the last century, a series of lameness detection methods related to locomotion and lameness scoring have been developed and applied on dairy farms (Whay, 2002b). In general, most detection systems classify the cow's gait into several levels and use this level to evaluate the severity of lameness (Sprecher et al., 1997b). Although these methods are easily applied on farms without any intrusive devices (Whay, 2002c), they are usually not sensitive when symptoms are not evident (Tadich et al., 2010). Moreover, automatic detection technologies such as wearable sensors (Haladjian et al., 2018), 3D-video recording (van Hertem et al., 2014), and accelerometers (O'Leary et al., 2020b) have been developed and applied to detect lameness, they are not effective before lameness symptoms appear and cannot distinguish different lameness phenotypes without the careful evaluation of the feet by the veterinarian. Therefore, the development of a novel method to diagnose early-stage lameness is necessary to prevent and properly treat the lameness of beef cattle.

. There is substantial evidence suggesting that molecular biomarkers can be used for early-stage disease diagnosis in both humans (Krishnan et al., 2018) and livestock (Jung et al., 2020). However, the understanding of the pathogenesis and regulatory mechanisms of lameness in cattle at the molecular level is very limited. MicroRNAs (miRNAs) are small 19-24 nucleotides single-strand non-coding RNA molecules that regulate gene expression through the degradation of the target messenger RNA (mRNA) and translation inhibition (O'Brien et al., 2018; Ye et al., 2019). Many studies have revealed the essential roles of miRNAs in the pathogenesis of human diseases, and specific miRNAs are uniquely expressed under different pathological conditions (L. Wang et al., 2019). For instance, the expression of miRNAs including miR-219-1, miR-207, miR-38 and miR-185 are significantly increased in metastatic liver tissues when compared with the healthy liver tissues (Budhu et al., 2008). Accumulating similar findings suggest the miRNAs expression levels

changes can reflect the animal physical condition changes. The miRNA expression profiles are regional and tissue-specific within an animal system and can change under different physiological conditions (Liang et al., 2007). In addition, miRNAs can also be found in body fluids, including blood, serum, saliva, and urine, where they are identified as circulating miRNAs (circ-miRNA). Blood and plasma are the most studied body fluids for disease detection and disease biomarker discovery such as in various cancer diagnoses (Montani and Bianchi, 2016). Moreover, recent studies have reported that plasma and whole blood miRNA profiles are related to the heat-stress (Lee et al., 2020b) and early pregnancy in dairy cattle (Ioannidis and Donadeu, 2016b), with differentially expressed miRNAs specifically identified in cattle who suffered from heat-stress (bta-miR-19a up-regulated and bta-miR-2284a down-regulated) and pregnant cows (miR-26a and miR-1249 were up-regulated) when compared with healthy and open cattle, respectively. This suggests that the blood miRNAomes could reflect the animal's response to abnormal physiological conditions, which makes them a global indicator for disease and physiological conditions (Cui et al., 2016) and their potential as biomarkers for early-stage disease detection.

To date, there are no studies assessing the roles of miRNA in the pathogenesis of lameness in cattle. Thus, we hypothesized that blood miRNA profiles are different between lame and healthy cattle, and cattle with different lameness phenotypes have uniquely expressed miRNAs in the blood. Therefore, this study aimed to 1) profile expressed microRNAs in the blood of feedlot cattle diagnosed with three lameness phenotypes: DD, FR and TTN; 2) compare the miRNA profiles among three lameness phenotypes; 3) investigate whether phenotype-specific miRNAs can be selected as potential markers for lameness diagnosis.

2.1 Materials and methods

2.1.1 Ethical statement

All experimental protocols described in this chapter were reviewed and approved by the Lethbridge Research and Development Centre Animal care committee (Animal Use protocol Review 1817), and the procedures were conducted following the guidelines of the Canadian Council on Animal Care (2020).

2.1.2 Animal and sample selection

Between September 2018 and March 2020, 596 whole blood samples (3 ml per sample) were collected by venipuncture from a total of 363 lame and healthy feedlot cattle consisting of heifers (n=142; Average body weight = 464 kg) and steers (n = 221; Average body weight = 496 kg) using Tempus™ Blood RNA Tubes (Thermo Fisher Scientific, CA). The experimental animals were selected from five over-10,000 head capacity feedlots located in southern Alberta near Lethbridge. The lame cattle were observed and then pulled for lameness severity evaluation by the research group from Lethbridge Research and Development Centre based on M-stage scoring and the Gait score system. Whole blood samples were collected prior to any medical treatments (**Week 0**, n = 369), one week after treatment (**Week 1**, n = 104), two weeks after treatment (**Week 2**, n = 74), and three weeks after treatment (**Week 3**, n = 24) and more than three weeks treatment until the animal was healed after treatment (up to 25 weeks, n = 25). Beef cattle diagnosed with lameness were treated after the first-time whole blood sample collection with different therapies based on their lameness phenotypes according to the protocol (Table 2.1).

To generate microRNA expression profiles in the whole blood of feedlot cattle diagnosed with three lameness phenotypes without treatment effect, blood samples collected from 106 cattle at

Week 0 were selected for downstream RNA-seq analysis, including HC (n = 12), DD (n = 24), FR (n = 40), TTN (n = 13), as well as FRDD (n = 17).

2.1.3 Total RNA extraction and quality determination

Total RNA was extracted using a Preserved Blood RNA Purification Kit I (Norgen, Thorold, ON, CA) according to the manufacturer's instructions. Briefly, 4.5 ml of the blood and Stabilizing Reagent mix was transferred to a new Falcon 50 ml Conical Centrifuge Tube (Fisher Scientific, US) and mixed with 3 mL of Tempus TM Blood RNA Tube Diluent by a vigorous vortex. The mixture was centrifuged at 5000 × g for 30 min at 4 °C to separate the RNA pellet. The supernatant was discarded, and the RNA pellet was lysed by chemical disruption using Lysis Solution. Next, 300 µl ethanol was added to the lysed solution and all content was transferred to a new Mini Spin column provided in the kit followed by a two-time wash using the washing solution provided in the kit. After the washing step, total RNA was eluted by 50 µl Elution Solution provided in the kit.

The concentration of total RNA was determined using UV spectrum and absorbance on a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). The RNA quality and integrity were assessed by the RNA Integrity Number (RIN) using a 2200 TapeStation Instrument with RNA ScreenTapes (Agilent Technologies, Santa Clara, CA), and only samples with RIN > 7 were subjected to the miRNA library construction.

2.1.4 miRNA library construction and miRNA-sequencing

Total RNA (1.0 µg) extracted from each blood sample was used to construct miRNA RNA-seq libraries using QIAseq miRNA Library Kit (96) (Qiagen, CA) according to the manufacturer's instructions. Briefly, the total RNA was subjected to 3' adapter ligation to increase the length of miRNA for sequencing, followed by 5' adapter ligation (3' adapter:

AACTGTAGGCACCATCAAT; 5' adapter: GTTCAGAGTTCTACAGTCCGACGATC). The reverse transcription of ligated total RNA was carried out on a GeneAmp PCR System 9700 (Invitrogen, Carlsbad, CA, USA) using the reagents provided in the QIAseq miRNA Library Kit with the following steps: Incubation for one hour at 50 ºC, 15 min at 70 ºC, and held at 4 ºC. PCR enrichment was performed as follows: Held at 95 ºC for 15 mins, followed by 11 cycles of 95 ºC for 15 seconds, annealing at 60 ºC for 30 seconds and. Extension at 72 ºC for 15 seconds was finally held at 72 ºC for 2 mins. The PCR enriched miRNA cDNA libraries were cleaned using QIAseq miRNA Next Generation Sequencing (QMN) Beads (Qiagen, CA) on a magnetic stand. Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) was used for miRNA cDNA libraries quantity measurement. The cDNA library was sequenced at Génome Québec (Montréal, Canada) using an Illumina HiSeq 2000 system (Illumina) with 50 bases single end reads.

2.1.5 miRNA sequence data analysis

The generated raw sequences were subjected to a web-based tool: sRNAtoolbox (https://arn.ugr.es/srnatoolbox/) for the low-quality read removal, 3' adapter trimming and small RNA sequence length distribution analysis (Aparicio-Puerta et al., 2019). SRNAtoolbox is an online web-based tool that can implement automatic processing of miRNA expression profiling and downstream analysis. To identify known miRNAs, sequences that passed quality control with lengths larger than 15 nt were aligned to the corresponding miRNA precursor sequences in the bovine miRNA database (miRBase v22.2, http://www.mirbase.org/) using sRNAtoolbox with default parameters (alignment type: bowtie seed alignment; seed length for alignment: 20; minimum read count: 2; minimum read length: 15; allowed number of mismatches: 2; the maximum number of multiple mappings: 10) following the pipeline published by Sun et al., (2019). The read counts were normalized to RPM (reads per million mapped reads) and were used for

downstream analysis using R studio (Version 1.4.1717, RStudio Team, 2020).

2.1.6 Differential expression analysis

To investigate whether blood miRNA profiles differ between HC and lameness phenotypes, principal component analysis (PCA) was performed to compare the overall grouping patterns of miRNA profiles based on the RPM matrix using the DEseq2 and ggplot2 R packages in R studio. Firstly, reads of miRNA were normalized using counts per million reads mapped (RPM), with RPM > 1 being considered as expressed. Lameness phenotype specifically expressed miRNA were defined as those with an RPM > 1 in at least 60% of all samples within one phenotype group but not expressed in HC and other phenotype groups. The differentially expressed (DE) miRNAs between different lameness phenotype groups and the HC group were identified using the DEseq2 package (Love et al., 2014a) in the R studio. Specifically, the normalized miRNA expression was compared following: HC vs DD; HC vs TTN; HC vs FR; DD vs TTN; DD vs FR and FR vs TTN using the DEseq 2 package (Love et al., 2014b) in R studio. The DE miRNAs were identified between two groups with the threshold of $\log_2$ fold change < -1 or >1 and false discovery rate (FDR) < 0.05 based on Benjamini and Hochberg's multiple testing correction (Benjamini and Hochberg, 1995).

2.1.7 Target gene prediction and functional analysis

The target genes for DE miRNAs and phenotype-specific miRNAs were predicted using TargetScan (Release 7.2, http://www.targetscan.org/vert_72/, Agarwal et al., 2015). The predicted functions of DE miRNAs were identified based on their predicted target genes using Database for Annotation, Visualization and Integrated Discovery (DAVID, version 6.8, https://david.ncifcrf.gov/). The miRNA-related genes were grouped into functional clusters, and a

threshold of FDR < 0.05 was applied to the enrichment of functional annotation clusters. The clusters were sorted by enrichment score, and the groups with the highest enrichment score in the output list were subjected to the following analysis.

2.1.8 Validation of differentially expressed miRNAs expression using RT-qPCR

To validate the identified DE miRNAs and lameness phenotype-specific miRNAs from transcriptome comparison, three DE (bta-miR-6119-3p, bta-miR-1, bta-miR-133a) and three phenotype-specific miRNAs (bta-miR-200a, bta-miR-2904 and bta-miR-483) were selected for validation using stem-loop RT-qPCR with Taqman miRNA assays (Applied Biosystems, Carlsbad, CA). Briefly, 10 ng of total RNA were reversely transcribed to cDNAs using commercial miRNA RT primers (Applied Biosystems, Carlsbad, CA) and then subjected to RT-qPCR on a ViiA 7 Real-Time PCR System (Applied Biosystems, Carlsbad, CA). Specifically, the RT-qPCR program was used as follows: incubation at 95 ℃ for 10 minutes, followed by 40 cycles of denaturation at 95 ℃ for 15 seconds, annealing and extending at 60 ℃ for 60 seconds. Two miRNAs were used as references for miRNA RT-qPCR: bta-miR-93, selected based on a previous study (Zhao et al., 2016a); and bta-miR-16b, selected based on RNA-seq results with the most stable expression level. The relative expression level of each targeted miRNA was calculated using the ΔΔCt method (relative expression = ΔCt of selected miRNA- ΔCt of reference miRNA). One-way ANOVA was applied to the normally distributed relative expression data of miRNA to compare the difference between different lameness phenotypes with a threshold of $p < 0.05$ was considered the significant difference.

2.3 Results

2.3.1 Profiling of miRNAs in blood of lame and healthy beef cattle

A total of 709 million reads were obtained from RNA sequencing with an average of 9.4 ± 5.31 million reads per sample, and the sequence length distribution of the reads revealed most of the sequences had a length of 21-23 nt (Figure 2.1). Among them, 563 million out of 709 million reads were mapped to the known miRNA database (miRbase; Release 22.1). In total, 321 known miRNAs were identified from all samples, with 273 in the HC group, 280 in the DD group, 289 in the TTN group, 280 in the FR group and 286 in the FRDD group, respectively. Of the expressed miRNAs, 254 were detected in blood samples of all groups (Figure 2.2A), with the top 10 most expressed miRNAs accounting for more than 80% of the total number of reads. Among them, bta-miR-486 was the most highly expressed miRNA followed by bta-let-7 (bta-let-7b and bta-let-7a-5p) and bta-miR-16b families (Figure 2.2B). Principal component analysis showed no difference in the whole miRNA profiles between healthy and lame animals and among different lameness phenotype groups (Figure 2.3).

The expression of some miRNAs was only detected in particular lameness phenotypes, which were defined as lameness phenotype-specific miRNAs: seven from the HC group, four from the DD group, three from the FR group, seven from the TTN group and seven from the FRDD group, respectively. The average expression level of lameness phenotype-specific miRNAs ranged from 1.16 ± 0.38 RPM to 7.46 ± 6.20 RPM (Table 2.1).

2.3.2 Identification of lameness phenotype differentially expressed miRNAs

When the expression of miRNAs was further compared between each lameness phenotype and the HC group, three, seven, six, and fourteen DE miRNAs were identified for DD vs HC, FR vs HC,

TTN vs HC, and FRDD vs HC comparisons, respectively (Table 2.2). Among these DE miRNAs, the expression of three (bta-miR-340, bta-miR-2408 and bta-miR-6119-3p) identified in the DD group were all down-regulated compared to those from the HC group. Expression of bta-miR-6119-3p was down-regulated in all lameness phenotypes compared to the HC group. The expression of bta-miR-133a and bta-miR-206 were up-regulated in TTN and FR groups but represented a similar expression level in FRDD and DD cases when compared with HC animals. The expression of two DE miRNAs: bta-miR-340 and bta-miR-2408 were down-regulated in the TTN and DD groups compared to HC. The expression of another down-regulated miRNA: bta-miR-125a was identified in FR and FRDD groups compared to HC (Table 2.2).

Further DE analysis between lameness phenotype groups revealed the DE miRNAs between lameness phenotype groups, with five, twenty, six, twelve, seven and fifteen DE miRNAs were identified for DD vs FR, DD vs FRDD, DD vs TTN, FR vs TTN and FRDD vs TTN, respectively (Table 2.3). Most DE miRNAs identified between lameness phenotype groups had the same up- or down-regulation trends when they were compared between HC and lameness groups. For example, bta-miR-92b was down-regulated in the FRDD group when compared to the HC group, and it was also down-regulated when compared with FR, DD and TTN groups (Table 2.3). Moreover, bta-miR-6119-3p miRNAs were identified as down-regulated DE miRNA in all lameness phenotypes.

2.3.3 Functional predictions of differentially expressed miRNAs and phenotype-specific miRNAs

The identified lameness phenotype-specific miRNAs and DE miRNAs were subjected to functional analysis to explore their roles in lameness. Based on the target genes generated using TargetScan, the most significant enriched function annotations (FDR < 0.05) with the highest

enrichment score were identified by DAVID. Significantly enriched functions were identified for 13 out of 22 DE miRNAs, including six down-regulated and seven upregulated DE miRNAs. For the down-regulated miRNAs identified in different lameness phenotype groups, five significant enriched function categories were identified, including Zinc-finger, Voltage-gated channel, Src homology-3 domain, Cadherin-like and MAD homology 1 for four out of six down-regulated miRNAs (bta-miR-340 in DD and TTN group, -125a in FR and FRDD group, -6119-3p in all three lameness phenotype groups and -2408 in DD and TTN group) (Table 2.4). While significantly enriched functions of the other two down-regulated miRNAs (bta-miR-211 and -92b) were different from others which related to the cadherin conservation sites and a CCAAT box-binding transcription factor. As for the up-regulated DE miRNAs identified in different lameness phenotype groups, only one out of seven (bta-miR-30a) was predicted to play essential roles in the Zinc-finger protein and zinc-iron binding functions, which was similar to most of the down-regulated DE miRNAs. While the enriched predicted functions of the other six up-regulated DE miRNAs were related to serine/threonine-protein kinase, protein kinase, FoxO signaling pathway, SH3 domain and insulin resistance (Table 2.5). However, no significant enriched function was identified for lameness phenotype-specific miRNAs.

2.3.4 Validation of selected differentially expressed and lameness phenotype-specific miRNAs using reverse transcription-quantitative real-time PCR

Bta-miR-1 (TTN vs HC), bta-miR-133a (TTN vs HC) and bta-miR-6119-3p (DD, FR, TTN vs HC) from DE miRNAs between lameness phenotypes and HC group (Table 2.2); bta-miR-2904, bta-miR-200a and bta-miR-483 from lameness phenotype-specific miRNAs (Table 2.1) were selected for further validation using reverse transcription-quantitative real-time PCR (RT-qPCR) analysis. The expression of bta-miR-133a was significantly higher (p = 0.007) in the TTN group when

compared to the HC group, which was consistent with the RNA-seq based comparison (Figure 2.4A). The expression of bta-miR-483 was significantly higher in the TTN group (p = 0.007) compared with the FR group, which was consistent with the trend identified by RNA-seq (Figure 2.4B), although the expression level of bta-miR-483 was not significantly different between these two groups from RNA-seq. From the comparison between other groups, the qPCR results showed the expression of bta-miR-483 was significantly down-regulated in the FR compared to the DD group (p = 0.014), while bta-miR-6119-3p was up-regulated in the FR compared to the HC group (p = 0.045). However, the opposite regulatory trend was observed using RNA-seq since the expression level of bta-miR-483 was higher in FR compared to DD cattle (Figure 2.4C), and bta-miR-6119-3p was significantly up-regulated in the HC group (Figure 2.4D).

2.4 Discussion

Although miRNAs have been identified as potential biomarkers of various diseases in different mammalian species (Duttagupta et al., 2011; Miretti et al., 2020), studies focusing on miRNAs in beef cattle are still limited, especially regarding their expression profiles in the blood and their profiles related to cattle disease and health-related traits. In this study, we identified 321 miRNAs expressed in the whole blood of beef steers and heifers, which is similar to previously reported bovine blood-specific miRNAs (~320) (Ioannidis and Donadeu, 2016c). A previous study revealed that the most highly expressed miRNA in bovine blood was bta-miR-486 (Sun et al., 2019a), which was also confirmed in the current study. In addition, the top ten most highly expressed miRNAs were similar to those reported in cattle sera and exosomes (Zhao et al., 2016b). These suggest that the bovine blood miRNAome is highly consistent among studies.

Although bta-miR-486 is one of the top 10 highly expressed miRNAs in bovine blood from our study, its expression levels differ in other bovine tissue, including liver and muscle (Sun et al.,

2019b). Previous studies also suggested its expression was associated with different traits. Specifically, the differential expression of bta-miR-486 has been reported in different studies, which was linked with various physiological conditions and critical functions. For instance, the expression of bta-miR-486 was significantly down-regulated in buffalo sera when the animals were infected by *Fasciola gigantica* (Guo and Guo, 2019). The differential expression of bta-miR-486 has also been reported in serum when cattle were challenged with the bovine viral diarrhea virus (Taxis et al., 2017). Its expression has also been found in skeletal muscle, and its enriched target gene function has been shown to affect feed efficiency in Nelore cattle (de Oliveira et al., 2018a). These findings suggest that bta-miR-486 is one of the circulating miRNAs in the bovine blood and is related to various physiological states in cattle.

Among all identified DE miRNAs between healthy and lame cattle, expression of bta-miR-6119-3p was down-regulated in whole blood of all lameness groups compared with the HC group at **Week 0**. Only a few studies reported the differential expression of bta-miR-6119-3p under particular physiological conditions. Husseini reported that bta-miR-6119-5p, which is processed from the same pre-miRNA with bta-miR-6119-3p, was up-regulated with a 3.4 foldchange (p = 0.21) in liver tissues of high RFI Angus bulls compared to that in low RFI cattle, but no significant enriched function or targeted mRNA of bta-miR-6119 was reported (Al-Husseini et al., 2016a). It is unknown about the potential regulatory roles of bta-miR-6119 related to reduced feed efficiency, further studies are needed to explore the potential function of this miRNA in blood. Functional analysis showed that the main predicted functions of bta-miR-6119-3p were related to the Zinc-finger domain, one of the most frequently utilized DNA-binding motifs (Cassandri et al., 2017). The zinc finger domain is one of the transcriptional factors that regulate many cellular processes (Ladomery and Dellaire, 2002). The higher expression of this miRNA in the liver tissue, suggests

the potentially suppressed expression of genes which regulate the metabolic homeostasis including glucose and lipid metabolism, therefore leading to lower feed efficiency (Al-Husseini et al., 2016b). It is known that when cattle suffer from lameness, their movement is reduced due to the pain, as a result, their energy consumption is lower than healthy cattle who can walk freely. The downregulation of bta-miR-6119-3p could be the result of lower feed intake due to lameness and the possible reason for this regulation may relate to the reduction of extra energy consumption from movement. However, further studies are required to verify the role of this miRNA in regulating the potential relationship between feed intake and energy consumption.

It has also been reported that Zinc-finger proteins family member ZNF750 has functions in the pathogenesis of seborrhea-like dermatitis and psoriatic in humans (Birnbaum et al., 2006), and the promotion of ZNF750 is potentially related to skin diseases. Since miRNA is highly conserved between different species and the predicted function of bta-miR-6119-3p is related to the zinc-finger domain, it is speculated that this miRNA may have similar regulatory roles in skin diseases, including dermatitis on the skin between two digitals in cattle hoofs. As one of the most abundant transcription factors, zinc-finger proteins also play essential roles in muscle differentiation (Cassandri et al., 2017). The decreased SMYD1, one of the Zinc-finger proteins, has been reported to reduce the muscle cell myofiber formation and depress the muscle's special gene expression (Nagandla et al., 2016). In lame cattle, the muscle development function was affected due to restricted movement and reduced feed intake. Thus, the differential expression of bta-miR-6119-3p may be related to the muscle degeneration caused by different phenotypes of lameness. In addition to mediating the generation of skin diseases, bta-miR-6119-3p has a potential role in significantly reducing virus titers following infection with foot mouth disease (Sahu et al., 2020) and bovine respiratory disease (Johnston et al., 2021) by targeting the DE gene *BAK1*, which can

regulate the interferon response to virus infection. Lower expression levels of bta-miR-6119-3p in all lameness phenotypes may suggest the miRNA-mediated systemic inflammation reaction happened as the result of lameness. For other down-regulated DE miRNAs identified in lameness groups, most of them have predicted functions in the inhibition of cell growth, for instance, the miR-340 was reported to have functions related to colorectal cancer cells growth inhibition, with its expression level significantly down-regulated in colon tissue cell from human who survival over 5 years after colon cancer diagnosis (Sun et al., 2012). Similarly, another miRNA: miR-125a, a down-regulated DE miRNA identified in FR and FRDD groups, its expression level was decreased in Acute Myeloid Leukemia patient samples, and its predicted function was related to the cell cycle inhibition (Ufkin et al., 2014). The above evidence indicates that some of the down-regulated DE miRNAs in this may play essential roles in the cell cycle and growth inhibition in humans. In other words, their down-regulation might reduce the inhibition function, and even promote cell growth and progression, which indicates the potential self-healing in lame cattle.

The expression levels of bta-miR-1, -206 and -133a were significantly up-regulated in the TTN compared to the HC group. The bta-miR-1 and bta-miR-206 belong to the same miRNA family with similar sequences and predicted functions (Dai et al., 2016). These two miRNAs and bta-miR-133a were all reported to have functions in skeletal muscle development and progression (J Sun et al., 2015a; Siracusa et al., 2018; Hao et al., 2021a). The expression of these three miRNAs was determined as muscle-specific in previous studies. Sun et al. compared the expression level of bta-miR-1, -133a and -206 in different beef cattle tissues using stem-loop RT-qPCR and reported that those three miRNAs were not detected in liver, lung, kidney, brain and some other tissues, but were highly expressed in muscle and heart tissues (Sun et al., 2014). Two comprehensive analyses of miRNA profiles in cattle blood demonstrated that bta-miR-1 and bta-miR-206 were not

detectable (Ioannidis and Donadeu, 2018a), while bta-miR-133a was identified in plasma but not in blood cells (Sun et al., 2019b). Bta-miR-1 targets the Histone Deacetylase 4 gene to promote muscle development while miR-133a increases muscle cell growth by suppressing the Serum Response Factor gene (Rosenberg et al., 2006). In the current study, the possible reason for detecting those previously reported muscle-specific miRNAs in the blood is that muscle-specific miRNAs were compensatorily increased and released to blood from muscle by exosome emission, and finally led to muscle loss by reduced ambulation. In addition, the predicted functions of up-regulated DE miRNAs in the blood of lame cattle are involved in traumatic muscle stress (Zhao et al., 2012)and intramuscular adipogenesis (bta-miR-497 and bta-miR-210 from the FRDD group) (Ren et al., 2020), further suggesting that miRNAs may be involved in body weight or muscle loss after cattle affected by lameness. Future studies are needed to evaluate the expression of these miRNAs in the wound and swap samples from the lame cattle to verify our speculations.

In summary, based on the functional analysis, some of the down-regulated DE miRNAs between HC and other lameness phenotypes including bta-miR-125a and bta-miR-340 have reported functions involved in cell growth inhibition, suggesting that the downregulation of these miRNAs exerts an effect on cell growth promotion, which may be related to the lesion self-repair when lameness happens. Another down-regulated DE miRNA: bta-miR-6119-3p which was identified as down-regulated in all lameness phenotypes when compared to the HC group, was reported to play essential roles in the pathogenesis of human skin diseases. Also, two main phenotypes of lameness: DD and FR are specific skin diseases that happen on cattle hoofs, which suggests the downregulation of bta-miR-6119-3p may play similar functions in lameness as miRNAs are highly conserved among different species.

The potential functions of up-regulated miRNAs in different lameness phenotypes might be

related to the muscle cell development function in lameness phenotype groups, especially since some of them were not detected in bovine blood in the previous studies; this was probably related to the reaction to body muscle loss due to the low feed intake and restrict movement, suggesting the exosome emission may happen and deliver the miRNAs from muscle to whole blood.

In addition to the identification of DE miRNAs between lame and healthy cattle, we also observed that expression of some miRNAs was only detected in the blood of a specific lameness phenotype. It is noticeable that many of the lameness phenotype-specific miRNAs were low expressed, and no functions were predicted for most of them. However, it was reported that some of the lameness phenotype-specific miRNAs were differentially expressed in the cattle blood under different physiological conditions, which provides evidence of their potential regulatory roles in the corresponding conditions. For example, DD phenotype-specific miRNA bta-miR-2904 has been identified to be differently expressed in bovine milk and bovine granulosa cells, respectively, when under inflammation stress or when cattle were challenged with *Staphylococcus aureus* (Ma et al., 2019) or under heat stress (Menjivar et al., 2021). For the FR-specific miRNA, bta-miR-200a has been found to be up-regulated in mastitis and systemic lupus erythematosus models (Luoreng et al., 2018c). The bta-miR-10b was reported to be secreted by bovine embryos and negatively affected embryo quality (Lin et al., 2019). Wang et al. reported that the expression of bta-miR-2887 was down-regulated in the intestinal tissue of cattle with *Escherichia coli* O157:H7 fecal shedding and was implicated in affecting hematological system development (Wang et al., 2021a). And bta-miR-494 was identified as a response to gram-negative bacterial infection in bovine mammary epithelial cells with a significant downregulation (Lawless et al., 2013a). Although no significant enriched functions were predicted for lameness phenotype-specific miRNAs by the functional analysis in this study, based on the previous literature, it suggests that

these miRNAs could have target genes or related functions involved in immune and infection responses, proving that their specific expression may be due to their response to diagnosed lameness. Muscle-specific miRNAs with functions specifically related to muscle development were also identified in the FR and TTN group, which suggests that the muscle growth may be promoted under these conditions, and their existence in blood potentially suggests the miRNAs circulation by exosome emission from muscle to blood. In addition, the lameness phenotype-specific miRNAs were reported to express differentially under disease conditions in different bovine tissues, which suggests the identification of these miRNAs in bovine blood could be related to the existing health problem as the result of lameness. However, further research is needed to identify the target gene for the selected DE and phenotype-specific miRNAs to prove their roles in lameness pathogenesis.

Further RT-qPCR validation revealed that only bta-miR-133a showed a consistent altered expression trend between RT-qPCR and RNA-seq compared to the HC and TTN group, suggesting that miR-133a can be a potential miRNA marker for TTN. As for the other selected miRNAs, their expression levels were not significantly different between the groups throughout RT-qPCR and in some cases showed the opposite trend which might be caused by their low expression level (Cristino et al., 2011). Further analysis on the validation of more DE and group-specific miRNAs is needed to provide further evidence of their potential for biomarkers of different lameness phenotypes.

2.5 Conclusions

As the first study profiling the blood miRNA of healthy cattle and those diagnosed with different phenotypes of lameness, the results from the current study provided the baseline miRNAome data to study beef cattle lameness. This study revealed that although overall miRNAomes did not differ,

some specific miRNAs were differentially expressed among different lameness groups. Their differential expression may help distinguish the lameness phenotype based on the identified DE and lameness phenotype-specific miRNAs. Some of the identified lameness phenotype-specific miRNAs have potential immune and inflammation response functions. However, the inclusion of larger sample size and more DE miRNAs is needed for future validation using qPCR to verify the accuracy of potential miRNA markers for different lameness phenotypes. Moreover, functional validation analysis is required to determine the relationship between the DE miRNAs and their regulatory roles in biological processes behind different phenotypes of lameness pathogenesis and to what extent the miRNAs change contributes to this health issues of cattle.

2.6 Reference

Al-Husseini, W., Y. Chen, C. Gondro, R. M. Herd, J. P. Gibson, and P. F. Arthur. 2016a. Characterization and Profiling of Liver microRNAs by RNA-sequencing in Cattle Divergently Selected for Residual Feed Intake. Asian-Australasian Journal of Animal Sciences. 29:1371. doi:10.5713/AJAS.15.0605. Available from: /pmc/articles/PMC5003961/

Al-Husseini, W., Y. Chen, C. Gondro, R. M. Herd, J. P. Gibson, and P. F. Arthur. 2016b. Characterization and Profiling of Liver microRNAs by RNA-sequencing in Cattle Divergently Selected for Residual Feed Intake. Asian-Australasian Journal of Animal Sciences. 29:1371. doi:10.5713/AJAS.15.0605. Available from: /pmc/articles/PMC5003961/

Birnbaum, R. Y., A. Zvulunov, D. Hallel-Halevy, E. Cagnano, G. Finer, R. Ofir, D. Geiger, E. Silberstein, Y. Feferman, and O. S. Birk. 2006. Seborrhea-like dermatitis with psoriasiform elements caused by a mutation in ZNF750, encoding a putative C2H2 zinc finger protein. Nature Genetics 2006 38:7. 38:749–751. doi:10.1038/NG1813. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/ng1813

Budhu, A., H. L. Jia, M. Forgues, C. G. Liu, D. Goldstein, A. Lam, K. A. Zanetti, Q. H. Ye, L. X. Qin, C. M. Croce, Z. Y. Tang, and W. W. Xin. 2008. Identification of metastasis-related microRNAs in hepatocellular carcinoma. Hepatology. 47:897–907. doi:10.1002/HEP.22160. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/hep.22160

Cassandri, M., A. Smirnov, F. Novelli, C. Pitolli, M. Agostini, M. Malewicz, G. Melino, and G. Raschellà. 2017. Zinc-finger proteins in health and disease. Cell Death Discovery 2017 3:1. 3:1–12. doi:10.1038/cddiscovery.2017.71. Available from: https://www.nature.com/articles/cddiscovery201771

Chen, Y. 2021. The Economic Impact of Alternative Antimicrobial Management Strategies in Western Beef Feedlots: An Agent-based Model. doi:10.7939/R3-YYQM-V483. Available from: https://era-library-ualberta-ca.login.ezproxy.library.ualberta.ca/items/afd49fd8-acbd-4452-869f-4a76e758cae7

Cortes, J. A., S. Hendrick, E. Janzen, E. A. Pajor, and K. Orsel. 2021a. Economic impact of digital dermatitis, foot rot, and bovine respiratory disease in feedlot cattle. Translational Animal Science.

5:1–10. doi:10.1093/TAS/TXAB076. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/5/2/txab076/6278306

Cortes, J. A., S. Hendrick, E. Janzen, E. A. Pajor, and K. Orsel. 2021b. Economic impact of digital dermatitis, foot rot, and bovine respiratory disease in feedlot cattle. Translational Animal Science. 5:1–10. doi:10.1093/TAS/TXAB076. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/5/2/txab076/6278306

Cristino, A. S., E. D. Tanaka, M. Rubio, M. D. Piulachs, and X. Belles. 2011. Deep Sequencing of Organ- and Stage-Specific microRNAs in the Evolutionarily Basal Insect Blattella germanica (L.) (Dictyoptera, Blattellidae). PLOS ONE. 6:e19350. doi:10.1371/JOURNAL.PONE.0019350. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0019350

Cui, R., J. Xu, X. Chen, and W. Zhu. 2016. Global miRNA expression is temporally correlated with acute kidney injury in mice. PeerJ. 4:e1729. doi:10.7717/PEERJ.1729. Available from: https://peerj.com/articles/1729

Dai, Y., Y. M. Wang, W. R. Zhang, X. F. Liu, X. Li, X. bin Ding, and H. Guo. 2016. The role of microRNA-1 and microRNA-206 in the proliferation and differentiation of bovine skeletal muscle satellite cells. In Vitro Cell Dev Biol Anim. 52:27–34. doi:10.1007/S11626-015-9953-4. Available from: https://pubmed.ncbi.nlm.nih.gov/26424132/

Davis-Unger, J., E. A. Pajor, K. Schwartzkopf-Genswein, S. Marti, C. Dorin, E. Spackman, and K. Orsel. 2017. Economic impacts of lameness in feedlot cattle. Translational Animal Science. 1:467–479. doi:10.2527/TAS2017.0052. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/1/4/467/4780403

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Transl Anim Sci. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://pubmed.ncbi.nlm.nih.gov/32704830/

Duttagupta, R., R. Jiang, J. Gollub, R. C. Getts, and K. W. Jones. 2011. Impact of Cellular miRNAs on Circulating miRNA Biomarker Signatures. PLOS ONE. 6:e20769. doi:10.1371/JOURNAL.PONE.0020769. Available from: https://journals-plos-

org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0020769

Guo, X., and A. Guo. 2019. Profiling circulating microRNAs in serum of Fasciola gigantica-infected buffalo. Molecular and Biochemical Parasitology. 232:111201. doi:10.1016/J.MOLBIOPARA.2019.111201.

Haladjian, J., J. Haug, S. Nüske, and B. Bruegge. 2018. A Wearable Sensor System for Lameness Detection in Dairy Cattle. Multimodal Technologies and Interaction 2018, Vol. 2, Page 27. 2:27. doi:10.3390/MTI2020027. Available from: https://www.mdpi.com/2414-4088/2/2/27/htm

Hao, D., Xiaogang Wang, Xiao Wang, B. Thomsen, Y. Yang, X. Lan, Y. Huang, and H. Chen. 2021. MicroRNA bta-miR-365-3p inhibits proliferation but promotes differentiation of primary bovine myoblasts by targeting the activin A receptor type I. Journal of Animal Science and Biotechnology. 12:1–15. doi:10.1186/S40104-020-00528-0/TABLES/2. Available from: https://jasbsci-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s40104-020-00528-0

van Hertem, T., S. Viazzi, M. Steensels, E. Maltz, A. Antler, V. Alchanatis, A. A. Schlageter-Tello, K. Lokhorst, E. C. B. Romanini, C. Bahr, D. Berckmans, and I. Halachmi. 2014. Automatic lameness detection based on consecutive 3D-video recordings. Biosystems Engineering. 119:108–116. doi:10.1016/J.BIOSYSTEMSENG.2014.01.009.

van Huyssteen, M., H. W. Barkema, S. Mason, and K. Orsel. 2020. Association between lameness risk assessment and lameness and foot lesion prevalence on dairy farms in Alberta, Canada. Journal of Dairy Science. 103:11750–11761. doi:10.3168/JDS.2019-17819.

Ioannidis, J., and F. X. Donadeu. 2016a. Circulating miRNA signatures of early pregnancy in cattle. BMC Genomics. 17:1–12. doi:10.1186/S12864-016-2529-1/FIGURES/6. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-016-2529-1

Ioannidis, J., and F. X. Donadeu. 2016b. Circulating miRNA signatures of early pregnancy in cattle. BMC Genomics. 17:1–12. doi:10.1186/S12864-016-2529-1/FIGURES/6. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-016-2529-1

Ioannidis, J., and F. X. Donadeu. 2018. Comprehensive analysis of blood cells and plasma identifies tissue-specific miRNAs as potential novel circulating biomarkers in cattle. BMC Genomics. 19. doi:10.1186/S12864-018-4646-5. Available from: /pmc/articles/PMC5894187/

Johnston, D., B. Earley, M. S. McCabe, J. Kim, J. F. Taylor, K. Lemon, M. McMenamy, C. Duffy, S. L. Cosby, and S. M. Waters. 2021. Elucidation of the Host Bronchial Lymph Node miRNA Transcriptome Response to Bovine Respiratory Syncytial Virus. Frontiers in Genetics. 12. doi:10.3389/FGENE.2021.633125/FULL. Available from: /pmc/articles/PMC8100685/

Jung, G., E. Hernández-Illán, L. Moreira, F. Balaguer, and A. Goel. 2020. Epigenetics of colorectal cancer: biomarker and therapeutic potential. Nature Reviews Gastroenterology & Hepatology 2020 17:2. 17:111–130. doi:10.1038/S41575-019-0230-Y. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41575-019-0230-y

Kawaguchi, T., L. Yan, Q. Qi, X. Peng, E. M. Gabriel, J. Young, S. Liu, and K. Takabe. 2017. Overexpression of suppressive microRNAs, miR-30a and miR-200c are associated with improved survival of breast cancer patients. Scientific Reports. 7. doi:10.1038/S41598-017-16112-Y. Available from: /pmc/articles/PMC5698306/

Krishnan, H., J. Rayes, T. Miyashita, G. Ishii, E. P. Retzbach, S. A. Sheehan, A. Takemoto, Y.-W. Chang, K. Yoneda, J. Asai, L. Jensen, L. Chalise, A. Natsume, and G. S. Goldberg. 2018. Podoplanin: An emerging cancer biomarker and therapeutic target. Cancer Science. 109:1292–1299. doi:10.1111/CAS.13580. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/cas.13580

Ladomery, M., and □□□ G Dellaire#. 2002. Multifunctional zinc finger proteins in development and disease. Annals of Human Genetics. 66:331–342. doi:10.1017/S0003480002001215. Available from: https://www-cambridge-org.login.ezproxy.library.ualberta.ca/core/journals/annals-of-human-genetics/article/multifunctional-zinc-finger-proteins-in-development-and-disease/4FF9EA3338E54D0B61942E0B0D5B5EAE

Lawless, N., A. B. K. Foroushani, M. S. McCabe, C. O'Farrelly, and D. J. Lynn. 2013. Next Generation Sequencing Reveals the Expression of a Unique miRNA Profile in Response to a Gram-Positive Bacterial Infection. PLOS ONE. 8:e57543.

doi:10.1371/JOURNAL.PONE.0057543. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0057543

Lee, J., Soohyun Lee, J. Son, H. Lim, E. Kim, D. Kim, S. Ha, T. Hur, Seunghwan Lee, and I. Choi. 2020. Analysis of circulating-microRNA expression in lactating Holstein cows under summer heat stress. PLOS ONE. 15:e0231125. doi:10.1371/JOURNAL.PONE.0231125. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0231125

Li, W., E. O'hara, H. Sun, K. S. Schwartzkopf-Genswein, L. L. Guan, L. Li, X. Liu, L. Jiang, H. Yu, S. Nie, M. Xie, and J. Gong. 57 President Oral Presentation Pick: The exploration of blood microRNA profiling in feedlot cattle with different lameness phenotypes. J. Anim. Sci. 98. Available from: https://academic.oup.com/jas/article/98/Supplement_4/40/6011538

Liang, Y., D. Ridzon, L. Wong, and C. Chen. 2007. Characterization of microRNA expression profiles in normal human tissues. BMC Genomics 2007 8:1. 8:1–20. doi:10.1186/1471-2164-8-166. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/articles/10.1186/1471-2164-8-166

Lin, X., K. C. Pavani, K. Smits, D. Deforce, B. Heindryckx, A. van Soom, and L. Peelman. 2019. Bta-miR-10b Secreted by Bovine Embryos Negatively Impacts Preimplantation Embryo Quality. Frontiers in Genetics. 10:757. doi:10.3389/FGENE.2019.00757/BIBTEX.

Love, M. I., W. Huber, and S. Anders. 2014a. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology. 15:1–21. doi:10.1186/S13059-014-0550-8/FIGURES/9. Available from: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-014-0550-8

Love, M. I., W. Huber, and S. Anders. 2014b. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology. 15:1–21. doi:10.1186/S13059-014-0550-8/FIGURES/9. Available from: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-014-0550-8

Luoreng, Z. M., X. P. Wang, C. G. Mei, and L. sen Zan. 2018. Expression profiling of peripheral blood miRNA using RNAseq technology in dairy cows with Escherichia coli-induced mastitis.

Scientific Reports 2018 8:1. 8:1–10. doi:10.1038/S41598-018-30518-2. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-30518-2

Ma, S., C. Tong, E. M. Ibeagha-Awemu, and X. Zhao. 2019. Identification and characterization of differentially expressed exosomal microRNAs in bovine milk infected with Staphylococcus aureus. BMC Genomics. 20:1–13. doi:10.1186/S12864-019-6338-1/FIGURES/5. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-019-6338-1

Menjivar, N. G., S. Gebremedhn, D. Tesfaye, N. G. Menjivar, S. Gebremedhn, and D. Tesfaye. 2021. 108 Preferential loading of thermal stress-associated microRNAs into extracellular vesicles: attempt to mitigate effects of heat stress in bovine granulosa cells. Reproduction, Fertility and Development. 34:291–291. doi:10.1071/RDV34N2AB108. Available from: https://www-publish-csiro-au.login.ezproxy.library.ualberta.ca/rd/RDv34n2Ab108

Miretti, S., C. Lecchi, F. Ceciliani, and M. Baratta. 2020. MicroRNAs as Biomarkers for Animal Health and Welfare in Livestock. Frontiers in Veterinary Science. 7:985. doi:10.3389/FVETS.2020.578193/BIBTEX.

Montani, F., and F. Bianchi. 2016. Circulating Cancer Biomarkers: The Macro-revolution of the Micro-RNA. EBioMedicine. 5:4–6. doi:10.1016/J.EBIOM.2016.02.038.

Nagandla, H., S. Lopez, W. Yu, T. L. Rasmussen, H. O. Tucker, R. J. Schwartz, and M. D. Stewart. 2016. Defective myogenesis in the absence of the muscle-specific lysine methyltransferase SMYD1. Developmental Biology. 410:86–97. doi:10.1016/J.YDBIO.2015.12.005.

Norring, M., J. Häggman, H. Simojoki, P. Tamminen, C. Winckler, and M. Pastell. 2014. Short communication: Lameness impairs feeding behavior of dairy cows. Journal of Dairy Science. 97:4317–4321. doi:10.3168/JDS.2013-7512.

O'Brien, J., H. Hayder, Y. Zayed, and C. Peng. 2018. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Frontiers in Endocrinology. 9:402. doi:10.3389/FENDO.2018.00402/BIBTEX.

O'Leary, N. W., D. T. Byrne, A. H. O'Connor, and L. Shalloo. 2020. Invited review: Cattle lameness detection with accelerometers. Journal of Dairy Science. 103:3895–3911.

doi:10.3168/JDS.2019-17123.

de Oliveira, P. S. N., L. L. Coutinho, P. C. Tizioto, A. S. M. Cesar, G. B. de Oliveira, W. J. da S. Diniz, A. O. de Lima, J. M. Reecy, G. B. Mourão, A. Zerlotini, and L. C. A. Regitano. 2018. An integrative transcriptome analysis indicates regulatory mRNA-miRNA networks for residual feed intake in Nelore cattle. Scientific Reports 2018 8:1. 8:1–12. doi:10.1038/S41598-018-35315-5. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-35315-5

Ren, L., Q. Li, X. Hu, Q. Yang, M. Du, Y. Xing, Y. Wang, J. Li, and L. Zhang. 2020. A Novel Mechanism of bta-miR-210 in Bovine Early Intramuscular Adipogenesis. Genes (Basel). 11. doi:10.3390/GENES11060601. Available from: /pmc/articles/PMC7349823/

Rosenberg, M. I., S. A. Georges, A. Asawachaicharn, E. Analau, and S. J. Tapscott. 2006. MyoD inhibits Fstl1 and Utrn expression by inducing transcription of miR-206. Journal of Cell Biology. 175:77–85. doi:10.1083/JCB.200603039. Available from: http://www.jcb.org/cgi/doi/10.1083/jcb.200603039

Sahu, T. K., A. K. S. Gurjar, P. K. Meher, C. Varghese, S. Marwaha, G. P. Rao, A. Rai, N. Guleria, S. H. Basagoudanavar, A. Sanyal, and A. R. Rao. 2020. Computational insights into RNAi-based therapeutics for foot and mouth disease of Bos taurus. Scientific Reports 2020 10:1. 10:1–13. doi:10.1038/S41598-020-78541-6. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-020-78541-6

Siracusa, J., N. Koulmann, A. Sourdrille, C. Chapus, C. Verret, S. Bourdon, M. E. Goriot, and S. Banzet. 2018. Phenotype-specific response of circulating miRNAs provides new biomarkers of slow or fast muscle damage. Frontiers in Physiology. 9:684. doi:10.3389/FPHYS.2018.00684/BIBTEX.

Sprecher, D. J., D. E. Hostetler, and J. B. Kaneene. 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology. 47:1179–1187. doi:10.1016/S0093-691X(97)00098-8.

Sun, H. Z., Y. Chen, and L. L. Guan. 2019a. MicroRNA expression profiles across blood and different tissues in cattle. Scientific Data 2019 6:1. 6:1–8. doi:10.1038/sdata.2019.13. Available

from: https://www.nature.com/articles/sdata201913

Sun, H. Z., Y. Chen, and L. L. Guan. 2019b. MicroRNA expression profiles across blood and different tissues in cattle. Scientific Data 2019 6:1. 6:1–8. doi:10.1038/sdata.2019.13. Available from: https://www.nature.com/articles/sdata201913

Sun, J., T. S. Sonstegard, C. Li, Y. Huang, Z. Li, X. Lan, C. Zhang, C. Lei, X. Zhao, and H. Chen. 2015. Altered microRNA expression in bovine skeletal muscle with age. Animal Genetics. 46:227–238. doi:10.1111/AGE.12272. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/age.12272

Sun, J., B. Zhang, X. Lan, C. Zhang, C. Lei, and H. Chen. 2014. Comparative Transcriptome Analysis Reveals Significant Differences in MicroRNA Expression and Their Target Genes between Adipose and Muscular Tissues in Cattle. PLOS ONE. 9:e102142. doi:10.1371/JOURNAL.PONE.0102142. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0102142

Sun, Y., X. Zhao, Y. Zhou, and Y. Hu. 2012. MiR-124, miR-137 and miR-340 regulate colorectal cancer growth via inhibition of the Warburg effect. Oncology Reports. 28:1346–1352. doi:10.3892/OR.2012.1958/HTML. Available from: http://www.spandidos-publications.com.login.ezproxy.library.ualberta.ca/10.3892/or.2012.1958/abstract

Tadich, N., E. Flor, and L. Green. 2010. Associations between hoof lesions and locomotion score in 1098 unsound dairy cows. The Veterinary Journal. 184:60–65. doi:10.1016/J.TVJL.2009.01.005.

Taxis, T. M., F. v. Bauermann, J. F. Ridpath, and E. Casas. 2017. Circulating MicroRNAs in serum from cattle challenged with bovine viral diarrhea virus. Frontiers in Genetics. 8:91. doi:10.3389/FGENE.2017.00091/BIBTEX.

Thomas, L. H., P. D. Wood, and J. M. Longland. 1978. The Influence of Disease on the Performance of Beef Cattle. British Veterinary Journal. 134:152–161. doi:10.1016/S0007-1935(17)33539-X.

Thomsen, P. T., L. Munksgaard, and F. A. Togersen. 2008. Evaluation of a Lameness Scoring System for Dairy Cows. Journal of Dairy Science. 91:119–126. doi:10.3168/JDS.2007-0496.

Tunstall, J., K. Mueller, D. Grove-White, J. W. H. Oultram, and H. M. Higgins. 2021. Lameness in Beef Cattle: A Cross-Sectional Descriptive Survey of On-Farm Practices and Approaches. Frontiers in Veterinary Science. 0:490. doi:10.3389/FVETS.2021.657299.

Ufkin, M. L., S. Peterson, X. Yang, H. Driscoll, C. Duarte, and P. Sathyanarayana. 2014. miR-125a regulates cell cycle, proliferation, and apoptosis by targeting the ErbB pathway in acute myeloid leukemia. Leukemia Research. 38:402–410. doi:10.1016/J.LEUKRES.2013.12.021.

Wang, L., Z.-H. You, X. Chen, Y.-M. Li, Y.-N. Dong, L.-P. Li, and K. Zheng. 2019. LMTRDA: Using logistic model tree to predict MiRNA-disease associations by fusing multi-source information of sequences and similarities. PLOS Computational Biology. 15:e1006865. doi:10.1371/JOURNAL.PCBI.1006865. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/ploscompbiol/article?id=10.1371/journal.pcbi.1006865

Wang, O., M. Zhou, Y. Chen, T. A. McAllister, G. Plastow, K. Stanford, B. Selinger, and L. L. Guan. 2021. MicroRNAomes of Cattle Intestinal Tissues Revealed Possible miRNA Regulated Mechanisms Involved in Escherichia coli O157 Fecal Shedding. Frontiers in Cellular and Infection Microbiology. 11. doi:10.3389/FCIMB.2021.634505/FULL. Available from: /pmc/articles/PMC7959717/

Whay, H. 2002a. Locomotion scoring and lameness detection in diary cattle. In Practice. 24:444–449. doi:10.1136/INPRACT.24.8.444.

Whay, H. 2002b. Locomotion scoring and lameness detection in dairy cattle. In Practice. 24:444–449. doi:10.1136/INPRACT.24.8.444. Available from: https://onlinelibrary.wiley.com/doi/full/10.1136/inpract.24.8.444

Ye, J., M. Xu, X. Tian, S. Cai, and S. Zeng. 2019. Research advances in the detection of miRNA. Journal of Pharmaceutical Analysis. 9:217–226. doi:10.1016/J.JPHA.2019.05.004.

Zhao, C., F. Tian, Y. Yu, G. Liu, L. Zan, M. S. Updike, and J. Song. 2012. MiRNA-dysregulation associated with tenderness variation induced by acute stress in Angus cattle. Journal of Animal Science and Biotechnology. 3:1–8. doi:10.1186/2049-1891-3-12/FIGURES/5. Available from: https://jasbsci.biomedcentral.com/articles/10.1186/2049-1891-3-12

Zhao, K., G. Liang, X. Sun, and L. L. Guan. 2016a. Comparative miRNAome analysis revealed

different miRNA expression profiles in bovine sera and exosomes. BMC Genomics. 17:1–12. doi:10.1186/S12864-016-2962-1/TABLES/6. Available from: https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-016-2962-1

Zhao, K., G. Liang, X. Sun, and L. L. Guan. 2016b. Comparative miRNAome analysis revealed different miRNA expression profiles in bovine sera and exosomes. BMC Genomics. 17:1–12. doi:10.1186/S12864-016-2962-1/TABLES/6. Available from: https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-016-2962-1

2.7 Figures and tables

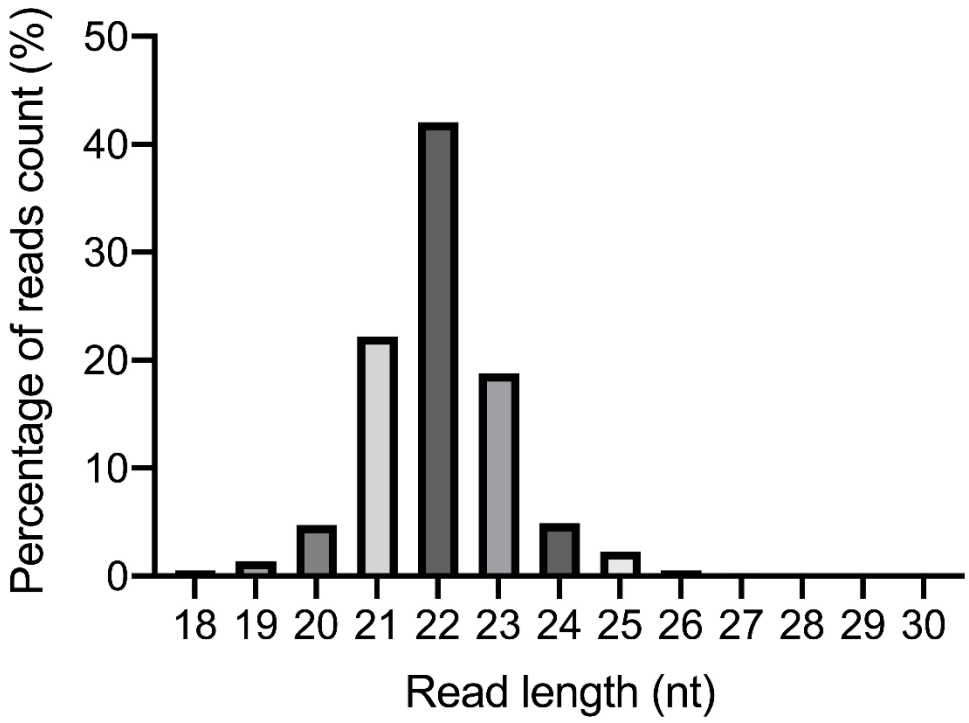

Figure 2.1 Length distribution of identified small RNA reads from blood samples collected from beef steers generated from RNA-seq. The X axis represents the read length of sequence reads and the Y axis represents their percentage.

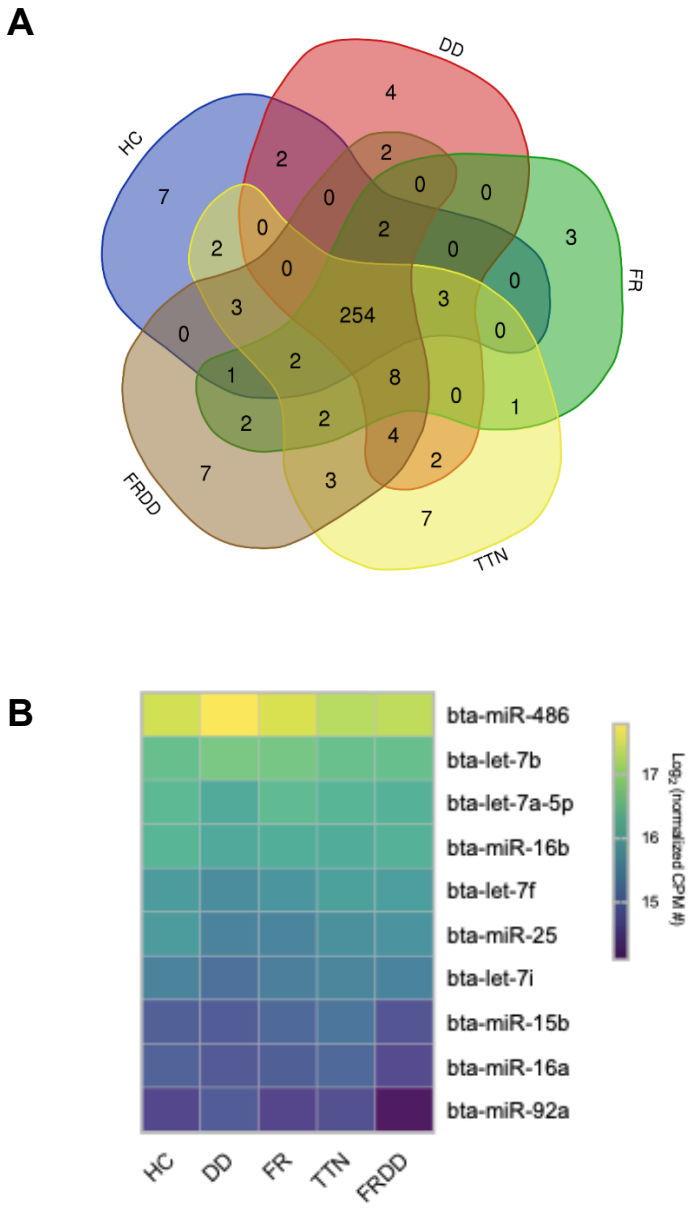

Figure 2.2 Overview of miRNAs expressed in from blood samples collected from beef steers using miRNA sequencing. (A) Comparison of the number of expressed miRNAs detected in health control (HC), digital dermatitis (DD), foot rot (FR), toe tip necrosis (TTN) and foot rot & digital dermatitis combined (FRDD). Colors represent different groups. (B) Comparative expression of top 10 highly expressed miRNAs in health control (HC), digital dermatitis (DD), foot rot (FR), toe tip necrosis (TTN) and foot rot & digital dermatitis combined (FRDD) using Heatmap function in GraphPad Prism 9. Colors represent different normalized reads per million (RPM) as indicated by the color bar.

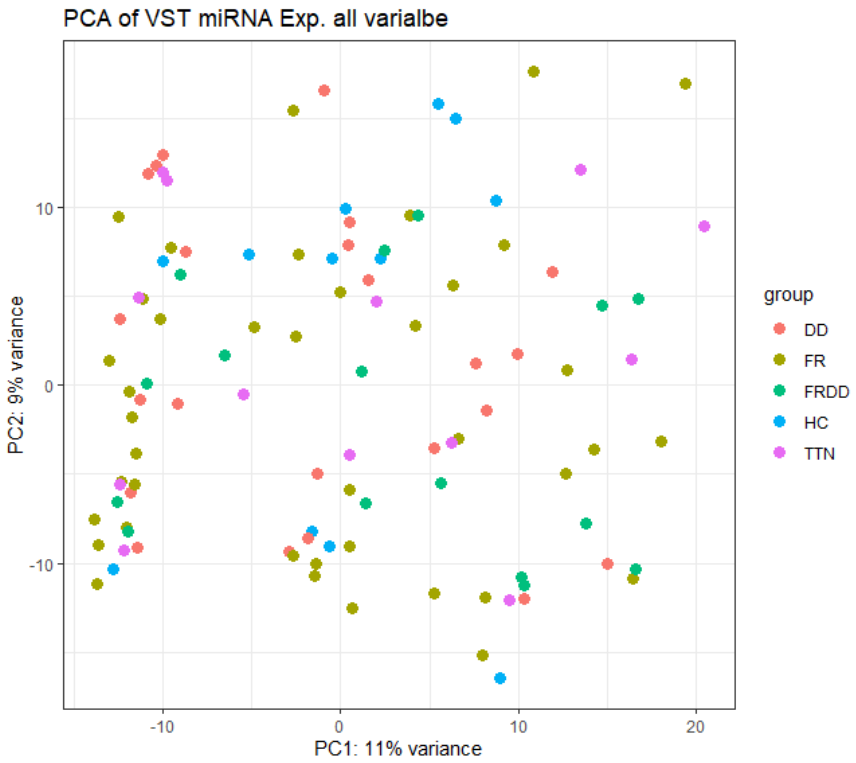

Figure 2.3 PCA plot of all the identified miRNAs. Comparison of overall miRNA profiles detected in health control (HC), digital dermatitis (DD), foot rot (FR), toe tip necrosis (TTN) and foot rot & digital dermatitis combined (FRDD). Each dot represents each sample, and the colors represent different groups. The X and Y-axis represent the first two principal components. The percentage value in the bracket represents the percentage of variance explained by that principal component. Nonsignificant difference had been identified from the miRNA profiles between each group.

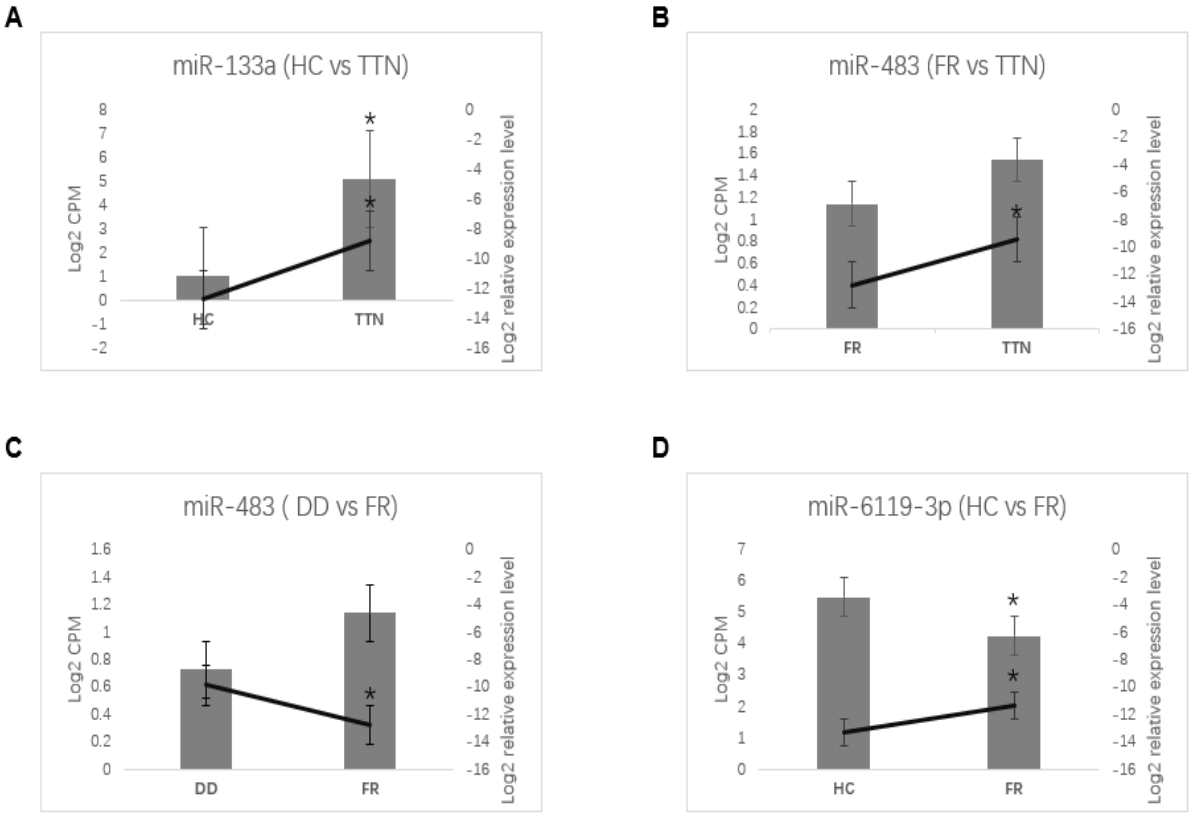

Figure 2.4 Expression of selected DE and phenotype specific miRNAs between each group detected by RT-qPCR and RNA-seq (A) Expression of miR-133a between health control (HC) and toe tip necrosis (TTN) group; (B) Expression of miR-483 between foot rot (FR) and TTN group; (C) Expression of miR-483 between digital dermatitis (DD) group and FR group; (D) Expression of miR-6119-3p between HC and FR group. miRNA expression from RNA-seq represented by bars and values are shown on the left vertical axis as log_2RPM and the expression level from RT-qPCR represented by lines and values are shown on the right vertical axis as log2 relative expression level. * On the top of the bar or lines indicates significant (P <0.05) difference identified, data are presented as Mean ± STD

Table 2.1 Phenotype specific miRNAs and expression levels

	Phenotype specific miRNA	Average expression level (RPM)
HC	bta-miR-10b	1.28±0.47
	bta-miR-2903	1.78±0.76
	bta-miR-2285aj-5p	1.48±0.64
	bta-miR-10164-3p	1.70±0.88
DD	bta-miR-2887	3.04±3.49
	bta-miR-2320-3p	1.28±0.60
	bta-miR-2904	7.46±6.20
TTN	bta-miR-7861	1.16±0.38
	bta-miR-11998	1.60±0.53
	bta-miR-483	2.91±2.30
	bta-miR-2285dj	1.26±0.69
FR	bta-miR-2382-5p	1.56±0.80
	bta-miR-200a	3.60±2.82
FRDD	bta-miR-1388-5p	1.28±0.66
	bta-miR-494	1.69±1.01
	bta-miR-2483-5p	1.34±0.72

Table 2.2 Differentially expressed miRNAs between health control and different lameness phenotype groups

Group	DE miRNA	log2FoldChange	*P*adj
DD vs HC	bta-miR-6119-3p	-1.50	2.00E-04
	bta-miR-2408	-1.46	5.00E-04
	bta-miR-340	-1.01	7.00E-03
FR vs HC	bta-miR-6119-3p	-1.29	2.00E-04
	bta-miR-125a	-1.01	2.00E-04
	bta-miR-200c	1.14	1.00E-04
	bta-miR-30a-5p	1.17	6.00E-04
	bta-miR-10225a	1.31	4.00E-03
	bta-miR-206	1.81	4.00E-03
	bta-miR-133a	2.45	8.00E-04
FRDD vs HC	bta-miR-6119-3p	-1.75	3.00E-04
	bta-miR-1434-3p	-1.58	7.00E-03
	bta-miR-211	-1.23	2.00E-02
	bta-miR-1246	-1.09	3.00E-02
	bta-miR-125a	-1.09	3.00E-04
	bta-miR-484	-1.08	7.00E-04
	bta-miR-92b	-1.07	6.00E-05
	bta-miR-1306	-1.03	8.00E-03
	bta-miR-2378	1.03	4.00E-02
	bta-miR-338	1.04	9.00E-03
	bta-miR-497	1.12	3.00E-04
	bta-miR-210	1.17	1.00E-05
	bta-miR-345-5p	1.47	1.00E-09
	bta-miR-21-3p	1.71	2.00E-06
TTN vs HC	bta-miR-6119-3p	-1.36	7.00E-03
	bta-miR-2408	-1.22	2.00E-02
	bta-miR-340	-1.12	1.00E-02
	bta-miR-1	2.67	8.00E-03
	bta-miR-206	2.94	3.00E-05
	bta-miR-133a	4.32	1.00E-07

Table 2.3 Identified differential expressed miRNAs between lameness phenotype groups

Group	DE miRNA	log2FoldChange	*P*adj
DD vs FR	bta-miR-133a	-1.44	1.41E-02
	bta-miR-141	-1.31	7.48E-05
	bta-miR-200c	-1.12	7.39E-09
	bta-miR-206	-1.06	3.43E-02
	bta-miR-122	1.60	5.27E-04
DD vs FRDD	bta-miR-2363	-1.76	7.97E-03
	bta-miR-497	-1.23	1.01E-05
	bta-miR-21-3p	-1.17	7.80E-05
	bta-miR-339b	-1.11	9.10E-07
	bta-miR-339a	-1.07	1.10E-06
	bta-miR-10225a	-1.07	9.02E-03
	bta-miR-127	-1.01	5.93E-04
	bta-miR-877	1.00	1.84E-02
	bta-miR-2284z	1.01	2.47E-07
	bta-miR-374b	1.01	8.88E-08
	bta-miR-92a	1.04	1.04E-05
	bta-miR-2284aa	1.06	1.13E-07
	bta-miR-320a	1.20	1.17E-04
	bta-miR-451	1.27	8.88E-08
	bta-miR-2332	1.28	9.02E-03
	bta-miR-1	1.36	3.26E-02
	bta-miR-1249	1.45	2.06E-03
	bta-miR-92b	1.49	1.04E-07
	bta-miR-2404	1.83	2.59E-04
	bta-miR-1434-3p	1.93	1.90E-03
DD vs TTN	bta-miR-133a	-3.34	8.05E-07
	bta-miR-206	-2.19	9.61E-04
	bta-miR-211	-1.32	2.36E-02
	bta-miR-452	-1.29	2.30E-02
	bta-miR-2285bf	1.03	1.39E-02
	bta-miR-129-5p	1.17	3.43E-02
FR vs FRDD	bta-miR-2363	-1.70	4.08E-03
	bta-miR-199a-5p	-1.20	6.72E-03
	bta-miR-127	-1.18	6.69E-06
	bta-miR-339b	-1.18	1.18E-08
	bta-miR-339a	-1.14	1.18E-08
	bta-miR-21-5p	1.02	8.32E-12

	bta-miR-877	1.10	4.95E-03
	bta-miR-92b	1.18	6.67E-06
	bta-miR-1434-3p	1.50	1.19E-02
	bta-miR-200c	1.52	5.49E-13
	bta-miR-206	1.75	3.40E-04
	bta-miR-133a	1.83	1.66E-03
FR vs TTN	bta-miR-133a	-1.90	1.77E-02
	bta-miR-1	-1.80	1.98E-02
	bta-miR-122	-1.39	3.95E-02
	bta-miR-11986c	1.02	3.78E-02
	bta-miR-141	1.19	1.77E-02
	bta-miR-129	1.42	1.99E-02
	bta-miR-129-5p	1.43	4.13E-03
FRDD vs TTN	bta-miR-129-5p	-1.61	3.06E-04
	bta-miR-129	-1.51	5.39E-03
	bta-miR-21-3p	-1.15	2.15E-03
	bta-miR-11986c	-1.13	1.60E-02
	bta-miR-345-5p	-1.09	1.15E-03
	bta-miR-339b	-1.08	1.54E-04
	bta-miR-210	-1.08	5.59E-06
	bta-miR-339a	-1.01	2.56E-04
	bta-miR-92b	1.05	3.62E-03
	bta-miR-2404	1.62	1.10E-02
	bta-miR-211	1.80	5.18E-04
	bta-miR-1434-3p	2.18	4.15E-03
	bta-miR-1	2.64	2.80E-04
	bta-miR-206	2.88	5.59E-06
	bta-miR-133a	3.73	2.71E-07

Table 2.4 Significantly enriched functional categories of down-regulated differentially expressed miRNAs using TargetScan and DAVID

Group	miRNAs	Functional Annotation Clustering (With highest enrichment score)	Function	Reference
DD & TTN vs HC	bta-miR-340	Zinc-finger Zinc ion binding Metal-binding Zinc	Inhibition of cancer cells growth	Sun et al., 2012 Fernandez et al., 2015
FR & FRDD vs HC	bta-miR-125a	Voltage-gated channel Ion transport Zinc finger, C2H2-like Metal ion binding	Inhibition of cell cycle progression	Wu et al., 2011 Xiong et al., 2017
All groups vs HC	bta-miR-6119-3p	Zinc-finger Zinc ion binding Metal-binding Zinc	Formation of seborrhea-like dermatitis Formation of psoriatic epidermis	Cassandri et al., 2017
DD & TTN vs HC	bta-miR-2408	Src homology-3 domain CGMP-PKG signaling pathway Zinc-finger Zinc ion binding	Tumor suppressors Development of inflammatory disease	Ji et al., 2014
FRDD vs HC	bta-miR-211	Cadherin-like Cadherin conserved site Sterile alpha motif/pointed domain	Host immune regulation	Wang et al., 2021
FRDD vs HC	bta-miR-92b	MAD homology 1, Dwarfin-type CTF transcription factor/nuclear factor 1	*E. coli* lipopolysaccharide-mediated inflammatory injury	Jiang et al., 2021

Table 2.5 Significantly enriched functional categories of up-regulated differentially expressed miRNAs using TargetScan and DAVID

Group	miRNAs	Functional Annotation Clustering (With highest enrichment score)	Function	Reference
TTN vs HC	bta-miR-1	DNA-binding	Regulate skeletal muscle development	Valsecchi et al., 2015
TTN vs HC	bta-miR-206	SH3 domain BTB/POZ-like	Regulate muscle cell differentiation	Chen et al., 2006
TTN vs HC	bta-miR-133	Pleckstrin homology-like domain	Suppress tumor growth, cell migration and invasion	Kawaguchi et al., 2017
FR vs HC	bta-miR-30a	zinc ion binding K Homology domain		
FR vs HC	bta-miR-200c	Serine/threonine-protein kinase, active site		
FRDD vs HC	bta-miR-497	Protein kinase, catalytic domain Serine/threonine-protein kinase, active site	Muscle traumatic stress	Zhao, 2012
FRDD vs HC	bta-miR-210	FoxO signaling pathway Insulin resistance	Bovine early intramuscular adipogenesis	Ren et al., 2020

Chapter 3. Analysis of temporal expression of miRNAs in cattle blood revealed their relationship with lameness recovery patterns

3.1 Introduction

Beef cattle are more likely to get lameness when entering the feedlot due to changes in diet, water, housing, rough flooring surfaces, and a series of stressors including transportation, high population density and different handling strategies (Davis-Unger et al., 2019e). The causes of lameness are multi-factorial, including environmental factors like the muddy feedlot pens and nutritional factors such as low calcium and magnesium intake that make the hoofs soften and more susceptible (Smart, 1985; Marti et al., 2021). Compared to other common diseases that happen in the feedlot, the subclinical symptoms of lameness are hard to be observed and require different treatments (Qiao et al., 2021b). Foot rot is the most prevalent phenotype of lameness (75% of all cases) and is usually treated by injectable antibiotics including penicillin or broad-spectrum antibiotics (Currin et al., 2005). However, many operators may treat lame cattle as foot rot without close observation, which may lead to ineffective treatment, this suggests a detailed diagnostic method is needed for the varied causes and pathogenesis of different lameness phenotypes (Tunstall et al., 2019). In addition, some lameness phenotypes like digital dermatitis may not respond well to antibiotic treatment (Laven and Logue, 2006; Penny et al., 2017). Moreover, the lameness pathogenesis of different lameness phenotypes is still not well understood, especially in beef cattle (Huxley, 2013a). Furthermore, a large percentage of lame dairy cows had high relapse rates after treatments

(Thomas et al., 2016); only less than 16% was recovered. This suggests that proper diagnosis and treatments are essential to improve lameness detection, prognosis and recovery.

The previous research chapter identified that blood microRNAs, a group of non-coding RNAs, are associated with different lameness phenotypes. The expression of some miRNAs was only detected in the blood of cattle with specific lameness phenotype, and expression of some miRNAs was up- or down-regulated in the lame cattle compared to healthy ones. Specifically, 18 differentially expressed miRNA (DE miRNA) were identified in each lameness phenotype group when compared with healthy cattle, along with 16 lameness phenotype-specific miRNAs. These findings suggest that blood miRNAs have the potential to be diagnostic markers for lame cattle, other animals (Ioannidis and Donadeu, 2016b; Ioannidis and Donadeu, 2018b), and even in humans (Duttagupta et al., 2011; Cheng et al., 2014). Apart from disease diagnosis markers, the miRNA profiles may also function as markers of disease progression or recovery, because their expression-changing trends may reflect different physiological states associated with advancing disease or a return to the health status (Lau et al., 2013). For example, the different profiles of specific miRNAs, such as hsa-miR-503, -1307, -212 and -592, between healthy and people affected by early/advanced-stage breast cancer showed the relationship between miRNA and cancer prognosis, stages and severity (Sathipati and Ho, 2018). The serum miRNA profiles between humans suffering from liver injury and recovered individuals were compared using miRNA RT-qPCR, which demonstrated that a unique miRNA signature undergoes concordant changes in three types of recovery after liver transplantation and infection with the hepatitis C

virus (Salehi et al., 2020). Therefore, miRNA profiles in biological samples may be useful in identifying varying stages of disease progression and recovery. However, whether temporal differences in miRNA profiles can be detected in various stages of either lameness progression or recovery has not been studied.

In the present study, we hypothesized that the miRNA profiles change after lameness treatment and the profiles differ between recovered and unrecovered cattle for each lameness phenotype. Therefore, the aim of this study was to identify the temporal changes of miRNAs expression in blood over a two–week period following diagnosis and treatment for three types of lameness: digital dermatitis (DD), toe tip necrosis (TTN) and foot rot (FR) and DD combined (FRDD), respectively.

3.2 Material and methods

3.2.1 Whole Blood sample selection

A total of 596 whole blood samples were collected from 363 lames (n = 344) and healthy cattle (n = 19) by venipuncture and using Tempus™ Blood RNA Tubes (Thermo Fisher Scientific, CA) between the 2018 fall and the 2020 spring. The cattle used in this study were selected from five feedlots (> 10000 heads) located in southern Alberta and consisted of 142 heifers and 221 beef steers with different breeds, including Black/red Angus, Simmental, Charolais, Hereford and other mixed breeds, respectively. The blood sample collection followed the Animal Use protocol Review #1817 approved by the Lethbridge RDC Animal care committee. To identify the relationship between lameness recovery and miRNA expression patterns, 140 blood samples werecollected

from three lameness phenotype groups, including DD (n = 59), TTN (n = 31) and FRDD (n = 50) were selected for miRNA-seq in this study. To be more specific, the blood samples were collected on the day when lame cattle were pulled for treatment (defined as **Week 0**, **W0**); one week after diagnosis and treatment (defined as **Week 1**, **W1**) and 2 weeks after the first treatment (defined as **Week 2**, **W2**). Afterwards, the lame cattle were further classified into 18 different groups based on their monitored gait score, including recovered (RE) and unrecovered (UNR) across the three-time points (2 recovery patterns * 3 lameness phenotypes * 3 time points=18). Specifically, the gait score of each lame animal was categorized according to a 4-point scale (between 0 and 3) previously described by Webster et al. (2008), with 0 defined as no lameness and 3 defined as the most severe lameness. The cattle with gait scores showed a down-regulating trend among the three time points were dd as RE cattle, and UNR cattle's gait scores were increased or not changed from **W0** to **W2**. The sample number of each group is presented in Table 3.1, and the detailed sample information is listed in the appendix Table A1. FR group was not included in this study as all FR samples were collected in **W0** only.

3.2.2 Total RNA extraction, miRNA-seq library construction, and sequencing

Total RNA was extracted from 4.5 mL of whole blood using Preserved Blood RNA Purification Kit I (Norgen, Thorold, ON, CA) following the manufacturer's protocol. The concentration and quality of total RNA were measured following the procedures described in Chapter 2. Only the total RNA samples with RIN > 7.0 passed the quality control and were subjected to RNA-seq library construction.

One microgram of total RNA from each sample was selected for miRNA library construction using QIAseq miRNA Library Kit (Qiagen, CA) following the manufacturer's instructions. The library quantity was determined using Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) and was sent to Génome Québec (Montréal, Canada) for sequencing with 50 bases single-end reads on an Illumina HiSeq 2000 system (Illumina).

3.2.3 miRNA sequencing data analysis

Low-quality sequences from the raw sequencing data were filtered by quality control and the passed sequences were then aligned to the bovine miRNA database (miRBase, v22.2) (Kozomara et al., 2019b) using the web-based tool sRNAtoolbox (https://arn.ugr.es/srnatoolbox/, Aparicio-Puerta et al., 2019) with default parameters which were described in the previous chapter. The expression level of each miRNA was normalized by counts per million mapped reads (RPM) by the following method: RPM = (miRNA reads number/total mapped reads per library) × 1000000. To compare the overall miRNA profiles, principal component analysis (PCA) was performed using DEseq2 and ggplot2 packages in R studio (Version 1.4.1717, RStudio Team, 2020); PCA plots were generated based on the following groupings: 1. Based on the time points within the same phenotype; 2. Based on the recovery information within the same time point and phenotype group.

3.3.4. Identification of differentially expressed miRNAs

The miRNA with RPM > 1 in at least 60% of samples in one group was identified as expressed miRNA and used for differential expression (DE) analysis. Differentially expressed miRNAs were

identified using the DEseq2 package Release (3.14) (Love et al., 2014c) in RStudio. As for the threshold of the differential expression analysis, adjusted p-value (padj) using the Benjamini and Hochberg method, also represented as a false discovery rate (FDR) (Benjamini and Hochberg, 1995). The expression miRNAs with $\log_2$ foldchange (FC) < -1 or > 1 and FDR < 0.05 was considered as significantly different.

The identification of DE and lameness phenotype-temporal miRNAs were performed on the following comparisons: 1). The lameness phenotype-temporal miRNAs were first identified between different time points for each lameness phenotype to investigate the time effect on the miRNA profile; 2). The differential expression analysis was performed between RE and UNR groups within the same time-point and lameness phenotype to identify the RE-related or UNR-related miRNAs for each lameness phenotype.

3.2.5 Time-series clustering analysis

To identify the relationship between miRNAs expression and different recovery patterns in three lameness phenotypes, time-series clustering analysis was performed to cluster differentially expressed miRNAs along with time series using datasets categorized from recovered DD cattle (DDRE), unrecovered DD cattle (DDUNR), recovered FRDD cattle (FRDDRE), unrecovered FRDD cattle (FRDDUNR), recovered TTN cattle (TTNRE) and unrecovered TTN cattle (TTNUNR) respectively (see Table 3.1) using the R package Mfuzz (v2.30, Futschik M, Carlisle B, 2005). To be more specific, the miRNA expression patterns were generated within each group which considered all the time, recovery, and phenotype information.: all **W0** blood samples from

the cattle diagnosed with DD and identified recovered during this period were defined as the DD recovered group at **W0** (DDREW0) and samples from unrecovered cattle were defined as the DD unrecovered group at **W0** (DDUNRW0), while the samples collected at **Week 1** and **Week 2** were defined as DDREW1, DDUNRW1, DDREW2 and DDUNRW2 as well. Correspondingly, similar names were defined in the FRDD and TTN groups with recovery and time point information (FRDD: FRDDREW0; FRDDUNRW0; FRDDREW1; FRDDUNRW1 FRDDREW2; FRDDUNRW2. TTN: TTNREW0; TTNUNRW0; TTNREW1; TTNUNRW1; TTNREW2; TTNUNRW2). The average RPM of each miRNA within each time point (**W1**, **W2** and W3, respectively) in each lameness phenotype (DD, TTN and FRDD) was used to generate the miRNA expression changing pattern among three time points. Temporally expressed miRNAs assigned to each time series cluster were filtered by a membership score > 0.7 (Futschik and Carlisle, 2005), and miRNA with the higher score (maximum 1) was more likely to belong to the specific pattern. A changing miRNA expression pattern between two-time points with an up-regulated trend was defined as "U," while no change in the expression pattern was defined as "N", and the down-regulated trend was defined as "D". The overall change in miRNA expression patterns within the three-time points were defined as a combination of the two letters. For example, if a miRNA expression showed continuous up-regulation within two weeks (from **W0** to **W1** and to **W2**), the miRNA expression changing pattern was defined as "U-U".

3.2.6 Target gene prediction and functional analysis

Target gene prediction was performed for the selected miRNAs based on their time-series changing

patterns using TargetScan (Release 7.2, http://www.targetscan.org/vert_72/, Agarwal et al., 2015). The functional enrichment analysis of predicted target genes was then performed using Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/). To identify the significance of predicted functional categories, multiple testing corrected P-value determined by the Benjamini-Hochberg method was used, and a threshold of FDR < 0.05 was applied to the enriched functions.

3.3 Results

3.3.1 Profiling of miRNAs in the blood of cattle with different lameness phenotypes over a two-week period after diagnosis and treatment

A total of 1,212 million high-quality 50 bp single reads were obtained from the 140 bovine blood miRNA-seq libraries. After quality control and the application of sequence length filters, ~ 96% of reads were mapped to the bovine mature miRNA database (miRBase, Release 22.1), with less than 2% of reads mapped to the bovine miRNA hairpin sequences (miRBase v22) and less than 1% of reads mapped to tRNA, snoRNA and rRNA (Figure 3.1A). Size distributions revealed most of the sequences had a length of 21-23 nt which accounted for 85.19 % of total reads (Figure 3.1B). A total of 350 known miRNAs were identified with an expression of 307 miRNAs detected for the DD group, 318 for the TTN group and 304 for the FDRR group across all three-time points. PCA plots showed that overall miRNA profiles were not grouped based on the time points for the DD group (Figure 3.2A), the TTN (Figure 3.2B) and FRDD groups (Figure 3.2C).

3.3.2 Identification of temporally specific expressed miRNAs

The temporally specific expressed miRNAs for each lameness phenotype were then identified by comparing the number of expressed miRNAs between each time point within the same lameness phenotype (Figure 3.3). Among identified miRNAs for each lameness phenotype, 267 miRNAs were expressed at all 3-time points in the DD group, with 5, 13, and 3 miRNAs specifically expressed at **W0**, **W1**, and **W2**, respectively (Figure 3.3A). The number of time-related commonly expressed miRNAs in the TTN group was 277, with 2 miRNAs expressed at **W0**, 3 at **W1**, and 6 at **W2** (Figure 3.3 B). In the FRDD group, no **W0** specific miRNA was detected, but two miRNAs were identified at **W1** and 10 at **W2**, with 273 miRNAs commonly expressed at all three-time points (Figure 3.3C). Temporally specific miRNAs were identified between different time points within same lameness phenotype. As a result, 30 temporally specific miRNAs were identified in nine groups (DDW0, DDW1, DDW2, TTNW0, TTNW1, TTNW2, FRDDW0, FRDDW1, FRDDW2) and their expression levels were presented in Table 3.2. From all temporally expressed miRNAs, bta-miR-2285j was screened from more than one lameness phenotype group & time point including DDW1 (RPM = 1.60 ± 0.93), TTNW2 (RPM = 1.33 ± 0.68), and FRDDW2 (RPM = 1.72 ± 1.45) groups. There were two miRNAs, bta-miR-2397-3p and bta-miR-149-5p, also identified in DDW1 (bta-miR-2397-3p, RPM = 1.21 ± 0.57) and FRDDW1 (bta-miR-2397-3p, RPM = 1.34 ± 0.45), DDW1 (bta-miR-149-5p, RPM = 1.46 ± 0.85) and FRDDW2 (bta-miR-149-5, RPM = 1.21 ± 0.54), respectively. Other selected temporal-specific miRNAs were only identified once in one lameness phenotype group.

3.3.3 Identification of differentially expressed miRNAs between recovered and recovered lame cattle

The expression of miRNAs was further compared between RE and UNR groups at each time point within each lameness phenotype group. PCA plots showed that overall miRNA profiles were not different between RE and UNR groups for DD at any of the time points (Figure 3.4), as well as in the TTN group at **W0** and **W2** (Figure 3.5A, C), and in FRDD group at **W0** and **W1** (Figure 3.6A, B). However, significant separations were detected between RE and UNR groups for TTN at **W1** (Figure 3.5B), and for FDRR at **W2** (Figure 3.6C).

The expression of miRNAs was further compared between RE and UNR groups at different time points, and a total of 10 DE miRNAs were identified among all the groups (Figure 3.7). Two miRNAs: bta-miR-1 and bta-miR-211 were differentially expressed between recovered and unrecovered DD cattle on **W0** with Bta-miR-1 having a higher expression (log_2FC of DDREW0 vs DDUNRW0 = 3.89, *P*adj = 0.009) and bta-miR-211 showing lower expression in the UNR cattle (log_2FC of DDREW0 vs DDUNRW0 = -1.99, *P*adj = 0.352) (Fig 3.8A). The DE miRNAs were also identified in the FRDD group: three miRNAs were differently expressed between RE and UNR cattle on **W0** including bta-miR-2903 (log_2FC of FRDDREW0 vs FRDDUNRW0 = -2.23, *P*adj = 0.009); bta-miR-874 (log_2FC of FRDDREW0 vs FRDDUNRW0 = -1.05, *P*adj = 0.009) and bta-miR-33b (log_2FC of FRDDREW0 vs FRDDUNRW0 = 1.58, *P*adj = 0.038). Moreover, the lowest log2 foldchange of DE miRNA was detected in the FRDDW1 group, with bta-miR-339a being less expressed in UNR cattle (log_2FC of FRDDREW1 vs

FRDDUNRW1 = -6.37, *P*adj = 0.003) (Figure 3.7B). Another four DE miRNAs, bta-miR-1246 were identified at week1 with $\log_2$ FC = 7.68 and *P*adj = 0.0001) and DE three miRNAs at **Week 2** including bta-miR-9-5p ($\log_2$FC of TTNREW2 vs TTNUNRW2 = 1.78, *P*adj < 0.001), bta-miR-296-3p ($\log_2$FC of TTNREW2 vs TTNUNRW2 = -1.17, *P*adj = 0.016) and bta-miR-6523a ($\log_2$FC of TTNREW2 vs TTNUNRW2 = -1.31, *P*adj = 0.025) which were identified as DE miRNAs between RE and UNR cattle in the TTN group (Figure 3.7C). Expression of bta-miR-1246 and bta-miR-9-5p were upregulated in UNR cattle at **Week 1** and **Week 2**, respectively, while expression of another two miRNAs, bta-miR-296-3p and bta-miR-6523a, were downregulated in uncovered TTN cattle at **Week 2**.

3.3.4 Temporal changes in miRNAs profiles between recovered and unrecovered cattle diagnosed with lameness

As shown in Figure 3.8, 36 temporal change patterns in miRNAs expression were identified in both RE and UNR groups within each lameness phenotype, respectively. Specifically, no N-N pattern was detected for any miRNA profile, while D-D and U-D patterns were only detected for miRNAs of unrecovered DD cattle, and D-U patterns were only detected in REDD cattle. For the TTN cattle, the U-N pattern was UNR phenotype-specific, and for FRDD cattle, U-D and U-U patterns were only detected in UNR and RE cattle, respectively. The expressed miRNAs with a membership score > 0.7 in each changing pattern indicated above are presented in Table 3.3.

3.3.5 Functional predictions of differentially expressed miRNAs and special miRNA changing patterns between recovered and unrecovered cattle diagnosed with lameness

The significantly enriched functional categories of the 10 DE miRNAs between RE and UNR cattle were identified through the target gene prediction and functional analysis. Among these, a total of 6 functional categories were enriched from 5 DE miRNAs (bta-miR-1, -211, -1246, -2903, -33b) based on the threshold of FDR < 0.05 including transcription regulation, cadherin conserved site, zinc-finger, homeobox conserved site, protein kinase-like domain, and BTB/POZ-like domain (Table 3.4), no significant enriched functional categories were discovered for the other 5 DE miRNAs.

In addition, miRNAs identified with the changing patterns between RE and UNR cattle were subjected to further functional analysis. The significantly enriched function categories of the selected six miRNAs change patterns included D-D, U-D in DDUNR cattle; D-U in recovered DD cattle; U-N in TTNUNR cattle; U-D in FRDDUNR cattle and U-U in FRDDRE cattle. A total of 5 functional categories were identified from the six miRNA patterns identified between RE and UNR cattle, including Ras signaling pathway, BTB/POZ-like domain, zinc-finger, homeodomain, metazoan, and DNA-binding (Table 3.5). Among these, four out of six changing patterns were specifically identified in UNR cattle, two in DD, one in TTN and one in FRDD groups, while no specific miRNA change trends were observed in recovered cattle. The only exception was in DD cattle, with 4 miRNAs (bta-miR-12044, bta-miR-142-3p, bta-miR-181b and bta-miR-374b) identified within the D-U trend which predicted functions in the BTB/BOZ-like domain. The

BTB/BOZ-like and zinc-finger functional categories had been identified in most of the changing patterns except for the U-D pattern in the FRDD group. In addition, bta-miR-505 was the only miRNA that had been identified within the U-U changing trend in recovered FRDD cattle over the two-week evaluation period however, no significant enriched functions were found for this miRNA.

3.4 Discussions

No significant difference in overall miRNA profiles identified between the different time points indicates the overall composition of blood miRNA profiles did not change significantly over the two-week sample collection period. Although time point-specific miRNAs were identified, their expression levels were too low to affect the overall profile expression.

From the identified time point-specific miRNAs for each group, some miRNAs expressed in more than one-time point and/or one lameness phenotype. For example, bta-miR-2285j was detected in the DDW1, TTNW2, and FRDDW2 groups; bta-miR-2397-3p was identified in the DDW1 and FRDDW1 groups, and bta-miR-149-5p was found in DDW1 and FRDDW2 groups. Among them, only bta-miR-149-5p was reported to have the predicted function as a negative regulator of adipocyte differentiation in bovine primary preadipocytes (Khan et al., 2020; Guo et al., 2021), and the functions of the other two miRNAs have not been reported. In addition, the expression of bta-miR-30f was only detected in the FRDDW2 group. Actually, bta-miR-30f could act as a lipid deposit regulator in cattle which negatively affects the fat deposit by inhibiting the PPAR signaling pathway (Y. Guo et al., 2017). Therefore, the inhibition of adipocyte

differentiation may happen in the first and the second week when cattle were diagnosed as and treated for DD and FRDD, respectively. The possible explanation of this regulation may relate to the reduced feed intake of lame cattle which could lead to a decrease in the body fat deposit. Moreover, the miRNA bta-miR-2887 was specifically identified expressed at **W0** in the DD group. A similar finding was also reported in Chapter 2, where bta-miR-2887 was identified as a phenotype-specific miRNA in DD cattle. This miRNA is an immune response-related miRNA that was up-regulated in bovine serum when infected by *Mycobacterium avium* (Choi et al., 2021). Also, eczematous dermatitis was diagnosed in humans after the *Mycobacterium avium* infection (Inagaki et al., 2018), providing the potential linkage between the up-regulation of bta-miR-2887 and dermatitis. The possible reason for the specific identification of this miRNA in DD samples may be related to the different pathogenesis when compared with FR and TTN, which are defined as dermatitis or skin disease rather than local tissue rot or necrosis. Thus, bta-miR-2887 could be a biomarker for the diagnosis of specific lameness phenotypes like DD since the expression was not identified in other lameness phenotypes and timepoints. Besides, no significant enriched or reported functions were identified for other timepoint-specific miRNAs, which may be related to their relatively low expression level (< 4 RPM) and are rarely studied.

In addition, the expression of some miRNAs related to the recovery patterns of cattle was identified for each lameness phenotype. Unique miRNAs were identified in specific recovery patterns among different lameness phenotypes, which were only detected in the blood of RE cattle or UNR cattle. For example, the bta-miR-10225a was only expressed in RE cattle in the DD group

at all three time points. This miRNA has been reported as a novel detected miRNA in the bovine alveolar macrophage challenged by *Mycobacterium bovis* (Vegh et al., 2015). *Mycobacterium bovis* can affect both humans and cattle (Grange and Yates, 1994), and chronic dermatitis was reported in humans after foodborne and waterborne *Mycobacterium bovi* infection (Dark, 2013). The possible explanation of the specific expression of bta-miR-10225a in DDRE cattle blood may be related to the unique type of DD: dermatitis. However, further studies about the target gene and functions of bta-miR-10225a and its role in the skin health of cattle are needed. Expression of bta-miR-339a, -1 and -10179-5p were detected at all three time points for unrecovered DD cattle. Bta-miR-339a was reported as an insulin signaling pathway regulator in the skeletal muscle and liver of beef cattle, with its expression can positively regulate the residual feed intake (RFI) of cattle (de Oliveira et al., 2018b), in other words, the downregulation of bta-miR-339a can reduce the RFI (cattle with high feed efficiency). The higher or specific expression of this miRNA suggests that DDUNR cattle may have higher RFI (lower feed efficiency) due to an unhealthy state compared with healthy cattle. Another unrecovered cattle-specific miRNA, bta-miR-1, had a downregulating trend for two weeks. This miRNA family was reported in cattle muscle which acts as a muscle cell development promoter in various studies (Sun et al., 2015b; Hao et al., 2021b). However, the clinical sign of DD is skin and underlying tissue lesions rather than muscle damage. Therefore, the identification of bta-miR-1 in the blood of unrecovered DD cattle suggests the possibility of misdiagnosis of these cattle in terms of their lameness phenotypes and is also evidenced by why they were not recovered after treatment. Future study is needed to determine whether this miRNA

can be transferred by microvesicles (exosomes) from muscle to blood. Moreover, bta-miR-206 was specifically expressed in FRDDUNR cattle blood when compared with recovered cattle. The miR-206 shares the same seed sequence with miR-1, which was also found to act as the major regulator of muscle reconnection following injury in mice (Mitchelson and Qin, 2015). As miRNAs are highly conserved in mammals (Pedersen et al., 2007), the findings identified in other mammalian species could have similar implications in cattle (Zhao et al., 2016c). Therefore, the expression of this miRNA indicates the potential myofiber reconnection in DDUNR cattle. Two possible reasons for this finding are: the muscle reconnection may happen on the healthy legs since they were used more to support the body, or maybe actually the cattle were affected by another muscle-related lameness. In addition, the expressions of bta-miR-1 and bta-miR-206 were significantly up-regulated in the TTN group at **W0** compared with health control but not differentially expressed in the FR and DD group (Chapter 2), which suggests that DDUNR and FRDDUNR animals might also be misdiagnosed, and the actual cause of the lameness could be TTN.

As for the DE miRNAs identified between RE and UNR cattle within the same lameness phenotype, significant upregulation of bta-miR-1 and downregulation of bta-miR-211was identified in unrecovered DD cattle at **W0**. The bta-miR-211 was identified as downregulated miRNA in the FRDD group when compared with health control at **W0**. The functions of bta-miR-211 were predicted to be associated with hematological system development, and immune cell trafficking, and its up-regulation at the recto-anal junction in British × Continental feedlot steers

is reported to be involved in reducing the presence of *Escherichia coli* O157:H7 in the digestive tract and its shedding in feces (Wang et al., 2021b). In addition, up-regulation of blood miRNA bta-miR-211 was reported to inhibit inflammation in Chinese Holstein cows through the reduction of secretory IL-10 (S. Wang et al., 2018; Chen et al., 2020a). These studies suggest that the downregulation of this miRNA in the blood may reflect the systemic exacerbate inflammation and altered immune function that subsequently affects lameness recovery. For up-regulated DE miRNAs, bta-miR-1246 and bta-miR-9-5p were highly expressed in TTNUNR cattle at **Week 1** and **Week 2** post-treatment, respectively. Increased expression of this miRNA was found in cattle suffering from heat stress (Lee et al., 2020c) and *S. aureus* infection (Li et al., 2015a; Z.M. Luoreng et al., 2018b). In addition, significant up-regulation of bta-miR-1246 was associated with responses to Caprine parainfluenza virus type 3, the *S. aureus* (Li et al., 2015b) and *M. avium subsp. Paratuberculosis* infections in bovine MDBK cells (J. Li et al., 2018), mammary glands and macrophages (Wang et al., 2019), respectively. Although *S. aureus* and *M. avium subsp. Paratuberculosis* is not the main pathogen associated with cattle lameness, *S. aureus* has been reported to be one of the pathogens related to lameness in chickens (Kumar et al., 2011) and mastitis in dairy cows (Ekesi et al., 2021). This suggests that the suppressed immunity caused by lameness may lead to other infectious diseases caused by the above bacteria (Stokka et al., 2001b; Davis-Unger et al., 2019f), and the identification of these miRNAs suggests the possible presence of other infectious diseases. Therefore, suppress the immune system and this complication may lead to a delay in the recovery from the lameness.

In addition, miRNAs with unique temporal expression patterns were identified in RE and UNR cattle. Two miRNAs, miR-2468 and miR-494, had downregulated expression over the two weeks evaluation period after treatment in the unrecovered DD cattle. The bta-miR-494 might play an essential role in body fat deposition (Lawless et al., 2013b) and the response to gram-negative bacterial infections (Seong et al., 2016). It was identified that the expression level of bta-miR-486 was significantly reduced after a lipopolysaccharide (LPS) challenge in mouse lungs (Lawless et al., 2013b). It is known that the primary pathogens of different lameness phenotypes, including DD, FR and TTN are all gram-negative bacteria (*Spirochete* and *Fusobacterium*) (Walker et al., 1995a; Grant Bennett et al., 2009). Moreover, a higher concentration of LPS in the cattle rumen and hindgut led to ruminal and hindgut acidosis in cattle and made rumen epithelium at risk of injury (Gozho et al., 2005). It was also reported that higher LPS intake could induce the metabolic endotoxemia and results in inflammation and obesity in human (Chakraborti, 2015) and mice (Masaki et al., 2004). These findings suggest that UNR cattle may have more inflammation and fat metabolism in response to LPS produced by the pathogens, therefore harder to recover under the same treatment as the RE cattle. However, the role of blood miRNAs behind the response to systemic LPS changes in relation to lameness warrants future research.

Other miRNAs changing trends, including U-D and U-N were also detected in the UNR cattle, the U-D and U-N expression patterns of bta-let-7 families were identified in both unrecovered TTN and DD groups. This miRNA has been reported to have functions suppressing the immune response by negatively regulating the level of anti-inflammatory cytokines in cattle blood (Lawless

et al., 2013b). The bta-let-7 family has similar muscle-enriched expression patterns with the bta-miR-1/206 family, which functions in the regulation of skeletal muscle development (Sun et al., 2015c). In a human study, the upregulation of the bta-let-7 family was reported as a marker of cell cycle function damage, which may reduce the renewal and regeneration of muscle cells in older individuals (Drummond et al., 2011). Thus, the up-regulation of bta-let-7 family in the first week after treatment in UNR cattle may be the result of suppressed immune function and reduced muscle cell repair. However, lame cattle were not assessed for their muscle damage in this study. It is unclear whether the local infection/disease happened on the feet could affect the whole-body muscle cell development, as well as whether the leg muscle could be affected by feet disorders. Thus, it is meaningful to investigate the potential functions of bta-let-7 to identify the potential regulating roles on muscle development and the effect of lameness on other parts of the body. Only one RE-specific changing pattern was identified among all groups: the D-U trend in DDRE group, with four miRNAs including bta-miR-12044, bta-miR-142-3p, bta-miR-181b, and bta-miR-374b were specifically expressed in this trend. However, no significant related functions were enriched for all these miRNAs, therefore their roles in DD recovery cannot be speculated.

The identification of temporal expression patterns of these miRNAs in unrecovered cattle indicates their potential for disease recovery suppression by inducing inflammatory responses and reducing immune function and tissue repair therefore leading to unresolved lameness.

3.5 Conclusions

This is the first study to analyze the miRNAs expression changing trends in the blood of lame

cattle during the treatment period. We identified that the overall expression of blood miRNAs was not significantly different between the onset of the diagnosis of lameness as well as two weeks after treatment. The functions of the differentially expressed miRNAs between recovered and unrecovered cattle were mainly related to the immune response of inflammation and muscle cell progression development. In addition, the lameness phenotype-specific miRNAs identified in Chapter 2 were also identified in this study. However, some of them were identified in unrecovered cattle with different lameness phenotypes, which provided the conjecture of lameness misdiagnosis. Besides, the predicted functions of specific miRNA changing trends identified in unrecovered cattle showed a regulatory in the inhibition of immune function and body repair, suggesting the possible molecular regulatory mechanisms on the delay in the recovery from lameness after treatment. In summary, the comparisons between lame cattle miRNAs profiles reveal the role of specific miRNAs in lameness recovery under different recovery modes, which provides the basis for identifying lameness recovery conditions. In addition, the detection of different miRNA patterns provides ideas for evaluating the effectiveness specific lameness mitigation strategies in beef cattle. However, it still needs to determine to what extent the specific miRNAs can reflect the lameness recovery efficiency and their practicality in treating lameness.

3.6 References

Bennett, G., J. Hickford, R. Sedcole, and H. Zhou. 2009. Dichelobacter nodosus, Fusobacterium necrophorum and the epidemiology of footrot. Anaerobe. 15:173–176. doi:10.1016/J.ANAEROBE.2009.02.002.

Chen, L., R. Tian, H. Zhang, and X. Liu. 2020. Anti-mastitis SNV identification of NFκB1 in Chinese Holstein cows and the possible anti-inflammation role of NFκB1/p105 in bovine MECs. Acta Biochimica et Biophysica Sinica. 52:1191–1201. doi:10.1093/ABBS/GMAA118. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/abbs/article/52/11/1191/5983617

Cheng, L., R. A. Sharples, B. J. Scicluna, and A. F. Hill. 2014. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. Journal of Extracellular Vesicles. 3. doi:10.3402/JEV.V3.23743/SUPPL_FILE/ZJEV_A_11815528_SM0004.XLSX. Available from: https://www-tandfonline-com.login.ezproxy.library.ualberta.ca/doi/abs/10.3402/jev.v3.23743

Choi, S. W., S. Kim, H. T. Park, H. E. Park, J. S. Choi, and H. S. Yoo. 2021. MicroRNA profiling in bovine serum according to the stage of Mycobacterium avium subsp. paratuberculosi infection. PLOS ONE. 16:e0259539. doi:10.1371/JOURNAL.PONE.0259539. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0259539

Currin, J. F., W. D. Whittier, and N. Currin. 2005. Foot Rot in Beef Cattle. Available from: https://vtechworks.lib.vt.edu/handle/10919/50702

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019a. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Translational Animal Science. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/3/2/595/5377510

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019b. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Translational animal science. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://pubmed.ncbi.nlm.nih.gov/32704830/

Drummond, M. J., J. J. McCarthy, M. Sinha, H. M. Spratt, E. Volpi, K. A. Esser, and B. B. Rasmussen. 2011. Aging and microRNA expression in human skeletal muscle: A microarray and bioinformatics analysis. Physiological Genomics. 43:595–603. doi:10.1152/PHYSIOLGENOMICS.00148.2010/ASSET/IMAGES/LARGE/ZH701610354600 6.JPEG. Available from: https://journals-physiology-org.login.ezproxy.library.ualberta.ca/doi/abs/10.1152/physiolgenomics.00148.2010

Duttagupta, R., R. Jiang, J. Gollub, R. C. Getts, and K. W. Jones. 2011. Impact of Cellular miRNAs on Circulating miRNA Biomarker Signatures. PLOS ONE. 6:e20769. doi:10.1371/JOURNAL.PONE.0020769. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0020769

Ekesi, N. S., B. Dolka, A. A. K. Alrubaye, and D. D. Rhoads. 2021. Analysis of genomes of bacterial isolates from lameness outbreaks in broilers. Poultry Science. 100:101148. doi:10.1016/J.PSJ.2021.101148.

Guo, H., R. Khan, S. H. A. Raza, S. M. Suhail, H. Khan, S. B. Khan, A. H. A. El-Aziz, and L. Zan. 2021. RNA-Seq Reveals Function of Bta-miR-149-5p in the Regulation of Bovine Adipocyte Differentiation. Animals 2021, Vol. 11, Page 1207. 11:1207. doi:10.3390/ANI11051207. Available from: https://www.mdpi.com/2076-2615/11/5/1207/htm

Guo, Y., X. Zhang, W. Huang, and X. Miao. 2017. Identification and characterization of differentially expressed miRNAs in subcutaneous adipose between Wagyu and Holstein cattle. Scientific Reports 2017 7:1. 7:1–10. doi:10.1038/SREP44026. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/srep44026

Hao, D., Xiaogang Wang, Xiao Wang, B. Thomsen, Y. Yang, X. Lan, Y. Huang, and H. Chen. 2021. MicroRNA bta-miR-365-3p inhibits proliferation but promotes differentiation of primary bovine myoblasts by targeting the activin A receptor type I. Journal of Animal Science and Biotechnology. 12:1–15. doi:10.1186/S40104-020-00528-0/TABLES/2. Available from: https://jasbsci-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s40104-020-00528-0

Huxley, J. N. 2013. Impact of lameness and claw lesions in cows on health and production. Livestock Science. 156:64–70. doi:10.1016/J.LIVSCI.2013.06.012.

Ioannidis, J., and F. X. Donadeu. 2016. Circulating miRNA signatures of early pregnancy in cattle. BMC Genomics. 17:1–12. doi:10.1186/S12864-016-2529-1/FIGURES/6. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-016-2529-1

Ioannidis, J., and F. X. Donadeu. 2018. Comprehensive analysis of blood cells and plasma identifies tissue-specific miRNAs as potential novel circulating biomarkers in cattle. BMC Genomics. 19:1–11. doi:10.1186/S12864-018-4646-5/FIGURES/5. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-018-4646-5

Khan, R., S. H. A. Raza, Z. Junjvlieke, X. Wang, H. Wang, G. Cheng, C. Mei, I. Elsaeid Elnour, and L. Zan. 2020. Bta-miR-149-5p inhibits proliferation and differentiation of bovine adipocytes through targeting CRTCs at both transcriptional and posttranscriptional levels. Journal of Cellular Physiology. 235:5796–5810. doi:10.1002/JCP.29513. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/jcp.29513

Kozomara, A., M. Birgaoanu, and S. Griffiths-Jones. 2019. miRBase: from microRNA sequences to function. Nucleic Acids Research. 47:D155–D162. doi:10.1093/NAR/GKY1141. Available from: https://academic.oup.com/nar/article/47/D1/D155/5179337

Kumar, R., B. R. Yadav, and R. S. Singh. Antibiotic resistance and pathogenicity factors in Staphylococcus aureus isolated from mastitic Sahiwal cattle. J. Biosci. 36:175–188. doi:10.1007/s12038-011-9004-6. Available from: http://www.ias.ac.in/jbiosci

Lameness - Beef Cattle Research Council. Available from: https://www.beefresearch.ca/research-topic.cfm/lameness-64

Lau, P., K. Bossers, R. Janky, E. Salta, C. S. Frigerio, S. Barbash, R. Rothman, A. S. R. Sierksma, A. Thathiah, D. Greenberg, A. S. Papadopoulou, T. Achsel, T. Ayoubi, H. Soreq, J. Verhaagen, D. F. Swaab, S. Aerts, and B. de Strooper. 2013. Alteration of the microRNA network during the progression of Alzheimer's disease. EMBO Molecular Medicine. 5:1613–1634. doi:10.1002/EMMM.201201974. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/emmm.201201974

Laven, R. A., and D. N. Logue. 2006. Treatment strategies for digital dermatitis for the UK. The Veterinary Journal. 171:79–88. doi:10.1016/J.TVJL.2004.08.009.

Lawless, N., A. B. K. Foroushani, M. S. McCabe, C. O'Farrelly, and D. J. Lynn. 2013a. Next Generation Sequencing Reveals the Expression of a Unique miRNA Profile in Response to a Gram-Positive Bacterial Infection. PLOS ONE. 8:e57543. doi:10.1371/JOURNAL.PONE.0057543. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0057543

Lawless, N., A. B. K. Foroushani, M. S. McCabe, C. O'Farrelly, and D. J. Lynn. 2013b. Next Generation Sequencing Reveals the Expression of a Unique miRNA Profile in Response to a Gram-Positive Bacterial Infection. PLOS ONE. 8:e57543. doi:10.1371/JOURNAL.PONE.0057543. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0057543

Lee, J., Soohyun Lee, J. Son, H. Lim, E. Kim, D. Kim, S. Ha, T. Hur, Seunghwan Lee, and I. Choi. 2020. Analysis of circulating-microRNA expression in lactating Holstein cows under summer heat

stress. PLOS ONE. 15:e0231125. doi:10.1371/JOURNAL.PONE.0231125. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0231125

Li, J., L. Mao, W. Li, F. Hao, C. Zhong, X. Zhu, X. Ji, L. Yang, W. Zhang, M. Liu, and J. Jiang. 2018. Analysis of microRNAs expression profiles in madin-darby bovine kidney cells infected with caprine parainfluenza virus type 3. Frontiers in Cellular and Infection Microbiology. 8:93. doi:10.3389/FCIMB.2018.00093/BIBTEX.

Li, R., C. L. Zhang, X. X. Liao, D. Chen, W. Q. Wang, Y. H. Zhu, X. H. Geng, D. J. Ji, Y. J. Mao, Y. C. Gong, and Z. P. Yang. 2015a. Transcriptome MicroRNA Profiling of Bovine Mammary Glands Infected with Staphylococcus aureus. International Journal of Molecular Sciences 2015, Vol. 16, Pages 4997-5013. 16:4997–5013. doi:10.3390/IJMS16034997. Available from: https://www.mdpi.com/1422-0067/16/3/4997/htm

Li, R., C. L. Zhang, X. X. Liao, D. Chen, W. Q. Wang, Y. H. Zhu, X. H. Geng, D. J. Ji, Y. J. Mao, Y. C. Gong, and Z. P. Yang. 2015b. Transcriptome MicroRNA Profiling of Bovine Mammary Glands Infected with Staphylococcus aureus. International Journal of Molecular Sciences 2015, Vol. 16, Pages 4997-5013. 16:4997–5013. doi:10.3390/IJMS16034997. Available from: https://www.mdpi.com/1422-0067/16/3/4997/htm

Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology. 15:1–21. doi:10.1186/S13059-014-0550-8/FIGURES/9. Available from: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-014-0550-8

Luoreng, Z. M., X. P. Wang, C. G. Mei, and L. sen Zan. 2018. Comparison of microRNA Profiles between Bovine Mammary Glands Infected with Staphylococcus aureus and Escherichia coli. International Journal of Biological Sciences. 14:87. doi:10.7150/IJBS.22498. Available from: /pmc/articles/PMC5821052/

Marti, S., M. D. Jelinski, E. D. Janzen, M. J. Jelinski, C. L. Dorin, K. Orsel, E. A. Pajor, J. Shearer, S. T. Millman, and K. S. Schwartzkopf-Genswein. 2021. A prospective longitudinal study of risk factors associated with cattle lameness in southern alberta feedlots. Canadian Journal of Animal Science. 101:647–654. doi:10.1139/CJAS-2020-0128/ASSET/IMAGES/CJAS-2020-0128TAB3.GIF. Available from: https://cdnsciencepub-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1139/cjas-2020-0128

Menjivar, N. G., S. Gebremedhn, D. Tesfaye, N. G. Menjivar, S. Gebremedhn, and D. Tesfaye. 2021. 108 Preferential loading of thermal stress-associated microRNAs into extracellular vesicles: attempt to mitigate effects of heat stress in bovine granulosa cells. Reproduction, Fertility and Development. 34:291–291. doi:10.1071/RDV34N2AB108. Available from: https://www-publish-csiro-au.login.ezproxy.library.ualberta.ca/rd/RDv34n2Ab108

Mitchelson, K. R., and W.-Y. Qin. 2015. Roles of the canonical myomiRs miR-1, -133 and -206 in cell development and disease. World Journal of Biological Chemistry. 6:162. doi:10.4331/WJBC.V6.I3.162. Available from: /pmc/articles/PMC4549760/

de Oliveira, P. S. N., L. L. Coutinho, P. C. Tizioto, A. S. M. Cesar, G. B. de Oliveira, W. J. da S. Diniz, A. O. de Lima, J. M. Reecy, G. B. Mourão, A. Zerlotini, and L. C. A. Regitano. 2018. An integrative transcriptome analysis indicates regulatory mRNA-miRNA networks for residual feed intake in Nelore cattle. Scientific Reports 2018 8:1. 8:1–12. doi:10.1038/S41598-018-35315-5. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-35315-5

Pedersen, I. M., G. Cheng, S. Wieland, S. Volinia, C. M. Croce, F. v. Chisari, and M. David. 2007. Interferon modulation of cellular microRNAs as an antiviral mechanism. Nature 2007 449:7164. 449:919–922. doi:10.1038/nature06205. Available from: https://www.nature.com/articles/nature06205

Penny, C., S. Bradley, and D. Wilson. 2017. Lameness due to toe-tip necrosis syndrome in beef calves. Veterinary Record. 180:154–154. doi:10.1136/VR.J678. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1136/vr.j678

Qiao, Y., H. Kong, C. Clark, S. Lomax, D. Su, S. Eiffert, and S. Sukkarieh. 2021. Intelligent Perception-Based Cattle Lameness Detection and Behaviour Recognition: A Review. Animals 2021, Vol. 11, Page 3033. 11:3033. doi:10.3390/ANI11113033. Available from: https://www.mdpi.com/2076-2615/11/11/3033/htm

Salehi, S., O. D. Tavabie, S. Verma, M. J. W. McPhail, F. Farzaneh, W. Bernal, K. Menon, K. Agarwal, and V. R. Aluvihare. 2020. Serum MicroRNA Signatures in Recovery From Acute and Chronic Liver Injury and Selection for Liver Transplantation. Liver Transplantation. 26:811–822. doi:10.1002/LT.25781. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/lt.25781

Seong, J., • Hyojeong Yoon, and H. S. Kong. 2016. Identification of microRNA and target gene associated with marbling score in Korean cattle (Hanwoo). Genes & Genomics. 38. doi:10.1007/s13258-016-0401-y. Available from: www.targetscan.org

Shearer, J. K., and S. R. van Amstel. 2017. Traumatic Lesions of the Sole. Veterinary Clinics: Food Animal Practice. 33:271–281. doi:10.1016/J.CVFA.2017.02.001. Available from: http://www.vetfood.theclinics.com/article/S0749072017300099/fulltext

Smart, M. E. 1985. Nutritional Factors of Lameness and Metabolic Bone Disease in Cattle. Veterinary Clinics of North America: Food Animal Practice. 1:13–24. doi:10.1016/S0749-0720(15)31347-5.

Stokka, G. L., K. Lechtenberg, T. Edwards, S. MacGregor, K. Voss, D. Griffin, D. M. Grotelueschen, R. A. Smith, and L. J. Perino. 2001. Lameness in feedlot cattle. The Veterinary clinics of North America. Food animal practice. 17. doi:10.1016/S0749-0720(15)30062-1. Available from: https://pubmed.ncbi.nlm.nih.gov/11320695/

Sun, Jiajie, K. Aswath, S. G. Schroeder, J. D. Lippolis, T. A. Reinhardt, and T. S. Sonstegard. 2015. MicroRNA expression profiles of bovine milk exosomes in response to Staphylococcus aureus infection. BMC Genomics. 16:1–10. doi:10.1186/S12864-015-2044-9/FIGURES/3. Available from: https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-015-2044-9

Sun, J., T. S. Sonstegard, C. Li, Y. Huang, Z. Li, X. Lan, C. Zhang, C. Lei, X. Zhao, and H. Chen. 2015a. Altered microRNA expression in bovine skeletal muscle with age. Animal Genetics. 46:227–238. doi:10.1111/AGE.12272. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/age.12272

Sun, J., T. S. Sonstegard, C. Li, Y. Huang, Z. Li, X. Lan, C. Zhang, C. Lei, X. Zhao, and H. Chen. 2015b. Altered microRNA expression in bovine skeletal muscle with age. Animal Genetics. 46:227–238. doi:10.1111/AGE.12272. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/age.12272

Thomas, H. J., J. G. Remnant, N. J. Bollard, A. Burrows, H. R. Whay, N. J. Bell, C. Mason, and J. N. Huxley. 2016. Paper: Recovery of chronically lame dairy cows following treatment for claw horn lesions: a randomised controlled trial. The Veterinary Record. 178:116. doi:10.1136/VR.103394. Available from: /pmc/articles/PMC4752658/

Tunstall, J., K. Mueller, D. Grove-White, J. W. H. Oultram, and H. M. Higgins. 2021. Lameness in Beef Cattle: A Cross-Sectional Descriptive Survey of On-Farm Practices and Approaches. Frontiers in Veterinary Science. 8:490. doi:10.3389/FVETS.2021.657299/BIBTEX.

Vegh, P., D. A. Magee, N. C. Nalpas, K. Bryan, M. S. McCabe, J. A. Browne, K. M. Conlon, S. v. Gordon, D. G. Bradley, D. E. Machugh, and D. J. Lynn. 2015. MicroRNA profiling of the bovine alveolar macrophage response to Mycobacterium bovis infection suggests pathogen survival is enhanced by microRNA regulation of endocytosis and lysosome trafficking. Tuberculosis. 95:60–67. doi:10.1016/J.TUBE.2014.10.011.

Walker, R. L., D. H. Read, K. J. Loretz, and R. W. Nordhausen. 1995. Spirochetes isolated from dairy cattle with papillomatous digital dermatitis and interdigital dermatitis. Veterinary Microbiology. 47:343–355. doi:10.1016/0378-1135(95)00114-X.

Wang, O., M. Zhou, Y. Chen, T. A. McAllister, G. Plastow, K. Stanford, B. Selinger, and L. L. Guan. 2021a. MicroRNAomes of Cattle Intestinal Tissues Revealed Possible miRNA Regulated Mechanisms Involved in Escherichia coli O157 Fecal Shedding. Frontiers in Cellular and Infection Microbiology. 11. doi:10.3389/FCIMB.2021.634505/FULL. Available from: /pmc/articles/PMC7959717/

Wang, O., M. Zhou, Y. Chen, T. A. McAllister, G. Plastow, K. Stanford, B. Selinger, and L. L. Guan. 2021b. MicroRNAomes of Cattle Intestinal Tissues Revealed Possible miRNA Regulated Mechanisms Involved in Escherichia coli O157 Fecal Shedding. Frontiers in Cellular and Infection Microbiology. 11. doi:10.3389/FCIMB.2021.634505/FULL. Available from: /pmc/articles/PMC7959717/

Wang, S., Z. Li, Q. Chen, L. Wang, J. Zheng, Z. Lin, and W. Li. 2018. NF-κB-Induced MicroRNA-211 Inhibits Interleukin-10 in Macrophages of Rats with Lipopolysaccharide-Induced Acute Respiratory Distress Syndrome. Cellular Physiology and Biochemistry. 45:332–342. doi:10.1159/000486814. Available from: https://www-karger-com.login.ezproxy.library.ualberta.ca/Article/FullText/486814

Wang, Z., L. Cong Kong, B. Y. Jia, J. R. Chen, Y. Dong, X. Y. Jiang, and H. X. Ma. 2019. Analysis of the microRNA Expression Profile of Bovine Monocyte-derived Macrophages Infected with Mycobacterium avium subsp. Paratuberculosis Reveals that miR-150 Suppresses Cell Apoptosis by Targeting PDCD4. International Journal of Molecular Sciences 2019, Vol. 20, Page 2708. 20:2708. doi:10.3390/IJMS20112708. Available from: https://www.mdpi.com/1422-0067/20/11/2708/htm

Yerukala Sathipati, S., and S. Y. Ho. 2018. Identifying a miRNA signature for predicting the stage of breast cancer. Scientific Reports 2018 8:1. 8:1–11. doi:10.1038/S41598-018-34604-3.

Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-34604-3

Zhao, K., G. Liang, X. Sun, and L. L. Guan. 2016. Comparative miRNAome analysis revealed different miRNA expression profiles in bovine sera and exosomes. BMC Genomics. 17:1–12. doi:10.1186/S12864-016-2962-1/TABLES/6. Available from: https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-016-2962-1

3.7 Figures and tables

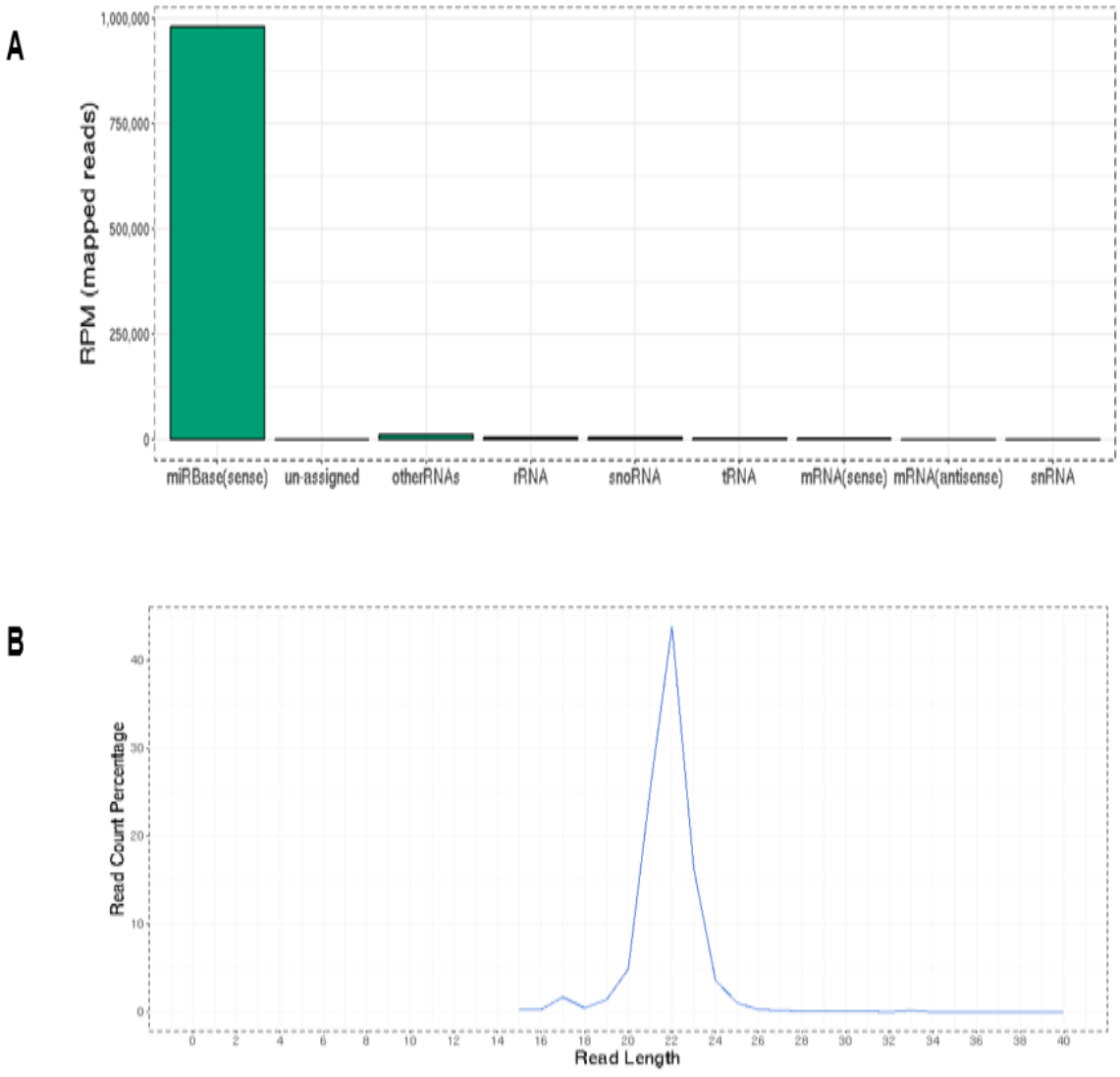

Figure 3.1 Profiling of small RNAs in blood samples collected from beef steer generated using RNA-seq (A) Relative abundance of different classes of small RNAs, y axis represents reads per million and x axis represents type of small RNA. (B) Size and frequency distribution of detected small RNAs (15-40 nt), y axis represents reads percentage and x axis represents length of identified reads

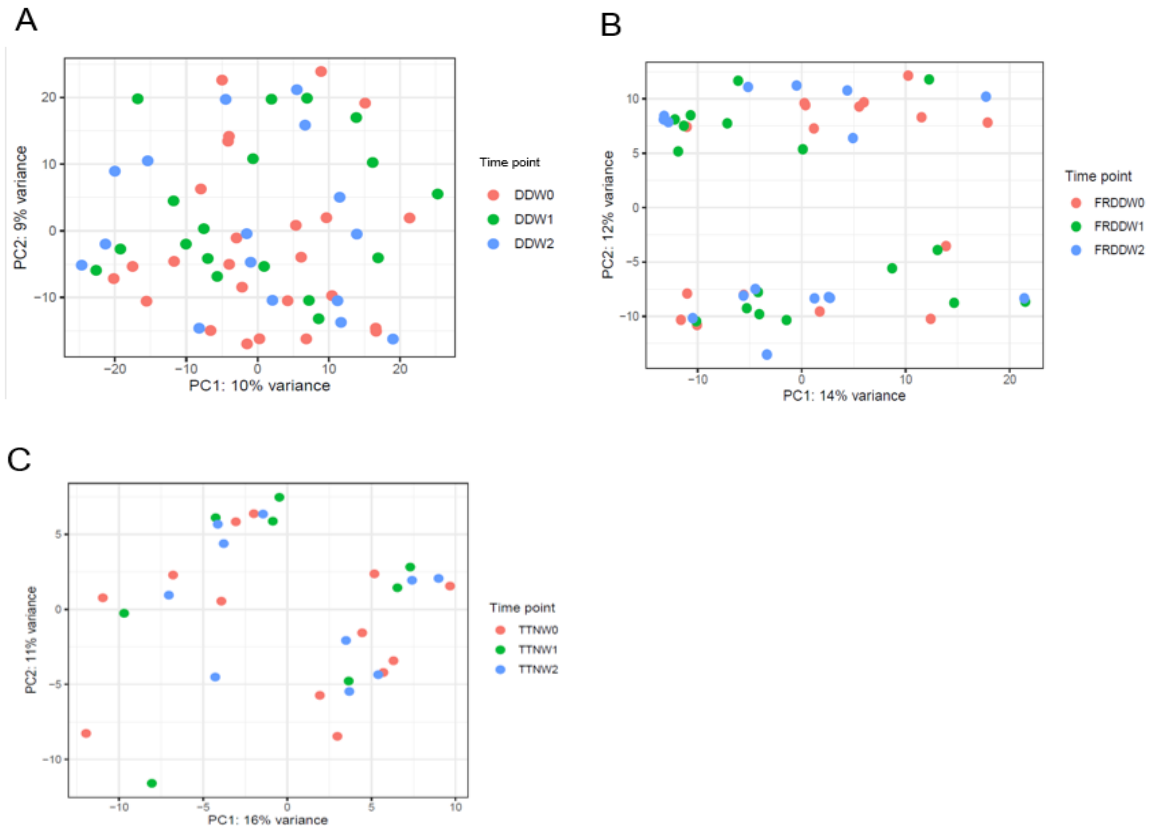

Figure 3.2 Principal component analysis of the overall miRNA profiles in different lameness phenotypes and under two weeks after treatment. (A) PCA plot based on DD group; DDW0 (red color) refers to DD samples collected at **Week 0**; DDW1 (green color) refers to DD samples collected at **Week 1**; DDW2 refers to DD samples collected at **Week 2** (B) PCA plot based on TTN group; TTNW0 (red color) refers to TTN samples collected at **Week 0**; TTNW1 (green color) refers to TTN samples collected at **Week 1**; TTNW2 refers to TTN samples collected at **Week 2** (C) PCA plot based on FRDD group; FRDDW0 (red color) refers to FRDD samples collected at **Week 0**; FRDDW1 (green color) refers to FRDD samples collected at **Week 1**; FRDDW2 refers to FRDD samples collected at **Week 2**

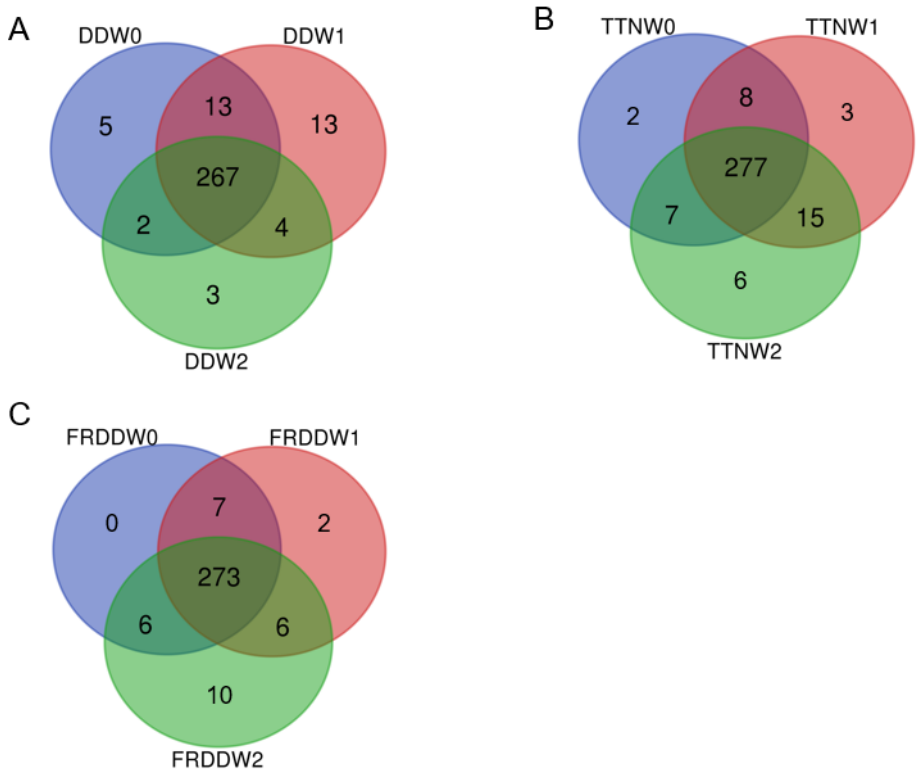

Figure 3.3 Overview of the identified miRNAs during the treatment period. (A). Numbers of expressed miRNA identified in the DD group within each time point (B). Numbers of expressed miRNA identified in the TTN group within each time point (C). Numbers of expressed miRNA identified in the FRDD group within each time point

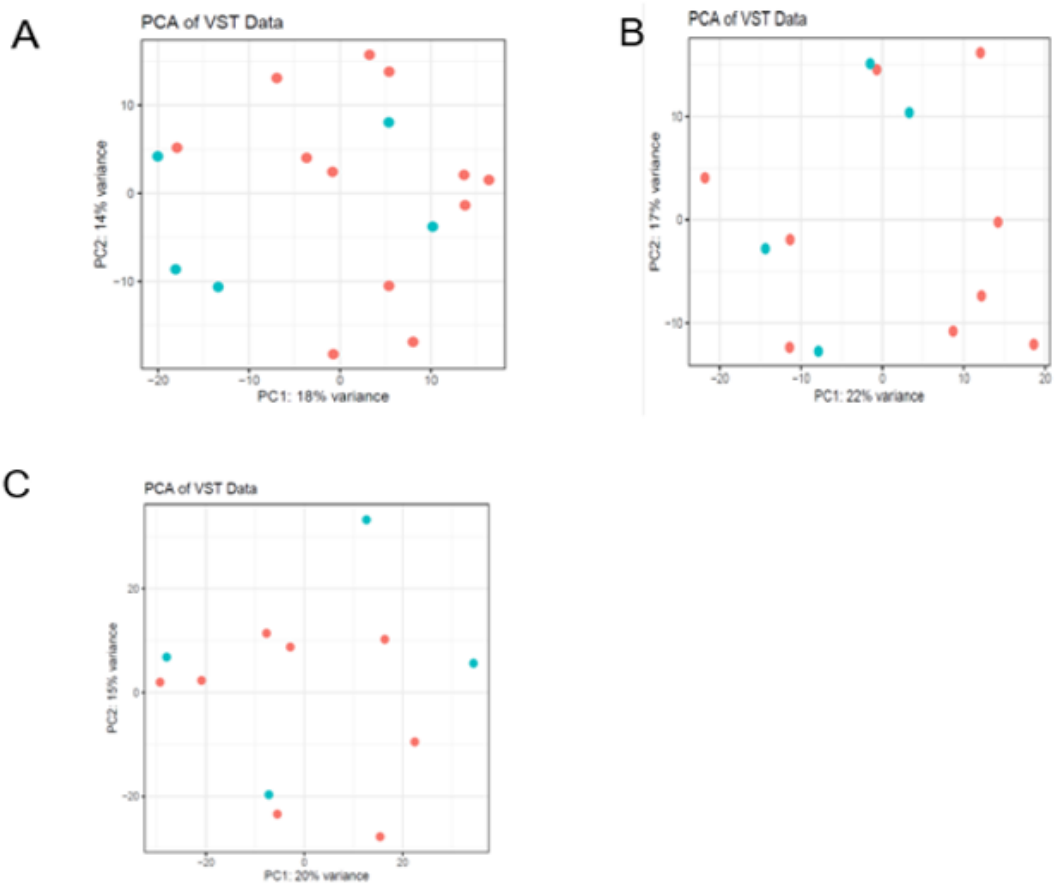

Figure 3.4 Principal component analysis of the total detected miRNAs in DD group with different time points and recovery patterns (A) comparison between recovered and unrecovered samples in **Week 0**. (B) comparison between recovered and unrecovered samples in **Week 1**. (C) comparison between recovered and unrecovered samples in **Week 2**. The X and Y-axis represent the first two principal components. The percentage value in the bracket represents the percentage of variance explained by that principal component. The blue color of dots represents recovered samples and red color represents unrecovered samples

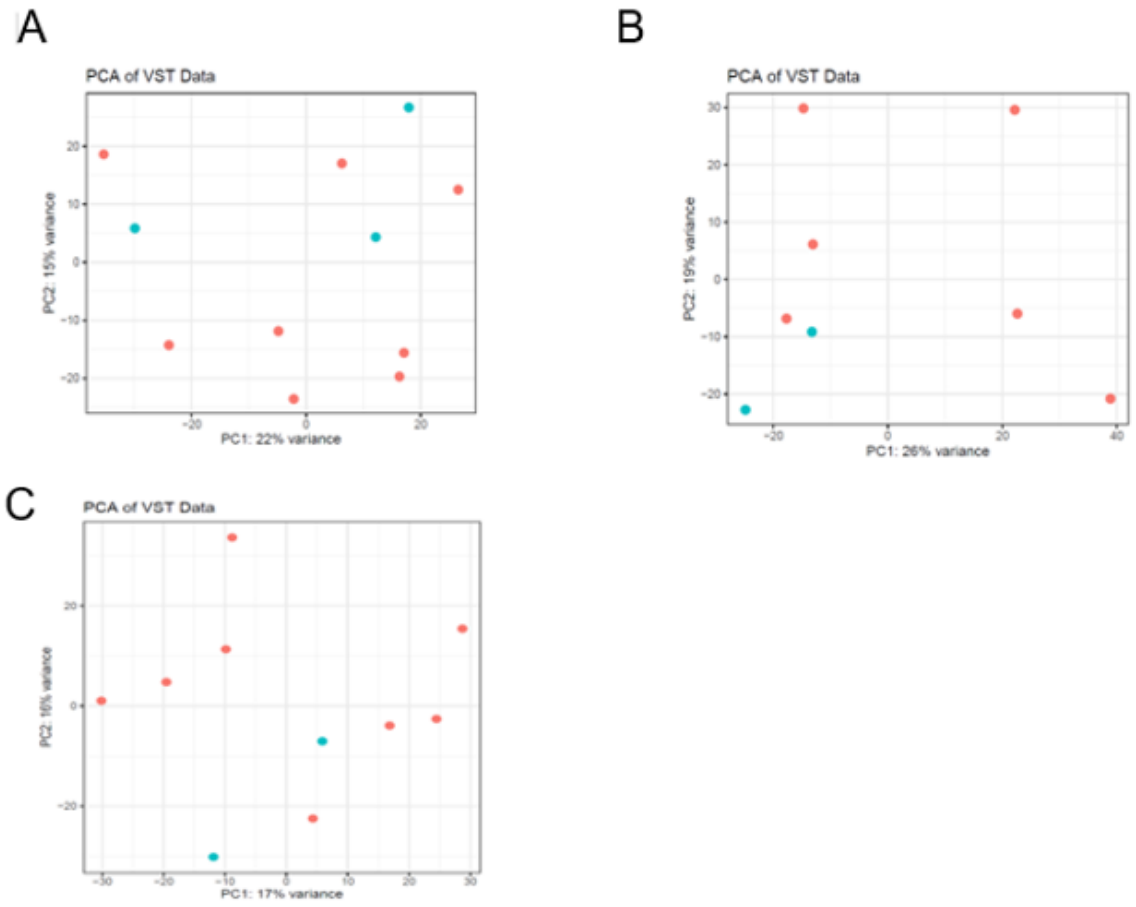

Figure 3.5 Principal component analysis of the total detected miRNAs in TTN group with different time points and recovery patterns (A) comparison between recovered and unrecovered samples in **Week 0**. (B) comparison between recovered and unrecovered samples in **Week 1**. (C) comparison between recovered and unrecovered samples in **Week 2**. The X and Y-axis represent the first two principal components. The percentage value in the bracket represents the percentage of variance explained by that principal component. The blue color of dots represents recovered samples and red color represents unrecovered samples

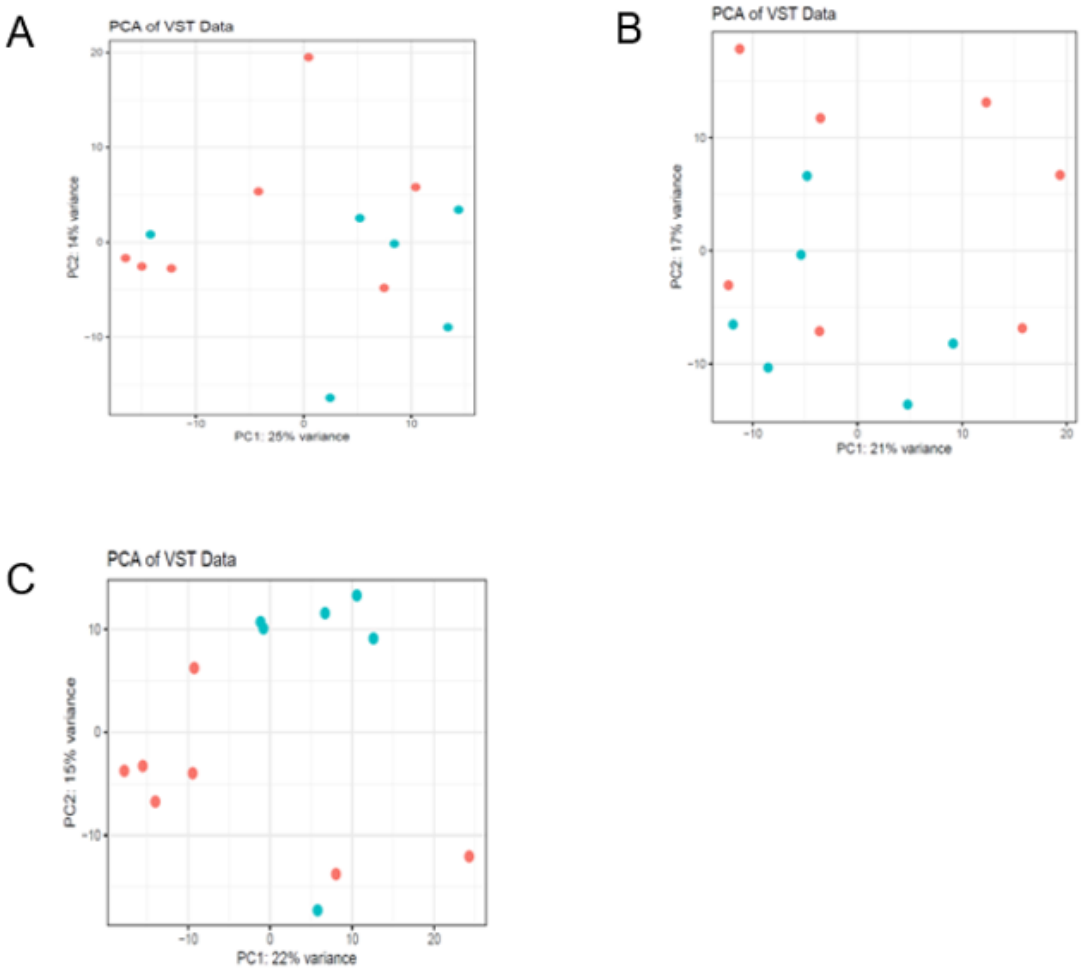

Figure 3.6 Principal component analysis of the total detected miRNAs in FRDD group with different time points and recovery patterns (A) comparison between recovered and unrecovered samples in **Week 0**. (B) comparison between recovered and unrecovered samples in **Week 1**. (C) comparison between recovered and unrecovered samples in **Week 2**. The X and Y-axis represent the first two principal components. The percentage value in the bracket represents the percentage of variance explained by that principal component. The blue color of dots represents recovered samples and red color represents unrecovered samples

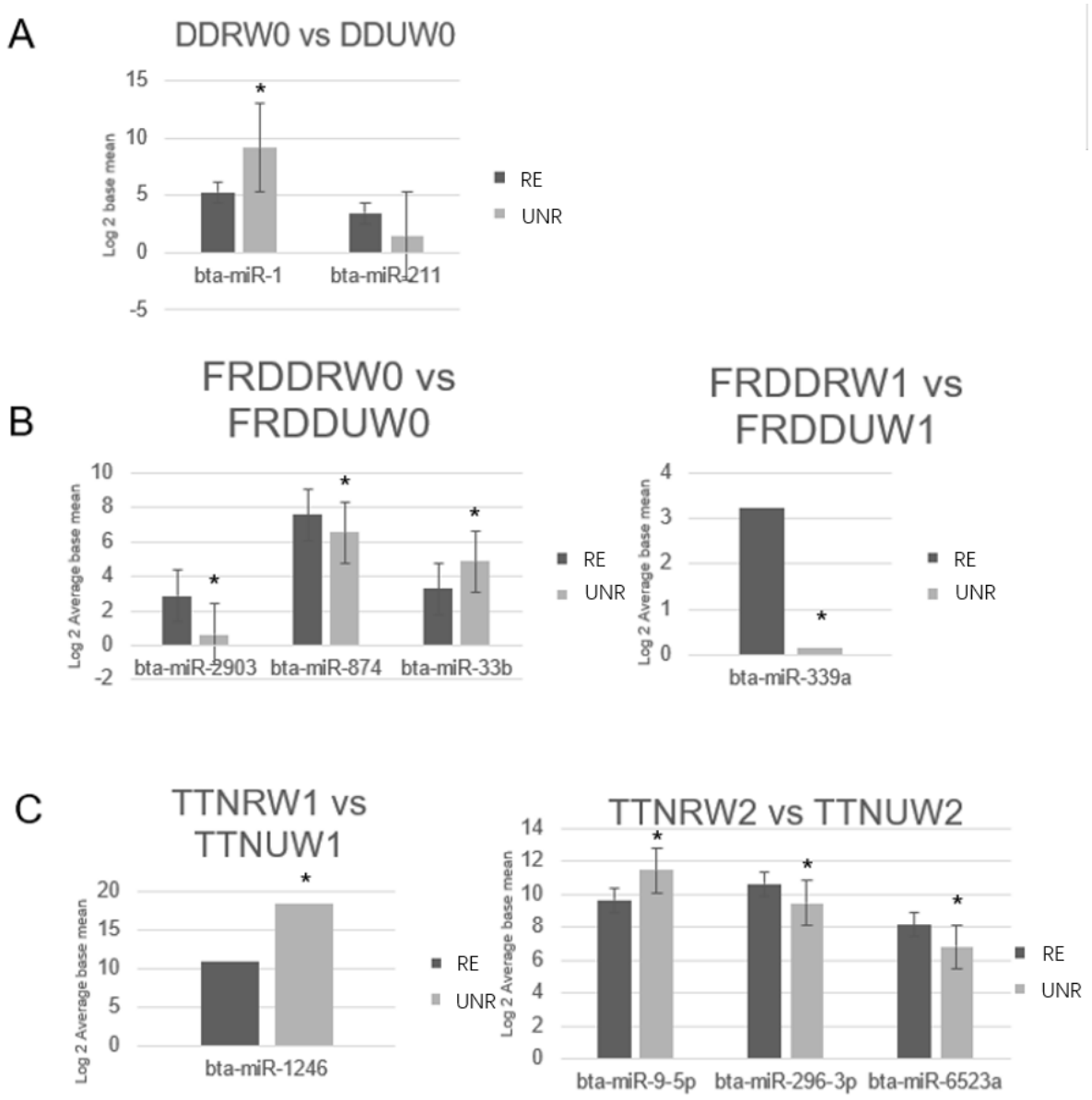

Figure 3.7 Expression of selected DE miRNAs between recovered and unrecovered cattle using miRNA-seq. The colors represent the recover pattern including recovered (RE) and unrecovered (UNR), and the y axis represents the average expression level normalized by $\log_2$ average base mean (A) Digital dermatitis group (DD); (B) Foot rot & Digital dermatitis combined group (FRDD); (C) Tor tip necrosis group (TTN)

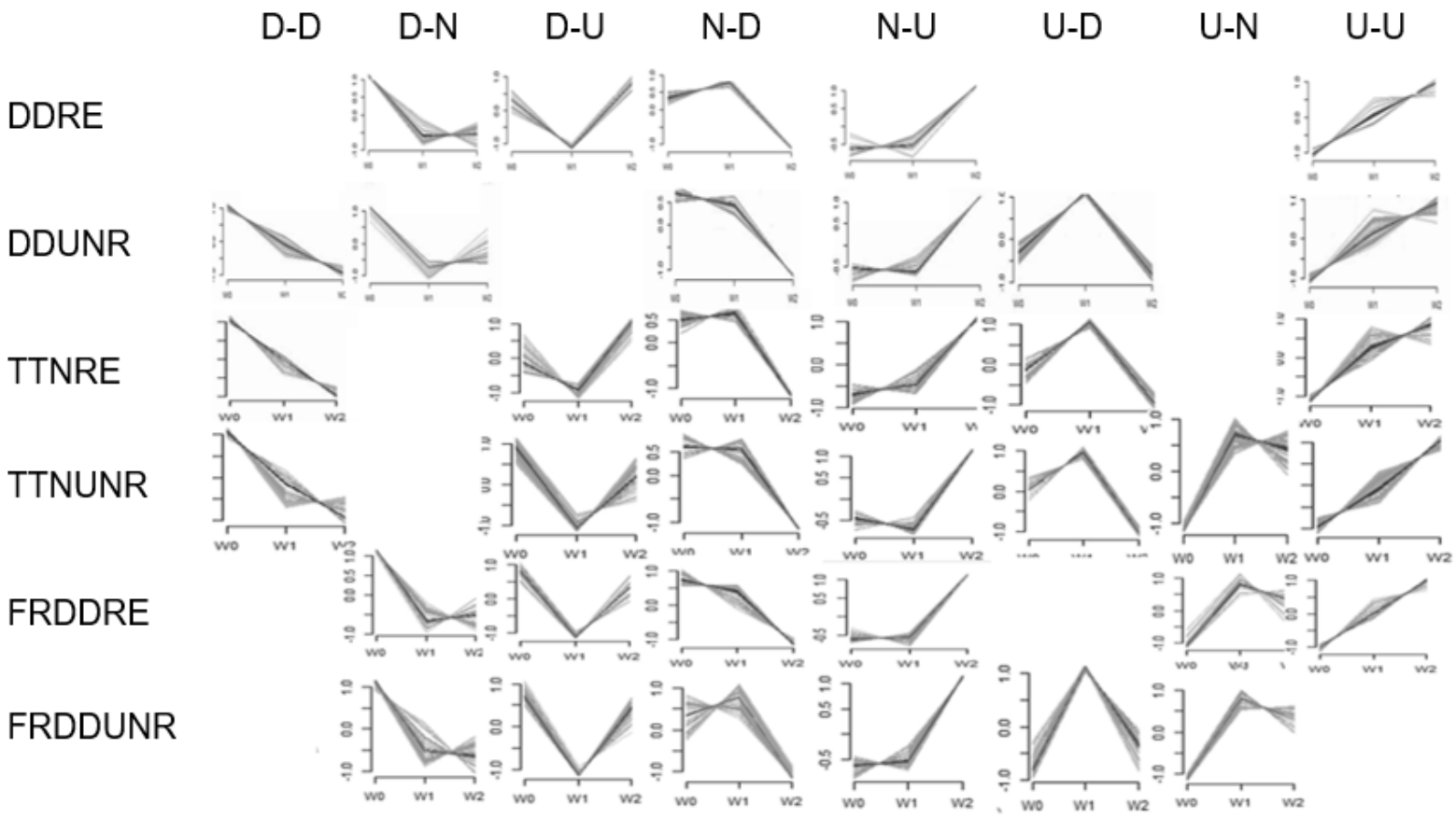

Figure 3.8 Temporally blood miRNA expression patterns for recovered and unrecovered cattle in three lameness phenotypes The letters in the X axis represent the miRNA regulating trends including downregulation (D), upregulation (U) and non-significant regulation (N). The Y axis represents different datasets including recovered DD group (DDRE), unrecovered DD group (DDUNR), recovered TTN group (TTNRE), unrecovered TTN group (TTNUNR, recovered FRDD group (FRDDRE) and unrecovered FRDD group (FRDDUNR)

Table 3.1 Number of samples under each classification

	DD			FRDD			TTN		
Week	**W0**	**W1**	**W2**	**W0**	**W1**	**W2**	**W0**	**W1**	**W2**
RE	**11**	**9**	**8**	**7**	**7**	**7**	**8**	**6**	**8**
UNR	**5**	**4**	**4**	**6**	**6**	**6**	**3**	**2**	**2**

The blood samples were collected from digital dermatitis (DD); foot rot & digital dermatitis combined (FRDD) and toe tip necrosis (TTN) cattle from **Week 0** (**W0**) to **Week 2** (**W2**). The blood samples were then defined as recovered group (RE) and unrecovered group (UNR) based on monitored gait score of the cattle

Table 3.2 Expression level of time point specific miRNA in three lameness phenotypes

	DDW0	DDW1	DDW2	TTNW0	TTNW1	TTNW2	FRDDW0	FRDDW1	FRDDW2
bta-miR-2887	3.95 ± 3.88	0	0	0	0	0	0	0	0
bta-miR-2320-3p	1.40 ± 0.72	0	0	0	0	0	0	0	0
bta-miR-2285bz	1.52 ± 0.81	0	0	0	0	0	0	0	0
bta-miR-339a	0	6.00 ± 3.03	0	0	0	0	0	0	0
bta-miR-545-3p	0	1.64 ± 1.05	0	0	0	0	0	0	0
bta-miR-2285aj-5p	0	1.37 ± 0.70	0	0	0	0	0	0	0
bta-miR-2285j	0	1.60 ± 0.93	0	0	0	1.33 ± 0.68	0	0	1.72 ± 1.45
bta-miR-2397-3p	0	1.21 ± 0.57	0	0	0	0	0	1.34 ± 0.45	0
bta-miR-33b	0	2.01 ± 1.14	0	0	0	0	0	0	0
bta-miR-149-5p	0	1.46 ± 0.85	0	0	0	0	0	0	1.21 ± 0.54
bta-miR-10179-5p	0	0	1.96 ± 1.32	0	0	0	0	0	0
bta-miR-7861	0	0	0	1.20 ± 0.46	0	0	0	0	0
bta-miR-582	0	0	0	1.58 ± 0.63	0	0	0	0	0
bta-miR-494	0	0	0	0	2.22± 1.12	0	0	0	0
bta-miR-10b	0	0	0	0	1.40 ± 0.79	0	0	0	0
bta-miR-199a-5p	0	0	0	0	1.15 ± 0.57	0	0	0	0
bta-miR-2440	0	0	0	0	0	1.28 ± 0.72	0	0	0
bta-miR-545-5p	0	0	0	0	0	1.46 ± 0.66	0	0	0
bta-miR-2285ak-5p	0	0	0	0	0	1.15 ± 0.57	0	0	0
bta-miR-1388-5p	0	0	0	0	0	1.75 ± 0.77	0	0	0
bta-miR-2411-3p	0	0	0	0	0	1.93 ± 1,45	0	0	0
bta-miR-2382-5p	0	0	0	0	0	0	0	1.94 ± 1.70	0
bta-miR-2448-5p	0	0	0	0	0	0	0	0	1.35 ± 0.75
bta-miR-2903	0	0	0	0	0	0	0	0	1.21 ± 0.38
bta-miR-30f	0	0	0	0	0	0	0	0	1.48 ± 0.54
bta-miR-11998	0	0	0	0	0	0	0	0	1.67 ± 1.10

bta-miR-7859	0	0	0	0	0	0	0	0	2.16 ± 1.06
bta-miR-2382-3p	0	0	0	0	0	0	0	0	1.49 ± 0.71
bta-miR-2285dj	0	0	0	0	0	0	0	0	1.62 ± 1.05
bta-miR-411a	0	0	0	0	0	0	0	0	1.40 ± 0.56

The expression level is represented by RPM, and identified from digital dermatitis (DD), toe tip necrosis (TTN), and foot rot & digital dermatitis combined (FRDD); and different time point including **Week 0** (**W0**), **Week 1** (**W1**) and **Week 2** (**W2**)

Table 3.3 Identified miRNAs in each expression changing pattern among all datasets

	D-D	D-N	D-U	N-D	N-N	N-U	U-D	U-N	U-U
DDRE		bta-miR-146b bta-miR-2313-3p	bta-miR-12044 bta-miR-142-3p bta-miR-181b bta-miR-374b	bta-miR-2408 bta-miR-361 bta-miR-362-5p bta-miR-363		bta-miR-12042 bta-miR-423-5p			bta-miR-126-5p bta-miR-20b bta-miR-26b bta-miR-338
DDUNR	bta-miR-2468 bta-miR-494	bta-miR-2285j bta-miR-2424		bta-miR-1839 bta-miR-27a-3p bta-miR-382 bta-miR-18a bta-miR-26a bta-miR-451		bta-miR-1296 bta-miR-423-3p bta-miR-6525 bta-miR-11986c bta-miR-2443	bta-let-7c bta-miR-139 bta-miR-144 bta-miR-30b-3p bta-miR-545-5p		bta-miR-122 bta-miR-449a bta-miR-7180
TTNRE	bta-miR-199a-3p bta-miR-499 bta-miR-7		bta-let-7a-5p bta-miR-2285ak-5p	bta-miR-196b bta-miR-29a bta-miR-421		bta-miR-10167-3p bta-miR-2346 bta-miR-2450a	bta-miR-185 bta-miR-29b bta-miR-340		bta-miR-1388-5p

	bta-miR-148a bta-miR-452				bta-miR-299	bta-miR-345-3p bta-miR-21-5p bta-miR-33a		
TTNUNR	bta-miR-2387 bta-miR-296-3p		bta-miR-1388-3p bta-miR-2448-3p bta-miR-324	bta-let-7a-5p	bta-miR-2397-3p bta-miR-582	bta-miR-130a bta-miR-2440 bta-miR-12027 bta-miR-7180	bta-let-7f bta-miR-2336 bta-miR-342 bta-miR-95	bta-miR-12044 bta-miR-147 bta-miR-9-5p
FRDDRE		bta-miR-11986c bta-miR-130b bta-miR-671	bta-miR-31 bta-miR-361	bta-miR-2903 bta-miR-6123	bta-miR-1388-3p bta-miR-15a bta-miR-181a bta-miR-301b bta-miR-7859 bta-miR-187 bta-miR-199b bta-miR-2285bf		bta-miR-11984 bta-let-7b bta-miR-423-3p	bta-miR-505
FRDDUNR		bta-miR-11991 bta-miR-12042	bta-miR-10174-3p bta-miR-16a	bta-miR-10a bta-miR-2431-3p	bta-miR-130b bta-miR-2284x	bta-miR-1307 bta-miR-7857-5p	bta-miR-197 bta-miR-2285p	

bta-miR-125b	bta-miR-29d-5p	bta-miR-362-5p	bta-miR-2285bf	bta-miR-99a-5p	bta-miR-6120-3p
bta-miR-145	bta-miR-205	bta-miR-532	bta-miR-30e-5p$bta-miR-30d		bta-miR-6123
bta-miR-1468	bta-miR-33a	bta-miR-671			bta-miR-744
bta-miR-181b	bta-miR-128				bta-miR-760-3p
bta-miR-2313-3p	bta-miR-148b				
bta-miR-2336					

The letters in the top represent the miRNA regulating trends including downregulation (D), upregulation (U) and non-significant regulation (N). And the letters on the left of the table represents different datasets including recovered DD group (DDRE), unrecovered DD group (DDUNR), recovered TTN group (TTNRE), unrecovered TTN group (TTNUNR, recovered FRDD group (FRDDRE) and unrecovered FRDD group (FRDDUNR)

Table 3.4 Significantly enriched function categories of DE miRNAs identified between RE and UNR cattle

DE miRNAs	Enriched functional category	FDR
bta-miR-1	Transcription regulation	1.20E-04
	Transcription	4.80E-04
	Nucleus	2.40E-03
	DNA-binding	1.40E-02
bta-miR-211	Cadherin-like	6.00E-03
	Cadherin conserved site	6.00E-03
	Cadherin	8.40E-03
	CA	3.70E-02
bta-miR-1246	Zinc-finger	2.90E-06
	Zinc	1.20E-04
	Metal-binding	8.10E-04
bta-miR-2903	Homeobox, conserved site	1.50E-06
	Homeobox	1.20E-06
	Homeodomain	2.00E-06
	Homeodomain-like	1.20E-05
	HOX	1.50E-05
bta-miR-33b	Protein kinase-like domain	9.10E-03
	Protein kinase, catalytic domain	2.10E-02
	ATP binding	1.50E-02
	Serine/threonine-protein kinase, active site	2.10E-02
	Protein kinase, ATP binding site	2.10E-02

Table 3.5 Significantly enriched functional categories of special miRNA changing trends detected among RE and UNR cattle using DAVID

Group	miRNAs	Cluster #	Predicted functions	Counts	FDR
D-D trend in DDU group	bta-miR-2468	Cluster 1	Ras signaling pathway	62	2.00E-05
	bta-miR-494		Rap1 signaling pathway	58	2.00E-05
			Melanoma	25	4.70E-04
		Cluster 2	BTB/POZ fold	52	4.10E-03
			BTB/POZ-like	49	4.10E-03
			BTB	47	4.60E-02
D-U trend in DDR group	bta-miR-12044	Cluster 1	BTB/POZ-like	48	1.10E-02
	bta-miR-142-3p		BTB/POZ fold	49	1.50E-02
	bta-miR-181b		BTB	47	5.20E-02
	bta-miR-374b				
U-D trend in DDU group	bta-let-7c	Cluster 1	BTB/POZ-like	53	8.40E-05
	bta-miR-139		BTB/POZ fold	55	1.20E-04
	bta-miR-144		BTB	53	7.40E-03
	bta-miR-30b-3p	Cluster 2	Zinc finger, C2H2	121	6.90E-05
	bta-miR-545-5p		metal ion binding	238	3.10E-04
			Zinc finger, C2H2-like	110	6.70E-04
			Zinc finger C2H2-type/integrase DNA-binding domain	95	4.30E-03
			nucleic acid binding	105	4.40E-02
			ZnF_C2H2	110	3.20E-01
U-N trend in TTNU group	bta-let-7f	Cluster 1	BTB/POZ fold	43	8.10E-04
	bta-miR-2336		BTB/POZ-like	41	8.10E-04
	bta-miR-342		BTB	39	7.50E-02
	bta-miR-95	Cluster 2	Zinc finger, C2H2	87	5.30E-03

			Zinc finger, C2H2-like	82	7.10E-03
			metal ion binding	167	1.30E-02
			Zinc finger C2H2-type/integrase DNA-binding domain	71	3.20E-02
			nucleic acid binding	78	9.00E-02
			ZnF_C2H2	82	4.30E-01
U-D trend in FRDDU group	bta-miR-1307	Cluster 1	Homeodomain, metazoa	13	4.90E-02
	bta-miR-7857-5p		Homeodomain-like	25	4.90E-02
	bta-miR-99a-5p		Homeobox, conserved site	18	5.60E-02
			Homeobox	20	4.60E-02
			Homeodomain	20	5.60E-02
			HOX	20	3.40E-01
			sequence-specific DNA binding	24	9.00E-01
			DNA-binding	39	1.00E+00
		Cluster 2	Wnt signaling pathway	16	6.40E-03
			Signaling pathways regulating pluripotency of stem cells	14	5.40E-02
			Hippo signaling pathway	11	8.50E-01
			Basal cell carcinoma	6	8.50E-01
U-U trend in FRDDR group	bta-miR-505				

Cluster 1 and 2 were generated based on the enrichment score provided by DAVID, the functional cluster with the highest enrichment score was defined as cluster 1, then followed by cluster

Chapter 4. General discussion

4.1 Summary of the main findings

There is substantial evidence supporting that the pain and distress associated with lameness in cattle and other livestock could lead to poor performance and reduced production (Whay et al., 1998; Warnick et al., 2001; Huxley, 2013b). Previous research has revealed different causes and the typical pathogens for different phenotypes of lameness (Walker et al., 1995b; Boettcher et al., 1998b; G. Bennett et al., 2009; Lean et al., 2013b), however, the molecular mechanisms of lameness pathogenesis in beef cattle are still unknown. This research investigated the relationship between blood miRNAomes and beef cattle lameness to determine if miRNAs could be the potential diagnostic markers for different lameness phenotypes in beef cattle. Two studies were performed, and the main findings are summarized in the following sections.

The results from Chapter 2 have provided the knowledge of 1) the overall expression of blood miRNAs in the beef steers/heifers and 2) the expression difference of miRNAs between healthy animals and cattle diagnosed with four phenotypes of lameness, including digital dermatitis (DD), foot rot (FR), toe tip necrosis (TTN) and FR & DD combined lameness (FRDD). The top ten most expressed miRNAs in the whole blood of beef steers are similar to those previously identified in the sera and exosomes of dairy cows (Zhao et al., 2016c), suggesting these miRNAs are the core miRNAs in the blood of cattle. However, future studies are needed to evaluate their functions to advance the knowledge of their roles in cattle biology. The overall blood miRNA expression

profiles were similar, however, differentially expressed miRNAs were detected between healthy and lame cattle. Among them, bta-miR-6119-3p was down-regulated in blood of all lame cattle. As reported, the downregulated expression of bta-miR-6119-3p in the blood of cattle was challenged with foot mouth disease virus (Johnston et al., 2021) and bovine respiratory disease virus (Sahu et al., 2020) when compared with healthy cattle. This suggests that the reduced expression of this miRNA might be related to the cattle's response to the infection due to lameness. In addition, the expression of some miRNAs was only detected in the blood of cattle with the particular lameness phenotype. Subsequent analysis revealed that their predicted functions were related to cell migration, traumatic muscle stress, and immune/infection reactions. As reported in the previous study, particular miRNAs rather than the overall miRNAome expression profiles can affect the gene expression in different biological processed (X. Wang et al., 2018). For example, the expression of three miRNAs, bta-miR-1, bta-miR-133a and bta-miR-206, which have been reported to be associated with muscle functions (Sun et al., 2015c; Sun et al., 2019c; Hao et al., 2021b), was up-regulated in the TTN group. Therefore, these lameness phenotype-specific miRNAs might be directly involved in various physiological responses to the corresponding lameness phenotype. However, it is unclear whether these miRNAs are also expressed in the tissues/toes of these cattle in response to different lameness phenotypes and can be circulated into the blood. Moreover, both RNA-seq and RT-qPCR analysis verified the similar differentially expression trend of bta-miR-133a and bta-miR-483. The expression of bta-miR-133a was significantly higher in TTN group when compared to HC group, and the expression level of bta-

miR-483 was higher than that in TTN group than that in FR group. This finding suggests that miRNA RT-qPCR could help distinguish different lameness phenotypes based on the expression change or difference. For example, the expression difference of bta-miR-133a suggests that it is TTN cattle associated as its expression level in TTN is significantly higher than HC and other lameness phenotypes (Chapter 2). Similarly, bta-miR-483 can also be selected as an auxiliary marker for FR cattle since its expression in FR cattle is significantly lower when compared with TTN cattle. Further investigation is needed to explore the roles of the differentially expressed miRNAs and the phenotype-specific miRNAs in the pathogenesis of cattle lameness.

In Chapter 3, I further studied the expression changes of miRNAs between cattle that recovered from lameness and those that did not recover; specific miRNA changing patterns were identified for each lameness phenotype. The specific miRNAs including bta-miR-211, bta-miR-1246 and bta-miR-9-5p, which have been reported to be involved in immune, infection and inflammation functions, were differentially expressed between recovered and unrecovered lame cattle. Bta-miR-211 was down-regulated and the other two were up-regulated in the blood of unrecovered cattle than the recovered ones. Previous studies revealed their expression levels change in cattle under different physiological states like *E. Coli* O157:H7 shedding (bta-miR-211) (Wang et al., 2021) and Johne's disease (bta-miR-1246 and bta-miR-9-5p) (Wang et al., 2019), with their functions directly related to inflammation inhibition and immunity suppression (Chen et al., 2020b). These suggest that the regulatory roles of miRNAs in the inflammation may lead to unhealed lameness conditions (Davis-Unger et al., 2019b). Also, the unique temporally changing

patterns of miRNAs were identified between recovered and unrecovered cattle during the treatment period. These miRNAs were reported to play crucial roles in affecting the body fat deposit function in Hanwoo longissimus muscle tissue (Seong et al., 2016) and gram-negative bacterial infection in bovine mammary epithelial cells (Lawless et al., 2013), respectively, suggesting that the failure or delay of recovery and reduced daily weight gain in unrecovered lame cattle could affect the expression of these miRNAs. Furthermore, the identified phenotype-related DE miRNAs from Chapter 2 were also identified in the unrecovered cattle when comparing the miRNA profiles between recovered and unrecovered cattle at the same time points, including TTN-related miRNAs: bta-miR-1 and bta-miR-206. However, these two miRNAs were not previously identified in the DD and FRDD groups. Such inconsistency brings the question that if the cattle were treated with ineffective methods, or they were misdiagnosed at the first time point. The data of causes for each lameness phenotype (such as histology and bacterial pathogens) should be collected and linked to the expression of lameness phenotype-specific miRNAs to draw more reliable conclusions.

4.2 Limitations and future directions

The limitations of the present study mainly reflected from the following aspects. First, although the potential functions of DE miRNAs were reported, their functions were predicted based on the predicted mRNA targets and the literature, which need to be validated using the luciferase reporter assay method to verify the miRNA-mRNA relationship. In addition, this thesis only studied the miRNAs and did not provide the miRNA-mediated regulatory network to explain the interaction

between miRNAs and other molecules in cattle blood and how they cooperatively regulate lameness. Furthermore, this study only investigated the miRNA profiles from blood samples, it is unclear whether the detected DE miRNAs in blood reflect the local responses in diseased foot tissue. In the meantime, no physiological measurement of cattle was included in the data analysis, therefore, it is unknown whether the DE miRNAs are the results of lameness or their physiological changes and/or the combined effect. Future studies are required to supplement more information, including the changes of mRNA expression and protein level in blood and diseased foot tissues to investigate the mechanisms behind lameness pathogenesis. To be more specific, the bacteria culture using an anaerobic/aerobic swab from the lesion for lame cattle is necessary to determine the pathogen of each lameness phenotype and used for miRNAome (together with mRNA and protein) analysis; biopsy as a secondary method should also be done. For the blood indices, more information related to the disease pathogenesis is required. For instance, the complete blood cell count as indicators for inflammation response, substance P as a pain marker, and the haptoglobin as an inflammation indicative should be included to help explain cattle's physiological response to lameness. Secondly, only six out of thirty-eight candidates were selected, and thirty-two cattle were used for RT-qPCR validation. As a result, the accuracy of the DE expression could be affected under a smaller sample size. Thus, the expression of all 38 DE miRNAs and 16 phenotype-specific miRNAs should be tested in the blood of all the samples collected (n = 596) from 363 lame cattle consisting of different lameness phenotypes and 19 healthy cattle to validate whether they are differentially expressed between healthy cattle and different lameness phenotypes as identified

from Chapter 2. Similarly, the temporal and recovery pattern related miRNAs should also be tested from 71 recovered cattle and 38 unrecovered cattle using miRNA RT-qPCR to determine whether their differential expression and changing patterns are related to the recovery pattern of the cattle.

There are some other limitations of the present study. The blood miRNAome varied significantly among individual animals within each group. Many factors might affect the expression of miRNAs in the blood such as diet, environment, genetics, animal physiological and metabolic conditions. The blood samples in this study were randomly selected from feedlot beef cattle with different breeds, including Black/Red Angus, Charolais, Simmental, Hereford and other breeds as well as their hybrids. Different breeds could lead to the variance in the blood miRNAome. 795 differential expressed miRNAs were identified from the colostrum exosome miRNAs between Holstein and Doğu Anadolu Kirmizisi cows (Özdemir, 2020). This finding suggests the miRNA compositions in body fluids can be affected by animal genetics although some of them are highly conserved among different mammalian species. Thus, the observed individualized expression of blood miRNAs might be potentially affected by different breeds. Apart from breeds, the sex difference should also be considered as the cattle used in this study included heifers and steers. Differentially expressed miRNAs including bta-miR-192 and bta-miR-101 have been identified in the serum from Korean native bulls, steers and heifers (bta-miR-192: $p < 0.085$ and bta-miR-101: $p < 0.070$) (Bae et al., 2015). In concert with this finding, deregulation of miRNAs was reported in human between males and females based on information provided from Cancer Genome Atlas database (L. Guo et al., 2017). These findings suggest that the expression of specific miRNAs

could be affected by sex; this should be taken into account in future experimental design and data analysis.

In addition, the sample collection time also affected the blood miRNAs profiles. All blood samples including two breeding cycles used in this study were collected from 2018 to 2020. As reported, different levels of sex hormones can affect the miRNA profiles; differentially expressed miRNAs between the ovine anestrus and the breeding season were identified in Tan sheep ovarian (Di et al., 2014). Moreover, season (environment) such as different temperatures can also affect the miRNA expression. It was reported that the blood miRNA profile in cattle might change under the higher temperature in summer. For example, 65 differentially expressed miRNAs in the blood of Frieswal dairy cow under summer heat stress were identified when compared with the same cow during the winter (Sengar et al., 2018). Also, there was a difference in blood miRNA profiles between the heat-stressed and healthy Holstein cows (Lee et al., 2020b). Moreover, the age information for all 363 cattle selected in this study is missing, the heifers and steers had different ages. As miRNAs play essential roles in gene expression regulation, their expression profiles may vary under different ages. In fact, the circulating skeletal muscle miRNA profiles in Piedmonts cattle were significantly different between different age groups; bta-miR-10b was significantly up-regulated and bta-miR-21-5p and bta-miR-211 significantly were down-regulated in the newborn cattle (Tewari et al., 2021). These miRNAs were also reported in Chapters 2 and 3 as phenotype-specific miRNAs and DE miRNAs, making it hard to distinguish if the differential expression identified in our study is due to lameness or age differences. As mentioned above, the

environmental difference should also be considered as the environmental condition is not the same among the six feedlots. Furthermore, the other health conditions of the lame cattle were not assessed. It is unknown if they were affected by other diseases like bovine respiratory diseases or rumen acidosis. Reported differentially expressed miRNAs in these two studies which function in immune and inflammation responses was also related to other potential diseases. Taken together, these suggest that the actual functions of the selected miRNAs await to be validated to take all above factors into account to investigate their roles in the pathogenesis of different phenotypes of lameness and their potentials to be selected as disease markers.

Further studies to investigate the relationship between miRNA and beef cattle lameness require better experimental design to minimize or control the above discussed factors. For example, the cattle should be born in the same breeding season, breed and sex, and also need to be fed in the same pen in the feedlot to minimize the environmental effect. Most importantly, the different lameness phenotypes should not be selected from the cattle with naturally occurring diseases, and those phenotypes should be artificially induced to maximize the pathogenesis consistency.

4.3 Implications to the industry

To the best of our knowledge, this is the first study to reveal the potential regulatory roles of miRNAs in feedlot beef cattle lameness. This study provided the blood miRNA expression profiles of different phenotypes of lameness and revealed the potential relationship between blood miRNA profiles and different types of lameness. Moreover, the RT-qPCR results of selected candidate miRNAs proved their potential to be selected as miRNA markers, which could be useful as

diagnostic tools to distinguish different lameness phenotypes and evaluate the lameness treatment efficiency. The knowledge obtained from this research will help develop novel diagnostic tools with higher accuracy and efficiency for lameness detection and mitigation, thus improving animal health and welfare. If the developed diagnosis tool could be applied to the industry, the disease screening when cattle enter the feedlot could significantly reduce the cost of lameness diagnosis and treatments, which could also help the feedlot operators change their management strategy. In addition, the application of this method could vastly improve animal welfare because it can diagnose lameness at the early stage, and the cattle will not suffer from pain for a long time until their abnormal was observed by the farmers.

4.5 Reference

Bae, I. S., Chung, K. Y., Yi, J., Kim, T. il, Choi, H. S., Cho, Y. M., Choi, I., & Kim, S. H. (2015). Identification of Reference Genes for Relative Quantification of Circulating MicroRNAs in Bovine Serum. PLOS ONE, 10(3), e0122554. https://doi.org/10.1371/JOURNAL.PONE.0122554

Bennett, G., Hickford, J., Zhou, H., Laporte, J., & Gibbs, J. (2009). Detection of Fusobacterium necrophorum and Dichelobacter nodosus in lame cattle on dairy farms in New Zealand. Research in Veterinary Science, 87(3), 413–415. https://doi.org/10.1016/J.RVSC.2009.04.001

Boettcher, P. J., Dekkers, J. C. M., Warnick, L. D., & Wells, S. J. (1998). Genetic Analysis of Clinical Lameness in Dairy Cattle. Journal of Dairy Science, 81(4), 1148–1156. https://doi.org/10.3168/JDS.S0022-0302(98)75677-2

Chen, L., Tian, R., Zhang, H., & Liu, X. (2020). Anti-mastitis SNV identification of NFκB1 in Chinese Holstein cows and the possible anti-inflammation role of NFκB1/p105 in bovine MECs. Acta Biochimica et Biophysica Sinica, 52(11), 1191–1201. https://doi.org/10.1093/ABBS/GMAA118

Davis-Unger, J., Schwartzkopf-Genswein, K. S. G., Pajor, E. A., Hendrick, S., Marti, S., Dorin, C., & Orsel, K. (2019). Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Translational Animal Science, 3(2), 595–606. https://doi.org/10.1093/TAS/TXZ008

Di, R., He, J., Song, S., Tian, D., Liu, Q., Liang, X., Ma, Q., Sun, M., Wang, J., Zhao, W., Cao, G., Wang, J., Yang, Z., Ge, Y., & Chu, M. (2014). Characterization and comparative profiling of ovarian microRNAs during ovine anestrus and the breeding season. BMC Genomics, 15(1), 1–15. https://doi.org/10.1186/1471-2164-15-899/FIGURES/8

Guo, L., Zhang, Q., Ma, X., Wang, J., & Liang, T. (2017). miRNA and mRNA expression analysis reveals potential sex-biased miRNA expression. Scientific Reports 2017 7:1, 7(1), 1–10. https://doi.org/10.1038/SREP39812

Hao, D., Wang, X., Wang, X., Thomsen, B., Yang, Y., Lan, X., Huang, Y., & Chen, H. (2021). MicroRNA bta-miR-365-3p inhibits proliferation but promotes differentiation of primary bovine myoblasts by targeting the activin A receptor type I. Journal of Animal Science and Biotechnology, 12(1), 1–15. https://doi.org/10.1186/S40104-020-00528-0/TABLES/2

Huxley, J. N. (2013). Impact of lameness and claw lesions in cows on health and production. Livestock Science, 156(1–3), 64–70. https://doi.org/10.1016/J.LIVSCI.2013.06.012

Johnston, D., Earley, B., McCabe, M. S., Kim, J., Taylor, J. F., Lemon, K., McMenamy, M., Duffy, C., Cosby, S. L., & Waters, S. M. (2021). Elucidation of the Host Bronchial Lymph Node miRNA Transcriptome Response to Bovine Respiratory Syncytial Virus. Frontiers in Genetics, 12. https://doi.org/10.3389/FGENE.2021.633125/FULL

Lawless, N., Foroushani, A. B. K., McCabe, M. S., O'Farrelly, C., & Lynn, D. J. (2013). Next Generation Sequencing Reveals the Expression of a Unique miRNA Profile in Response to a Gram-Positive Bacterial Infection. PLOS ONE, 8(3), e57543. https://doi.org/10.1371/JOURNAL.PONE.0057543

Lean, I. J., Westwood, C. T., Golder, H. M., & Vermunt, J. J. (2013). Impact of nutrition on lameness and claw health in cattle. Livestock Science, 156(1–3), 71–87. https://doi.org/10.1016/J.LIVSCI.2013.06.006

Lee, J., Lee, S., Son, J., Lim, H., Kim, E., Kim, D., Ha, S., Hur, T., Lee, S., & Choi, I. (2020). Analysis of circulating-microRNA expression in lactating Holstein cows under summer heat stress. PLOS ONE, 15(8), e0231125. https://doi.org/10.1371/JOURNAL.PONE.0231125

Özdemir, S. (2020). Identification and comparison of exosomal microRNAs in the milk and colostrum of two different cow breeds. Gene, 743, 144609. https://doi.org/10.1016/J.GENE.2020.144609

Sahu, T. K., Gurjar, A. K. S., Meher, P. K., Varghese, C., Marwaha, S., Rao, G. P., Rai, A., Guleria, N., Basagoudanavar, S. H., Sanyal, A., & Rao, A. R. (2020). Computational insights into RNAi-

based therapeutics for foot and mouth disease of Bos taurus. Scientific Reports 2020 10:1, 10(1), 1–13. https://doi.org/10.1038/S41598-020-78541-6

Seong, J., Yoon, • Hyojeong, & Kong, H. S. (2016). Identification of microRNA and target gene associated with marbling score in Korean cattle (Hanwoo). Genes & Genomics, 38. https://doi.org/10.1007/s13258-016-0401-y

Sun, H. Z., Chen, Y., & Guan, L. L. (2019). MicroRNA expression profiles across blood and different tissues in cattle. Scientific Data 2019 6:1, 6(1), 1–8. https://doi.org/10.1038/sdata.2019.13

Sun, J., Sonstegard, T. S., Li, C., Huang, Y., Li, Z., Lan, X., Zhang, C., Lei, C., Zhao, X., & Chen, H. (2015). Altered microRNA expression in bovine skeletal muscle with age. Animal Genetics, 46(3), 227–238. https://doi.org/10.1111/AGE.12272

Walker, R. L., Read, D. H., Loretz, K. J., & Nordhausen, R. W. (1995). Spirochetes isolated from dairy cattle with papillomatous digital dermatitis and interdigital dermatitis. Veterinary Microbiology, 47(3–4), 343–355. https://doi.org/10.1016/0378-1135(95)00114-X

Wang, O., Zhou, M., Chen, Y., McAllister, T. A., Plastow, G., Stanford, K., Selinger, B., & Guan, L. L. (2021). MicroRNAomes of Cattle Intestinal Tissues Revealed Possible miRNA Regulated Mechanisms Involved in Escherichia coli O157 Fecal Shedding. Frontiers in Cellular and Infection Microbiology, 11. https://doi.org/10.3389/FCIMB.2021.634505/FULL

Wang, X., Zhang, P., Li, L., Che, D., Li, T., Li, H., Li, Q., Jia, H., Tao, S., Hua, J., Zeng, W., & Liao, M. (2018). miRNA editing landscape reveals miR-34c regulated spermatogenesis through structure and target change in pig and mouse. Biochemical and Biophysical Research Communications, 502(4), 486–492. https://doi.org/10.1016/J.BBRC.2018.05.197

Wang, Z., Cong Kong, L., Jia, B. Y., Chen, J. R., Dong, Y., Jiang, X. Y., & Ma, H. X. (2019). Analysis of the microRNA Expression Profile of Bovine Monocyte-derived Macrophages Infected with Mycobacterium avium subsp. Paratuberculosis Reveals that miR-150 Suppresses Cell

Apoptosis by Targeting PDCD4. International Journal of Molecular Sciences 2019, Vol. 20, Page 2708, 20(11), 2708. https://doi.org/10.3390/IJMS20112708

Warnick, L. D., Janssen, D., Guard, C. L., & Gröhn, Y. T. (2001). The Effect of Lameness on Milk Production in Dairy Cows. Journal of Dairy Science, 84(9), 1988–1997. https://doi.org/10.3168/JDS.S0022-0302(01)74642-5

Whay, H. R., Waterman, A. E., Webster, A. J. F., & O'Brien, J. K. (1998). The influence of lesion type on the duration ofhyperalgesia associated with hindlimb lameness in dairy cattle. The Veterinary Journal, 156(1), 23–29. https://doi.org/10.1016/S1090-0233(98)80058-0

Zhao, K., Liang, G., Sun, X., & Guan, L. L. (2016). Comparative miRNAome analysis revealed different miRNA expression profiles in bovine sera and exosomes. BMC Genomics, 17(1), 1–12. https://doi.org/10.1186/S12864-016-2962-1/TABLES/6

Bibliography (All sources used)

Akao, Y., Y. Nakagawa, and T. Naoe. 2007. MicroRNA-143 and -145 in Colon Cancer. https://home-liebertpub-com.login.ezproxy.library.ualberta.ca/dna. 26:311–320. doi:10.1089/DNA.2006.0550. Available from: https://www-liebertpub-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1089/dna.2006.0550

Alberta has the most beef cattle in Canada and the second largest total farm area. Available from: https://www150.statcan.gc.ca/n1/pub/95-640-x/2016001/article/14808-eng.htm

Alemu, A. W., B. D. Amiro, S. Bittman, D. MacDonald, and K. H. Ominski. 2016. A typological characterization of Canadian beef cattle farms based on a producer survey. Canadian Journal of Animal Science. 96:187–202. doi:10.1139/CJAS-2015-0060/ASSET/IMAGES/CJAS-2015-0060TAB6.GIF. Available from: https://cdnsciencepub-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1139/cjas-2015-0060

Al-Husseini, W., Y. Chen, C. Gondro, R. M. Herd, J. P. Gibson, and P. F. Arthur. 2016a. Characterization and Profiling of Liver microRNAs by RNA-sequencing in Cattle Divergently Selected for Residual Feed Intake. Asian-Australasian Journal of Animal Sciences. 29:1371. doi:10.5713/AJAS.15.0605. Available from: /pmc/articles/PMC5003961/

Al-Husseini, W., Y. Chen, C. Gondro, R. M. Herd, J. P. Gibson, and P. F. Arthur. 2016b. Characterization and Profiling of Liver microRNAs by RNA-sequencing in Cattle Divergently Selected for Residual Feed Intake. Asian-Australasian Journal of Animal Sciences. 29:1371. doi:10.5713/AJAS.15.0605. Available from: /pmc/articles/PMC5003961/

Allenstein, L. C. 1981. Lamenesses of cattle. The Canadian Veterinary Journal. 22:65. Available from: https://www-ncbi-nlm-nih-gov.login.ezproxy.library.ualberta.ca/pmc/articles/PMC1789870/

van Amstel, S. R., S. van Vuuren, and L. C. Tutt. 1995. DIGITAL DERMATITIS REPORT OF AN OUTBREAK. Journal of the South African ’ ~terinary Association. 66:177–181. doi:10.10520/AJA00382809_1548.

Anindo, M. I. K., and A. Yaqinuddin. 2012. Insights into the potential use of microRNAs as biomarker in cancer. International Journal of Surgery. 10:443–449. doi:10.1016/J.IJSU.2012.08.006.

Azzalini, E., E. de Martino, P. Fattorini, V. Canzonieri, G. Stanta, and S. Bonin. 2019. Reliability of miRNA Analysis from Fixed and Paraffin-Embedded Tissues. International Journal of Molecular Sciences 2019, Vol. 20, Page 4819. 20:4819. doi:10.3390/IJMS20194819. Available from: https://www.mdpi.com/1422-0067/20/19/4819/htm

Babatunde, S. M., S. Z. Ramanoon, W. M. S. Mossadeq, R. Mansor, and S. S. S. Hussain. 2019. Dairy Farmers' Perceptions of and Actions in Relation to Lameness Management. Animals 2019, Vol. 9, Page 270. 9:270. doi:10.3390/ANI9050270. Available from: https://www.mdpi.com/2076-2615/9/5/270/htm

Backes, C., E. Meese, and A. Keller. 2016. Specific miRNA Disease Biomarkers in Blood, Serum and Plasma: Challenges and Prospects. Molecular Diagnosis & Therapy 2016 20:6. 20:509–518. doi:10.1007/S40291-016-0221-4. Available from: https://link.springer.com/article/10.1007/s40291-016-0221-4

Bae, I. S., K. Y. Chung, J. Yi, T. il Kim, H. S. Choi, Y. M. Cho, I. Choi, and S. H. Kim. 2015. Identification of Reference Genes for Relative Quantification of Circulating MicroRNAs in Bovine Serum. PLOS ONE. 10:e0122554. doi:10.1371/JOURNAL.PONE.0122554. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0122554

Barceló, M., M. Castells, L. Bassas, F. Vigués, and S. Larriba. 2019. Semen miRNAs Contained in Exosomes as Non-Invasive Biomarkers for Prostate Cancer Diagnosis. Scientific Reports 2019 9:1. 9:1–16. doi:10.1038/S41598-019-50172-6. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-019-50172-6

Bartel, D. P. 2018. Metazoan MicroRNAs. Cell. 173:20. doi:10.1016/J.CELL.2018.03.006. Available from: /pmc/articles/PMC6091663/

Benjamini, Y., and Y. Hochberg. 1995. Controlling the False Discovery Rate: A Practical and

Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological). 57:289–300. doi:10.1111/J.2517-6161.1995.TB02031.X. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.2517-6161.1995.tb02031.x

Bennett, Grant, J. Hickford, R. Sedcole, and H. Zhou. 2009. Dichelobacter nodosus, Fusobacterium necrophorum and the epidemiology of footrot. Anaerobe. 15:173–176. doi:10.1016/J.ANAEROBE.2009.02.002.

Bennett, G., J. Hickford, H. Zhou, J. Laporte, and J. Gibbs. 2009. Detection of Fusobacterium necrophorum and Dichelobacter nodosus in lame cattle on dairy farms in New Zealand. Research in Veterinary Science. 87:413–415. doi:10.1016/J.RVSC.2009.04.001.

Bennett, R. 2003. The 'Direct Costs'of Livestock Disease: The Development of a System of Models for the Analysis of 30 Endemic Livestock Diseases in Great Britain. Journal of Agricultural Economics. 54:55–71. doi:10.1111/J.1477-9552.2003.TB00048.X. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/j.1477-9552.2003.tb00048.x

Birnbaum, R. Y., A. Zvulunov, D. Hallel-Halevy, E. Cagnano, G. Finer, R. Ofir, D. Geiger, E. Silberstein, Y. Feferman, and O. S. Birk. 2006. Seborrhea-like dermatitis with psoriasiform elements caused by a mutation in ZNF750, encoding a putative C2H2 zinc finger protein. Nature Genetics 2006 38:7. 38:749–751. doi:10.1038/NG1813. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/ng1813

Blackie, N., E. Bleach, J. Amory, and J. Scaife. 2011. Impact of lameness on gait characteristics and lying behaviour of zero grazed dairy cattle in early lactation. Applied Animal Behaviour Science. 129:67–73. doi:10.1016/J.APPLANIM.2010.10.006.

Boettcher, P. J., J. C. M. Dekkers, L. D. Warnick, and S. J. Wells. 1998a. Genetic Analysis of Clinical Lameness in Dairy Cattle. Journal of Dairy Science. 81:1148–1156. doi:10.3168/JDS.S0022-0302(98)75677-2.

Boettcher, P. J., J. C. M. Dekkers, L. D. Warnick, and S. J. Wells. 1998b. Genetic Analysis of Clinical Lameness in Dairy Cattle. Journal of Dairy Science. 81:1148–1156.

doi:10.3168/JDS.S0022-0302(98)75677-2.

Bras, A. L., H. W. Barkema, M. R. Woodbury, C. S. Ribble, J. Perez-Casal, and M. C. Windeyer. 2017. Clinical presentation, prevalence, and risk factors associated with Mycoplasma bovis-associated disease in farmed bison (Bison bison) herds in western Canada. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.2460/javma.250.10.1167. 250:1167–1175. doi:10.2460/JAVMA.250.10.1167.

Brase, J. C., M. Johannes, T. Schlomm, M. Fälth, A. Haese, T. Steuber, T. Beissbarth, R. Kuner, and H. Sültmann. 2011. Circulating miRNAs are correlated with tumor progression in prostate cancer. International Journal of Cancer. 128:608–616. doi:10.1002/IJC.25376. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/ijc.25376

Brazier, J. S. 2006. Human infections with Fusobacterium necrophorum. Anaerobe. 12:165–172. doi:10.1016/J.ANAEROBE.2005.11.003.

Brazle, F. K. 1993. Effect of Zinpro 100® in a mineral mixture on gain and incidence of footrot in steers grazing native grass pastures. Kansas Agricultural Experiment Station Research Reports. 745:145–146. doi:10.4148/2378-5977.2148. Available from: https://newprairiepress.org/kaesrr/vol0/iss1/745

Budhu, A., H. L. Jia, M. Forgues, C. G. Liu, D. Goldstein, A. Lam, K. A. Zanetti, Q. H. Ye, L. X. Qin, C. M. Croce, Z. Y. Tang, and W. W. Xin. 2008. Identification of metastasis-related microRNAs in hepatocellular carcinoma. Hepatology. 47:897–907. doi:10.1002/HEP.22160. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/hep.22160

Bunt, M. van de, K. J. Gaulton, L. Parts, I. Moran, P. R. Johnson, C. M. Lindgren, J. Ferrer, A. L. Gloyn, and M. I. McCarthy. 2013. The miRNA Profile of Human Pancreatic Islets and Beta-Cells and Relationship to Type 2 Diabetes Pathogenesis. PLOS ONE. 8:e55272. doi:10.1371/JOURNAL.PONE.0055272. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0055272

Cassandri, M., A. Smirnov, F. Novelli, C. Pitolli, M. Agostini, M. Malewicz, G. Melino, and G. Raschellà. 2017. Zinc-finger proteins in health and disease. Cell Death Discovery 2017 3:1. 3:1–

12. doi:10.1038/cddiscovery.2017.71. Available from: https://www.nature.com/articles/cddiscovery201771

Chakraborti, C. K. 2015. New-found link between microbiota and obesity. World Journal of Gastrointestinal Pathophysiology. 6:110. doi:10.4291/WJGP.V6.I4.110. Available from: /pmc/articles/PMC4644874/

Chakraborty, C., C. G. P. Doss, S. Bandyopadhyay, and G. Agoramoorthy. 2014. Influence of miRNA in insulin signaling pathway and insulin resistance: micro-molecules with a major role in type-2 diabetes. Wiley Interdisciplinary Reviews: RNA. 5:697–712. doi:10.1002/WRNA.1240. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/wrna.1240

Checkley, S. L., E. D. Janzen, J. R. Campbell, and J. J. McKinnon. 2005a. Efficacy of vaccination against Fusobacterium necrophorum infection for control of liver abscesses and footrot in feedlot cattle in western Canada. The Canadian Veterinary Journal. 46:1002. Available from: /pmc/articles/PMC1259145/

Checkley, S. L., E. D. Janzen, J. R. Campbell, and J. J. McKinnon. 2005b. Efficacy of vaccination against Fusobacterium necrophorum infection for control of liver abscesses and footrot in feedlot cattle in western Canada. The Canadian Veterinary Journal. 46:1002. Available from: /pmc/articles/PMC1259145/

Chen, C., D. A. Ridzon, A. J. Broomer, Z. Zhou, D. H. Lee, J. T. Nguyen, M. Barbisin, N. L. Xu, V. R. Mahuvakar, M. R. Andersen, K. Q. Lao, K. J. Livak, and K. J. Guegler. 2005. Real-time quantification of microRNAs by stem–loop RT–PCR. Nucleic Acids Research. 33:e179–e179. doi:10.1093/NAR/GNI178. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/nar/article/33/20/e179/1082564

Chen, L., R. Tian, H. Zhang, and X. Liu. 2020a. Anti-mastitis SNV identification of NFκB1 in Chinese Holstein cows and the possible anti-inflammation role of NFκB1/p105 in bovine MECs. Acta Biochimica et Biophysica Sinica. 52:1191–1201. doi:10.1093/ABBS/GMAA118. Available from: https://academic-oup-

com.login.ezproxy.library.ualberta.ca/abbs/article/52/11/1191/5983617

Chen, L., R. Tian, H. Zhang, and X. Liu. 2020b. Anti-mastitis SNV identification of NFκB1 in Chinese Holstein cows and the possible anti-inflammation role of NFκB1/p105 in bovine MECs. Acta Biochimica et Biophysica Sinica. 52:1191–1201. doi:10.1093/ABBS/GMAA118. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/abbs/article/52/11/1191/5983617

Chen, Y. 2021. The Economic Impact of Alternative Antimicrobial Management Strategies in Western Beef Feedlots: An Agent-based Model. doi:10.7939/R3-YYQM-V483. Available from: https://era-library-ualberta-ca.login.ezproxy.library.ualberta.ca/items/afd49fd8-acbd-4452-869f-4a76e758cae7

Choi, S. W., S. Kim, H. T. Park, H. E. Park, J. S. Choi, and H. S. Yoo. 2021. MicroRNA profiling in bovine serum according to the stage of Mycobacterium avium subsp. paratuberculosis infection. PLOS ONE. 16:e0259539. doi:10.1371/JOURNAL.PONE.0259539. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0259539

Cochetti, G., J. A. Rossi de Vermandois, V. Maulà, M. Giulietti, M. Cecati, M. del Zingaro, R. Cagnani, C. Suvieri, A. Paladini, and E. Mearini. 2020. Role of miRNAs in prostate cancer: Do we really know everything? Urologic Oncology: Seminars and Original Investigations. 38:623–635. doi:10.1016/J.UROLONC.2020.03.007.

Coenen-Stass, A. M. L., M. J. Pauwels, B. Hanson, C. M. Perez, M. Conceição, M. J. A. Wood, I. Mäger, and T. C. Roberts. 2019. Extracellular microRNAs exhibit sequence-dependent stability and cellular release kinetics. https://doi-org.login.ezproxy.library.ualberta.ca/10.1080/15476286.2019.1582956. 16:696–706. doi:10.1080/15476286.2019.1582956. Available from: https://www-tandfonline-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1080/15476286.2019.1582956

Condotta, I. C. F. S., T. M. Brown-Brandl, G. A. Rohrer, and K. O. Silva-Miranda. 2020. *Development of method for lameness detection based on depth image analysis*. ASABE 2020

Annual International Meeting. 1-. doi:10.13031/AIM.202001082. Available from: https://elibrary-asabe-org.login.ezproxy.library.ualberta.ca/azdez.asp?JID=&AID=51535&CID=virt2020&v=&i=&T=1

Cook, N. B. 2017a. A Review of the Design and Management of Footbaths for Dairy Cattle. Veterinary Clinics of North America: Food Animal Practice. 33:195–225. doi:10.1016/J.CVFA.2017.02.004.

Cook, N. B. 2017b. A Review of the Design and Management of Footbaths for Dairy Cattle. Veterinary Clinics: Food Animal Practice. 33:195–225. doi:10.1016/J.CVFA.2017.02.004. Available from: http://www.vetfood.theclinics.com/article/S0749072017300129/fulltext

Cortes, J. A., S. Hendrick, E. Janzen, E. A. Pajor, and K. Orsel. 2021a. Economic impact of digital dermatitis, foot rot, and bovine respiratory disease in feedlot cattle. Translational Animal Science. 5. doi:10.1093/TAS/TXAB076. Available from: /pmc/articles/PMC8244990/

Cortes, J. A., S. Hendrick, E. Janzen, E. A. Pajor, and K. Orsel. 2021b. Economic impact of digital dermatitis, foot rot, and bovine respiratory disease in feedlot cattle. Translational Animal Science. 5:1–10. doi:10.1093/TAS/TXAB076. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/5/2/txab076/6278306

Cortes, J. A., S. Hendrick, E. Janzen, E. A. Pajor, and K. Orsel. 2021c. Economic impact of digital dermatitis, foot rot, and bovine respiratory disease in feedlot cattle. Translational Animal Science. 5:1–10. doi:10.1093/TAS/TXAB076. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/5/2/txab076/6278306

Cristino, A. S., E. D. Tanaka, M. Rubio, M. D. Piulachs, and X. Belles. 2011. Deep Sequencing of Organ- and Stage-Specific microRNAs in the Evolutionarily Basal Insect Blattella germanica (L.) (Dictyoptera, Blattellidae). PLOS ONE. 6:e19350. doi:10.1371/JOURNAL.PONE.0019350. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0019350

Cui, R., J. Xu, X. Chen, and W. Zhu. 2016. Global miRNA expression is temporally correlated with acute kidney injury in mice. PeerJ. 4:e1729. doi:10.7717/PEERJ.1729. Available from:

https://peerj.com/articles/1729

Currin, J. F., W. D. Whittier, and N. Currin. 2005. Foot Rot in Beef Cattle. Available from: https://vtechworks.lib.vt.edu/handle/10919/50702

Currin, J. F., W. D. Whittier, and N. Currin. 2014. Foot Rot in Beef Cattle. Available from: https://vtechworks.lib.vt.edu/handle/10919/50702

Dai, Y., Y. M. Wang, W. R. Zhang, X. F. Liu, X. Li, X. bin Ding, and H. Guo. 2016. The role of microRNA-1 and microRNA-206 in the proliferation and differentiation of bovine skeletal muscle satellite cells. In Vitro Cell Dev Biol Anim. 52:27–34. doi:10.1007/S11626-015-9953-4. Available from: https://pubmed.ncbi.nlm.nih.gov/26424132/

Damiran, D., K. A. Larson, L. T. Pearce, N. E. Erickson, and B. H. A. Lardner. 2018. Effect of calving period on beef cow longevity and lifetime productivity in western Canada. Translational Animal Science. 2:S61–S65. doi:10.1093/TAS/TXY020. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/2/suppl_1/S61/5108326

Dark, M. J. 2013. Mycobacterial Species. Foodborne Infections and Intoxications. 337–342. doi:10.1016/B978-0-12-416041-5.00024-X.

Davis-Unger, J., E. A. Pajor, K. Schwartzkopf-Genswein, S. Marti, C. Dorin, E. Spackman, and K. Orsel. 2017a. Economic impacts of lameness in feedlot cattle. Translational Animal Science. 1:467–479. doi:10.2527/TAS2017.0052. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/1/4/467/4780403

Davis-Unger, J., E. A. Pajor, K. Schwartzkopf-Genswein, S. Marti, C. Dorin, E. Spackman, and K. Orsel. 2017b. Economic impacts of lameness in feedlot cattle. Translational Animal Science. 1:467–479. doi:10.2527/TAS2017.0052. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/1/4/467/4780403

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019a. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Translational Animal Science. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/3/2/595/5377510

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019b. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Transl Anim Sci. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://pubmed.ncbi.nlm.nih.gov/32704830/

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019c. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Translational Animal Science. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/3/2/595/5377510

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019d. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Translational Animal Science. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://academic.oup.com/tas/article/3/2/595/5377510

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019e. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Translational Animal Science. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/tas/article/3/2/595/5377510

Davis-Unger, J., K. S. G. Schwartzkopf-Genswein, E. A. Pajor, S. Hendrick, S. Marti, C. Dorin, and K. Orsel. 2019f. Prevalence and lameness-associated risk factors in Alberta feedlot cattle. Transl Anim Sci. 3:595–606. doi:10.1093/TAS/TXZ008. Available from: https://pubmed.ncbi.nlm.nih.gov/32704830/

Di, R., J. He, S. Song, D. Tian, Q. Liu, X. Liang, Q. Ma, M. Sun, Jiandong Wang, W. Zhao, G. Cao, Jinxin Wang, Z. Yang, Y. Ge, and M. Chu. 2014. Characterization and comparative profiling of ovarian microRNAs during ovine anestrus and the breeding season. BMC Genomics. 15:1–15. doi:10.1186/1471-2164-15-899/FIGURES/8. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/articles/10.1186/1471-2164-15-899

Distl, O., and A. Mair. 1993. Computerized analysis of pedobarometric forces in cattle at the ground surface/floor interface. Computers and Electronics in Agriculture. 8:237–250.

doi:10.1016/0168-1699(93)90036-Z.

Do, D. N., P.-L. Dudemaine, M. Mathur, P. Suravajhala, X. Zhao, and E. M. Ibeagha-Awemu. 2021. miRNA Regulatory Functions in Farm Animal Diseases, and Biomarker Potentials for Effective Therapies. International Journal of Molecular Sciences 2021, Vol. 22, Page 3080. 22:3080. doi:10.3390/IJMS22063080. Available from: https://www.mdpi.com/1422-0067/22/6/3080/htm

Dolecheck, K., and J. Bewley. 2018. Animal board invited review: Dairy cow lameness expenditures, losses and total cost. animal. 12:1462–1474. doi:10.1017/S1751731118000575. Available from: https://www-cambridge-org.login.ezproxy.library.ualberta.ca/core/journals/animal/article/animal-board-invited-review-dairy-cow-lameness-expenditures-losses-and-total-cost/1DD4186D5AC3943FC5DB8E6536C0277A

Döpfer, D., A. A. H. M. ter Huurne, J. L. Cornelisse, A. J. A. M. van Asten, A. Koopmans, F. A. Meijer, Y. H. Schukken, I. Szakáll, W. Klee, and R. B. Bosma. 1997. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and Campylobacter faecalis. Veterinary Record. 140:620–623. doi:10.1136/VR.140.24.620. Available from: https://bvajournals.onlinelibrary.wiley.com/doi/full/10.1136/vr.140.24.620

Drummond, M. J., J. J. McCarthy, M. Sinha, H. M. Spratt, E. Volpi, K. A. Esser, and B. B. Rasmussen. 2011. Aging and microRNA expression in human skeletal muscle: A microarray and bioinformatics analysis. Physiological Genomics. 43:595–603. doi:10.1152/PHYSIOLGENOMICS.00148.2010/ASSET/IMAGES/LARGE/ZH7016103546000 6.JPEG. Available from: https://journals-physiology-org.login.ezproxy.library.ualberta.ca/doi/abs/10.1152/physiolgenomics.00148.2010

Duttagupta, R., R. Jiang, J. Gollub, R. C. Getts, and K. W. Jones. 2011a. Impact of Cellular miRNAs on Circulating miRNA Biomarker Signatures. PLOS ONE. 6:e20769. doi:10.1371/JOURNAL.PONE.0020769. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0020769

Duttagupta, R., R. Jiang, J. Gollub, R. C. Getts, and K. W. Jones. 2011b. Impact of Cellular

miRNAs on Circulating miRNA Biomarker Signatures. PLOS ONE. 6:e20769. doi:10.1371/JOURNAL.PONE.0020769. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0020769

Ekesi, N. S., B. Dolka, A. A. K. Alrubaye, and D. D. Rhoads. 2021. Analysis of genomes of bacterial isolates from lameness outbreaks in broilers. Poultry Science. 100:101148. doi:10.1016/J.PSJ.2021.101148.

Elias, K. M., W. Fendler, K. Stawiski, S. J. Fiascone, A. F. Vitonis, R. S. Berkowitz, G. Frendl, P. Konstantinopoulos, C. P. Crum, M. Kedzierska, D. W. Cramer, and D. Chowdhury. 2017. Diagnostic potential for a serum miRNA neural network for detection of ovarian cancer. Elife. 6. doi:10.7554/ELIFE.28932.

Endres, M. I. 2017. The Relationship of Cow Comfort and Flooring to Lameness Disorders in Dairy Cattle. Veterinary Clinics: Food Animal Practice. 33:227–233. doi:10.1016/J.CVFA.2017.02.007. Available from: http://www.vetfood.theclinics.com/article/S0749072017300154/fulltext

Esquela-Kerscher, A., and F. J. Slack. 2006. Oncomirs — microRNAs with a role in cancer. Nature Reviews Cancer 2006 6:4. 6:259–269. doi:10.1038/NRC1840. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/nrc1840

Eulalio, A., E. Huntzinger, and E. Izaurralde. 2008. Getting to the Root of miRNA-Mediated Gene Silencing. Cell. 132:9–14. doi:10.1016/J.CELL.2007.12.024.

Evans, N. J., R. D. Murray, and S. D. Carter. 2016. Bovine digital dermatitis: Current concepts from laboratory to farm. The Veterinary Journal. 211:3–13. doi:10.1016/J.TVJL.2015.10.028.

Fabian, M. R., N. Sonenberg, and W. Filipowicz. 2010a. Regulation of mRNA Translation and Stability by microRNAs. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.1146/annurev-biochem-060308-103103. 79:351–379. doi:10.1146/ANNUREV-BIOCHEM-060308-103103. Available from: https://www-annualreviews-org.login.ezproxy.library.ualberta.ca/doi/abs/10.1146/annurev-biochem-060308-103103

Fabian, M. R., N. Sonenberg, and W. Filipowicz. 2010b. Regulation of mRNA Translation and

Stability by microRNAs. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.1146/annurev-biochem-060308-103103. 79:351–379. doi:10.1146/ANNUREV-BIOCHEM-060308-103103. Available from: https://www-annualreviews-org.login.ezproxy.library.ualberta.ca/doi/abs/10.1146/annurev-biochem-060308-103103

Feuz, D. M., and W. J. Umberger. 2003. Beef cow-calf production. Veterinary Clinics: Food Animal Practice. 19:339–363. doi:10.1016/S0749-0720(03)00023-9. Available from: http://www.vetfood.theclinics.com/article/S0749072003000239/fulltext

Filella, X., and L. Foj. 2017. miRNAs as novel biomarkers in the management of prostate cancer. Clinical Chemistry and Laboratory Medicine (CCLM). 55:715–736. doi:10.1515/CCLM-2015-1073. Available from: https://www-degruyter-com.login.ezproxy.library.ualberta.ca/document/doi/10.1515/cclm-2015-1073/html

Filipowicz, W., S. N. Bhattacharyya, and N. Sonenberg. 2008. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nature Reviews Genetics 2008 9:2. 9:102–114. doi:10.1038/NRG2290. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/nrg2290

Flower, F. C., and D. M. Weary. 2006. Effect of Hoof Pathologies on Subjective Assessments of Dairy Cow Gait. Journal of Dairy Science. 89:139–146. doi:10.3168/JDS.S0022-0302(06)72077-X.

Focus on Herd Health: Hoof Health - Issuu. Available from: https://issuu.com/ontariobeefmagazine/docs/ontbeef_feb2020_final_lr/s/10255914

Forero, D. A., Y. González-Giraldo, L. J. Castro-Vega, and G. E. Barreto. 2019. qPCR-based methods for expression analysis of miRNAs. Biotechniques. 67:192–199. doi:10.2144/BTN-2019-0065/ASSET/IMAGES/LARGE/FIGURE4.JPEG. Available from: https://www.future-science.com/doi/abs/10.2144/btn-2019-0065

Futschik, M. E., and B. Carlisle. 2005. Noise-robust soft clustering of gene expression time-course data. J Bioinform Comput Biol. 3:965–988. doi:10.1142/S0219720005001375. Available from: https://pubmed.ncbi.nlm.nih.gov/16078370/

Garbarino, E. J., J. A. Hernandez, J. K. Shearer, C. A. Risco, and W. W. Thatcher. 2004. Effect of Lameness on Ovarian Activity in Postpartum Holstein Cows. Journal of Dairy Science. 87:4123–4131. doi:10.3168/JDS.S0022-0302(04)73555-9.

Gasparello, J., C. Papi, M. Allegretti, E. Giordani, F. Carboni, S. Zazza, E. Pescarmona, P. Romania, P. Giacomini, C. Scapoli, R. Gambari, and A. Finotti. 2020. A Distinctive microRNA (miRNA) Signature in the Blood of Colorectal Cancer (CRC) Patients at Surgery. Cancers 2020, Vol. 12, Page 2410. 12:2410. doi:10.3390/CANCERS12092410. Available from: https://www.mdpi.com/2072-6694/12/9/2410/htm

Giraldez, Maria D., R. M. Spengler, A. Etheridge, P. M. Godoy, A. J. Barczak, S. Srinivasan, P. L. de Hoff, K. Tanriverdi, A. Courtright, S. Lu, J. Khoory, R. Rubio, D. Baxter, T. A. P. Driedonks, H. P. J. Buermans, E. N. M. Nolte-‘T Hoen, H. Jiang, K. Wang, I. Ghiran, Y. E. Wang, K. van Keuren-Jensen, J. E. Freedman, P. G. Woodruff, L. C. Laurent, D. J. Erle, D. J. Galas, and M. Tewari. 2018. Comprehensive multi-center assessment of accuracy, reproducibility and bias of small RNA-seq methods for quantitative miRNA profiling. Nat Biotechnol. 36:746. doi:10.1038/NBT.4183. Available from: /pmc/articles/PMC6078798/

Giraldez, Maria D, R. M. Spengler, A. Etheridge, P. M. Godoy, A. J. Barczak, S. Srinivasan, P. L. de Hoff, K. Tanriverdi, A. Courtright, S. Lu, J. Khoory, R. Rubio, D. Baxter, T. A. P. Driedonks, H. P. J. Buermans, E. N. M. Nolte-’t Hoen, H. Jiang, K. Wang, I. Ghiran, Y. E. Wang, K. van Keuren-Jensen, J. E. Freedman, P. G. Woodruff, L. C. Laurent, D. J. Erle, D. J. Galas, and M. Tewari. 2018. Comprehensive multi-center assessment of small RNA-seq methods for quantitative miRNA profiling. Nature Biotechnology 2018 36:8. 36:746–757. doi:10.1038/NBT.4183. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/nbt.4183

Golan-Gerstl, R., Y. E. Shiff, V. Moshayoff, D. Schecter, D. Leshkowitz, and S. Reif. 2017. Characterization and biological function of milk-derived miRNAs. Molecular Nutrition & Food Research. 61:1700009. doi:10.1002/MNFR.201700009. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/mnfr.201700009

Gomez, A., N. B. Cook, N. D. Bernardoni, J. Rieman, A. F. Dusick, R. Hartshorn, M. T. Socha, D.

H. Read, and D. Döpfer. 2012. An experimental infection model to induce digital dermatitis infection in cattle. Journal of Dairy Science. 95:1821–1830. doi:10.3168/JDS.2011-4754.

Gozho, G. N., J. C. Plaizier, D. O. Krause, A. D. Kennedy, and K. M. Wittenberg. 2005. Subacute Ruminal Acidosis Induces Ruminal Lipopolysaccharide Endotoxin Release and Triggers an Inflammatory Response. Journal of Dairy Science. 88:1399–1403. doi:10.3168/JDS.S0022-0302(05)72807-1.

Grange, J. M., and M. D. Yates. 1994. Zoonotic aspects of Mycobacterium bovis infection. Veterinary Microbiology. 40:137–151. doi:10.1016/0378-1135(94)90052-3.

Green, L., Z. E. Barker, J. Scaife, J. Amory, and M. Speijers. 2006. RISK FACTORS ASSOCIATED WITH FOOT LAMENESS IN DAIRY CATTLE AND A SUGGESTED APPROACH FOR LAMENESS REDUCTION. undefined.

Greenwood, P. L. 2021. Review: An overview of beef production from pasture and feedlot globally, as demand for beef and the need for sustainable practices increase. Animal. 15:100295. doi:10.1016/J.ANIMAL.2021.100295.

Griffin, D. 2014. The monster we don't see: subclinical BRD in beef cattle. Animal Health Research Reviews. 15:138–141. doi:10.1017/S1466252314000255. Available from: https://www-cambridge-org.login.ezproxy.library.ualberta.ca/core/journals/animal-health-research-reviews/article/monster-we-dont-see-subclinical-brd-in-beef-cattle/013BE1899D30E2807F7B4D1C9530BF7A

Griffin, D., L. Perino, and D. Hudson. 1993a. G93-1159 Feedlot Lameness. Available from: https://digitalcommons.unl.edu/extensionhist

Griffin, D., L. Perino, and D. Hudson. 1993b. G93-1159 Feedlot Lameness. Available from: https://digitalcommons.unl.edu/extensionhist

Guo, H., N. T. Ingolia, J. S. Weissman, and D. P. Bartel. 2010. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 2010 466:7308. 466:835–840. doi:10.1038/nature09267. Available from: https://www.nature.com/articles/nature09267

Guo, H., R. Khan, S. H. A. Raza, S. M. Suhail, H. Khan, S. B. Khan, A. H. A. El-Aziz, and L. Zan.

2021. RNA-Seq Reveals Function of Bta-miR-149-5p in the Regulation of Bovine Adipocyte Differentiation. Animals 2021, Vol. 11, Page 1207. 11:1207. doi:10.3390/ANI11051207. Available from: https://www.mdpi.com/2076-2615/11/5/1207/htm

Guo, L., Q. Zhang, X. Ma, J. Wang, and T. Liang. 2017. miRNA and mRNA expression analysis reveals potential sex-biased miRNA expression. Scientific Reports 2017 7:1. 7:1–10. doi:10.1038/SREP39812. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/srep39812

Guo, X., and A. Guo. 2019. Profiling circulating microRNAs in serum of Fasciola gigantica-infected buffalo. Molecular and Biochemical Parasitology. 232:111201. doi:10.1016/J.MOLBIOPARA.2019.111201.

Guo, Y., X. Zhang, W. Huang, and X. Miao. 2017. Identification and characterization of differentially expressed miRNAs in subcutaneous adipose between Wagyu and Holstein cattle. Scientific Reports 2017 7:1. 7:1–10. doi:10.1038/SREP44026. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/srep44026

Gupta, S. K., P. H. Maclean, S. Ganesh, D. Shu, B. M. Buddle, D. N. Wedlock, and A. Heiser. 2018. Detection of microRNA in cattle serum and their potential use to diagnose severity of Johne's disease. Journal of Dairy Science. 101:10259–10270. doi:10.3168/JDS.2018-14785.

Haladjian, J., J. Haug, S. Nüske, and B. Bruegge. 2018. A Wearable Sensor System for Lameness Detection in Dairy Cattle. Multimodal Technologies and Interaction 2018, Vol. 2, Page 27. 2:27. doi:10.3390/MTI2020027. Available from: https://www.mdpi.com/2414-4088/2/2/27/htm

Hannon, S. J., S. A. Brault, S. J. G. Otto, P. S. Morley, T. A. McAllister, C. W. Booker, and S. P. Gow. 2020. Feedlot Cattle Antimicrobial Use Surveillance Network: A Canadian Journey. Frontiers in Veterinary Science. 7:968. doi:10.3389/FVETS.2020.596042/BIBTEX.

Hansen, E. P., H. Kringel, S. M. Thamsborg, A. Jex, and P. Nejsum. 2016. Profiling circulating miRNAs in serum from pigs infected with the porcine whipworm, Trichuris suis. Veterinary Parasitology. 223:30–33. doi:10.1016/J.VETPAR.2016.03.025.

Hao, D., Xiaogang Wang, Xiao Wang, B. Thomsen, Y. Yang, X. Lan, Y. Huang, and H. Chen.

2021a. MicroRNA bta-miR-365-3p inhibits proliferation but promotes differentiation of primary bovine myoblasts by targeting the activin A receptor type I. Journal of Animal Science and Biotechnology. 12:1–15. doi:10.1186/S40104-020-00528-0/TABLES/2. Available from: https://jasbsci-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s40104-020-00528-0

Hao, D., Xiaogang Wang, Xiao Wang, B. Thomsen, Y. Yang, X. Lan, Y. Huang, and H. Chen. 2021b. MicroRNA bta-miR-365-3p inhibits proliferation but promotes differentiation of primary bovine myoblasts by targeting the activin A receptor type I. Journal of Animal Science and Biotechnology. 12:1–15. doi:10.1186/S40104-020-00528-0/TABLES/2. Available from: https://jasbsci-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s40104-020-00528-0

Hao, N.-B., Y.-F. He, X.-Q. Li, K. Wang, and R.-L. Wang. 2017. The role of miRNA and lncRNA in gastric cancer. Oncotarget. 8:81572. doi:10.18632/ONCOTARGET.19197. Available from: /pmc/articles/PMC5655310/

Härdi-Landerer, M. C., A. C. Stoffel, S. Dürr, and A. Steiner. 2019. [Footbath as treatment of footrot in sheep. Current -situation on Swiss sheep farms]. Schweizer Archiv fur Tierheilkunde. 161:377–386. doi:10.17236/SAT00210. Available from: https://europepmc-org.login.ezproxy.library.ualberta.ca/article/med/31145680

van Hertem, T., S. Viazzi, M. Steensels, E. Maltz, A. Antler, V. Alchanatis, A. A. Schlageter-Tello, K. Lokhorst, E. C. B. Romanini, C. Bahr, D. Berckmans, and I. Halachmi. 2014. Automatic lameness detection based on consecutive 3D-video recordings. Biosystems Engineering. 119:108–116. doi:10.1016/J.BIOSYSTEMSENG.2014.01.009.

Holman, D. B., E. Timsit, S. Amat, D. W. Abbott, A. G. Buret, and T. W. Alexander. 2017. The nasopharyngeal microbiota of beef cattle before and after transport to a feedlot. BMC Microbiology. 17:1–12. doi:10.1186/S12866-017-0978-6/TABLES/1. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12866-017-0978-6

Holzhauer, M., C. Hardenberg, C. J. M. Bartels, and K. Frankena. 2006. Herd- and Cow-Level

Prevalence of Digital Dermatitis in The Netherlands and Associated Risk Factors. Journal of Dairy Science. 89:580–588. doi:10.3168/JDS.S0022-0302(06)72121-X.

Hu, H., Q. Jia, J. Xi, B. Zhou, and Z. Li. 2020. Integrated analysis of lncRNA, miRNA and mRNA reveals novel insights into the fertility regulation of large white sows. BMC Genomics 2020 21:1. 21:1–15. doi:10.1186/S12864-020-07055-2. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-020-07055-2

Huang, T. H., J. J. Uthe, S. M. D. Bearson, C. Y. Demirkale, D. Nettleton, S. Knetter, C. Christian, A. E. Ramer-Tait, M. J. Wannemuehler, and C. K. Tuggle. 2011. Distinct Peripheral Blood RNA Responses to Salmonella in Pigs Differing in Salmonella Shedding Levels: Intersection of IFNG, TLR and miRNA Pathways. PLOS ONE. 6:e28768. doi:10.1371/JOURNAL.PONE.0028768. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0028768

Huxley, J. N. 2013a. Impact of lameness and claw lesions in cows on health and production. Livestock Science. 156:64–70. doi:10.1016/J.LIVSCI.2013.06.012.

Huxley, J. N. 2013b. Impact of lameness and claw lesions in cows on health and production. Livestock Science. 156:64–70. doi:10.1016/J.LIVSCI.2013.06.012.

van Huyssteen, M., H. W. Barkema, S. Mason, and K. Orsel. 2020. Association between lameness risk assessment and lameness and foot lesion prevalence on dairy farms in Alberta, Canada. Journal of Dairy Science. 103:11750–11761. doi:10.3168/JDS.2019-17819.

Inagaki, Y., T. Ito, T. Kato, Y. Ono, and M. Sawa. 2018. Disseminated Cutaneous Infection of Mycobacterium colombiense in a Patient with Myelodysplastic Syndrome. Internal Medicine. 57:423. doi:10.2169/INTERNALMEDICINE.7890-16. Available from: /pmc/articles/PMC5827328/

Ioannidis, J., and F. X. Donadeu. 2016a. Circulating miRNA signatures of early pregnancy in cattle. BMC Genomics 2016 17:1. 17:1–12. doi:10.1186/S12864-016-2529-1. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-016-2529-1

Ioannidis, J., and F. X. Donadeu. 2016b. Circulating miRNA signatures of early pregnancy in cattle. BMC Genomics. 17:1–12. doi:10.1186/S12864-016-2529-1/FIGURES/6. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-016-2529-1

Ioannidis, J., and F. X. Donadeu. 2016c. Circulating miRNA signatures of early pregnancy in cattle. BMC Genomics. 17:1–12. doi:10.1186/S12864-016-2529-1/FIGURES/6. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-016-2529-1

Ioannidis, J., and F. X. Donadeu. 2018a. Comprehensive analysis of blood cells and plasma identifies tissue-specific miRNAs as potential novel circulating biomarkers in cattle. BMC Genomics. 19. doi:10.1186/S12864-018-4646-5. Available from: /pmc/articles/PMC5894187/

Ioannidis, J., and F. X. Donadeu. 2018b. Comprehensive analysis of blood cells and plasma identifies tissue-specific miRNAs as potential novel circulating biomarkers in cattle. BMC Genomics. 19:1–11. doi:10.1186/S12864-018-4646-5/FIGURES/5. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-018-4646-5

Ioannidis, J., E. Sánchez-Molano, A. Psifidi, F. X. Donadeu, and G. Banos. 2018. Association of plasma microRNA expression with age, genetic background and functional traits in dairy cattle. Scientific Reports 2018 8:1. 8:1–10. doi:10.1038/S41598-018-31099-W. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-31099-w

Iorio, M. v., M. Ferracin, C.-G. Liu, A. Veronese, R. Spizzo, S. Sabbioni, E. Magri, M. Pedriali, M. Fabbri, M. Campiglio, S. Ménard, J. P. Palazzo, A. Rosenberg, P. Musiani, S. Volinia, I. Nenci, G. A. Calin, P. Querzoli, M. Negrini, and C. M. Croce. 2005. MicroRNA Gene Expression Deregulation in Human Breast Cancer. Cancer Research. 65:7065–7070. doi:10.1158/0008-5472.CAN-05-1783. Available from: https://cancerres-aacrjournals-org.login.ezproxy.library.ualberta.ca/content/65/16/7065

Isabel Gessner, J. W. U. Fries, Veronika Brune, and Sanjay Mathur. 2021. Magnetic nanoparticle-

based amplification of microRNA detection in body fluids for early disease diagnosis. Journal of Materials Chemistry B. 9:9–22. doi:10.1039/D0TB02165B. Available from: https://pubs-rsc-org.login.ezproxy.library.ualberta.ca/en/content/articlehtml/2021/tb/d0tb02165b

Jacobs, C., K. Orsel, and H. W. Barkema. 2017. Prevalence of digital dermatitis in young stock in Alberta, Canada, using pen walks. Journal of Dairy Science. 100:9234–9244. doi:10.3168/JDS.2017-13044.

Jamaluddin, M. S., S. M. Weakley, L. Zhang, P. Kougias, P. H. Lin, Q. Yao, and C. Chen. 2014. miRNAs: roles and clinical applications in vascular disease. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.1586/erm.10.103. 11:79–89. doi:10.1586/ERM.10.103. Available from: https://www-tandfonline-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1586/erm.10.103

Jelinski, M., K. Fenton, T. Perrett, and C. Paetsch. 2016a. Epidemiology of toe tip necrosis syndrome (TTNS) of North American feedlot cattle. The Canadian Veterinary Journal. 57:829. Available from: /pmc/articles/PMC4944558/

Jelinski, M., K. Fenton, T. Perrett, and C. Paetsch. 2016b. Epidemiology of toe tip necrosis syndrome (TTNS) of North American feedlot cattle. The Canadian Veterinary Journal. 57:829. Available from: /pmc/articles/PMC4944558/

Jelinski, M., K. Fenton, T. Perrett, and C. Paetsch. 2016c. Epidemiology of toe tip necrosis syndrome (TTNS) of North American feedlot cattle. The Canadian Veterinary Journal. 57:829. Available from: /pmc/articles/PMC4944558/

Jelinski, M., K. Fenton, T. Perrett, and C. Paetsch. 2016d. Epidemiology of toe tip necrosis syndrome (TTNS) of North American feedlot cattle. The Canadian Veterinary Journal. 57:829. Available from: /pmc/articles/PMC4944558/

Jeon, J., E. Olkhov-Mitsel, H. Xie, C. Q. Yao, F. Zhao, S. Jahangiri, C. Cuizon, S. Scarcello, R. Jeyapala, J. D. Watson, M. Fraser, J. Ray, K. Commisso, A. Loblaw, N. E. Fleshner, R. G. Bristow, M. Downes, D. Vesprini, S. Liu, B. Bapat, and P. C. Boutros. 2020. Temporal Stability and Prognostic Biomarker Potential of the Prostate Cancer Urine miRNA Transcriptome. JNCI:

Journal of the National Cancer Institute. 112:247–255. doi:10.1093/JNCI/DJZ112. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/jnci/article/112/3/247/5510559

Jiang, L., and J. Zhu. 2020. Review of MiRNA-Disease Association Prediction. Current Protein & Peptide Science. 21:1044–1053. doi:10.2174/1389203721666200210102751.

Johansen, I., and R. Andreassen. 2014. Validation of miRNA genes suitable as reference genes in qPCR analyses of miRNA gene expression in Atlantic salmon (Salmo salar). BMC Research Notes. 7:1–9. doi:10.1186/1756-0500-7-945/FIGURES/1. Available from: https://bmcresnotes-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/1756-0500-7-945

John, B., A. J. Enright, A. Aravin, T. Tuschl, C. Sander, and D. S. Marks. 2004. Human MicroRNA Targets. PLOS Biology. 2:e363. doi:10.1371/JOURNAL.PBIO.0020363. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosbiology/article?id=10.1371/journal.pbio.0020363

Johnston, D., B. Earley, M. S. McCabe, J. Kim, J. F. Taylor, K. Lemon, M. McMenamy, C. Duffy, S. L. Cosby, and S. M. Waters. 2021. Elucidation of the Host Bronchial Lymph Node miRNA Transcriptome Response to Bovine Respiratory Syncytial Virus. Frontiers in Genetics. 12. doi:10.3389/FGENE.2021.633125/FULL. Available from: /pmc/articles/PMC8100685/

Johnston, J. D., D. J. R. Eichhorn, S. A. Kontulainen, S. D. Noble, and M. D. Jelinski. 2019a. Investigation of white line separation under load in bovine claws with and without toe-tip necrosis. American Journal of Veterinary Research. 80:736–742. doi:10.2460/AJVR.80.8.736. Available from: https://avmajournals-avma-org.login.ezproxy.library.ualberta.ca/view/journals/ajvr/80/8/ajvr.80.8.736.xml

Johnston, J. D., D. J. R. Eichhorn, S. A. Kontulainen, S. D. Noble, and M. D. Jelinski. 2019b. Investigation of white line separation under load in bovine claws with and without toe-tip necrosis. https://doi-org.login.ezproxy.library.ualberta.ca/10.2460/ajvr.80.8.736. 80:736–742. doi:10.2460/AJVR.80.8.736.

Jung, G., E. Hernández-Illán, L. Moreira, F. Balaguer, and A. Goel. 2020. Epigenetics of colorectal cancer: biomarker and therapeutic potential. Nature Reviews Gastroenterology & Hepatology

2020 17:2. 17:111–130. doi:10.1038/S41575-019-0230-Y. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41575-019-0230-y

Kara, N. K., A. Galic, and M. Koyuncu. 2011. Effects of stall type and bedding materials on lameness and hygiene score and effect of lameness on some reproductive problems in dairy cattle. http://www-tandfonline-com.login.ezproxy.library.ualberta.ca/action/journalInformation?show=aimsScope&journalCode=taar20#.VsXoziCLRhE. 39:334–338. doi:10.1080/09712119.2011.607890. Available from: https://www-tandfonline-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1080/09712119.2011.607890

Kausche, F., E. Robb, R. Bergman, and M. Eysker. 2003. A comprehensive review of ceftiofur sodium and hydrochloride formulations for treatment of acute bovine foot rot. Veterinary Therapeutics : Research in Applied Veterinary Medicine. 4:83–93. Available from: https://europepmc-org.login.ezproxy.library.ualberta.ca/article/med/12756639

Khan, R., S. H. A. Raza, Z. Junjvlieke, X. Wang, H. Wang, G. Cheng, C. Mei, I. Elsaeid Elnour, and L. Zan. 2020. Bta-miR-149-5p inhibits proliferation and differentiation of bovine adipocytes through targeting CRTCs at both transcriptional and posttranscriptional levels. Journal of Cellular Physiology. 235:5796–5810. doi:10.1002/JCP.29513. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/jcp.29513

Kosaka, N., H. Iguchi, and T. Ochiya. 2010. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Science. 101:2087–2092. doi:10.1111/J.1349-7006.2010.01650.X. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/j.1349-7006.2010.01650.x

Kozomara, A., M. Birgaoanu, and S. Griffiths-Jones. 2019a. miRBase: from microRNA sequences to function. Nucleic Acids Research. 47:D155–D162. doi:10.1093/NAR/GKY1141. Available from: https://academic.oup.com/nar/article/47/D1/D155/5179337

Kozomara, A., M. Birgaoanu, and S. Griffiths-Jones. 2019b. miRBase: from microRNA sequences to function. Nucleic Acids Research. 47:D155–D162. doi:10.1093/NAR/GKY1141. Available

from: https://academic.oup.com/nar/article/47/D1/D155/5179337

Krishnan, H., J. Rayes, T. Miyashita, G. Ishii, E. P. Retzbach, S. A. Sheehan, A. Takemoto, Y.-W. Chang, K. Yoneda, J. Asai, L. Jensen, L. Chalise, A. Natsume, and G. S. Goldberg. 2018. Podoplanin: An emerging cancer biomarker and therapeutic target. Cancer Science. 109:1292–1299. doi:10.1111/CAS.13580. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/cas.13580

Kumar, P., Z. Dezso, C. MacKenzie, J. Oestreicher, S. Agoulnik, M. Byrne, F. Bernier, M. Yanagimachi, K. Aoshima, and Y. Oda. 2013a. Circulating miRNA Biomarkers for Alzheimer's Disease. PLOS ONE. 8:e69807. doi:10.1371/JOURNAL.PONE.0069807. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0069807

Kumar, P., Z. Dezso, C. MacKenzie, J. Oestreicher, S. Agoulnik, M. Byrne, F. Bernier, M. Yanagimachi, K. Aoshima, and Y. Oda. 2013b. Circulating miRNA Biomarkers for Alzheimer's Disease. PLOS ONE. 8:e69807. doi:10.1371/JOURNAL.PONE.0069807. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0069807

Kumar, R., H. A. Lardner, J. J. McKinnon, D. A. Christensen, D. Damiran, and K. Larson. 2012. Comparison of alternative backgrounding systems on beef calf performance, feedlot finishing performance, carcass traits, and system cost of gain. The Professional Animal Scientist. 28:541–551. doi:10.15232/S1080-7446(15)30403-4.

Kumar, R., B. R. Yadav, and R. S. Singh. Antibiotic resistance and pathogenicity factors in Staphylococcus aureus isolated from mastitic Sahiwal cattle. J. Biosci. 36:175–188. doi:10.1007/s12038-011-9004-6. Available from: http://www.ias.ac.in/jbiosci

Kupsco, A., D. Prada, D. Valvi, L. Hu, M. S. Petersen, B. Coull, P. Grandjean, P. Weihe, and A. A. Baccarelli. 2021. Human milk extracellular vesicle miRNA expression and associations with maternal characteristics in a population-based cohort from the Faroe Islands. Scientific Reports 2021 11:1. 11:1–17. doi:10.1038/S41598-021-84809-2. Available from: https://www-nature-

com.login.ezproxy.library.ualberta.ca/articles/s41598-021-84809-2

Kutwin, P., T. Konecki, E. M. Borkowska, M. Traczyk-Borszyńska, and Z. Jabłonowski. 2018. Urine miRNA as a potential biomarker for bladder cancer detection – a meta-analysis. Central European Journal of Urology. 71:177. doi:10.5173/CEJU.2018.1605. Available from: /pmc/articles/PMC6051360/

Ladomery, M., and □□□ G Dellaire#. 2002. Multifunctional zinc finger proteins in development and disease. Annals of Human Genetics. 66:331–342. doi:10.1017/S0003480002001215. Available from: https://www-cambridge-org.login.ezproxy.library.ualberta.ca/core/journals/annals-of-human-genetics/article/multifunctional-zinc-finger-proteins-in-development-and-disease/4FF9EA3338E54D0B61942E0B0D5B5EAE

Ladomery, M. R., D. G. Maddocks, and I. D. Wilson. 2011. MicroRNAs: their discovery, biogenesis, function and potential use as biomarkers in non-invasive prenatal diagnostics. International Journal of Molecular Epidemiology and Genetics. 2:253. Available from: /pmc/articles/PMC3166153/

Lau, P., K. Bossers, R. Janky, E. Salta, C. S. Frigerio, S. Barbash, R. Rothman, A. S. R. Sierksma, A. Thathiah, D. Greenberg, A. S. Papadopoulou, T. Achsel, T. Ayoubi, H. Soreq, J. Verhaagen, D. F. Swaab, S. Aerts, and B. de Strooper. 2013. Alteration of the microRNA network during the progression of Alzheimer's disease. EMBO Molecular Medicine. 5:1613–1634. doi:10.1002/EMMM.201201974. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/emmm.201201974

Laven, R. A., and D. N. Logue. 2006. Treatment strategies for digital dermatitis for the UK. The Veterinary Journal. 171:79–88. doi:10.1016/J.TVJL.2004.08.009.

Lawless, N., A. B. K. Foroushani, M. S. McCabe, C. O'Farrelly, and D. J. Lynn. 2013a. Next Generation Sequencing Reveals the Expression of a Unique miRNA Profile in Response to a Gram-Positive Bacterial Infection. PLOS ONE. 8:e57543. doi:10.1371/JOURNAL.PONE.0057543. Available from: https://journals-plos-

org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0057543

Lawless, N., A. B. K. Foroushani, M. S. McCabe, C. O'Farrelly, and D. J. Lynn. 2013b. Next Generation Sequencing Reveals the Expression of a Unique miRNA Profile in Response to a Gram-Positive Bacterial Infection. PLOS ONE. 8:e57543. doi:10.1371/JOURNAL.PONE.0057543. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0057543

Lawless, N., A. B. K. Foroushani, M. S. McCabe, C. O'Farrelly, and D. J. Lynn. 2013c. Next Generation Sequencing Reveals the Expression of a Unique miRNA Profile in Response to a Gram-Positive Bacterial Infection. PLOS ONE. 8:e57543. doi:10.1371/JOURNAL.PONE.0057543. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0057543

Lean, I. J., C. T. Westwood, H. M. Golder, and J. J. Vermunt. 2013a. Impact of nutrition on lameness and claw health in cattle. Livestock Science. 156:71–87. doi:10.1016/J.LIVSCI.2013.06.006.

Lean, I. J., C. T. Westwood, H. M. Golder, and J. J. Vermunt. 2013b. Impact of nutrition on lameness and claw health in cattle. Livestock Science. 156:71–87. doi:10.1016/J.LIVSCI.2013.06.006.

Leão, R., T. van Agthoven, A. Figueiredo, M. A. S. Jewett, K. Fadaak, J. Sweet, A. E. Ahmad, L. Anson-Cartwright, P. Chung, A. Hansen, P. Warde, P. Castelo-Branco, M. O'Malley, P. L. Bedard, L. H. J. Looijenga, and R. J. Hamilton. 2018. Serum miRNA Predicts Viable Disease after Chemotherapy in Patients with Testicular Nonseminoma Germ Cell Tumor. Journal of Urology. 200:126–135. doi:10.1016/J.JURO.2018.02.068.

Lee, J., Soohyun Lee, J. Son, H. Lim, E. Kim, D. Kim, S. Ha, T. Hur, Seunghwan Lee, and I. Choi. 2020a. Analysis of circulating-microRNA expression in lactating Holstein cows under summer heat stress. PLOS ONE. 15:e0231125. doi:10.1371/JOURNAL.PONE.0231125. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0231125

Lee, J., Soohyun Lee, J. Son, H. Lim, E. Kim, D. Kim, S. Ha, T. Hur, Seunghwan Lee, and I. Choi. 2020b. Analysis of circulating-microRNA expression in lactating Holstein cows under summer heat stress. PLOS ONE. 15:e0231125. doi:10.1371/JOURNAL.PONE.0231125. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0231125

Lee, J., Soohyun Lee, J. Son, H. Lim, E. Kim, D. Kim, S. Ha, T. Hur, Seunghwan Lee, and I. Choi. 2020c. Analysis of circulating-microRNA expression in lactating Holstein cows under summer heat stress. PLOS ONE. 15:e0231125. doi:10.1371/JOURNAL.PONE.0231125. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0231125

Lee, R. C., R. L. Feinbaum, and V. Ambros. 1993. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 75:843–854. doi:10.1016/0092-8674(93)90529-Y.

Lee, T., K. Good, W. Jamieson, M. Quinn, and A. Krishnamurthy. 2017. Cattle and Carnivore Coexistence in Alberta: The Role of Compensation Programs. Rangelands. 39:10–16. doi:10.1016/J.RALA.2016.11.002.

Leidinger, P., C. Backes, S. Deutscher, K. Schmitt, S. C. Mueller, K. Frese, J. Haas, K. Ruprecht, F. Paul, C. Stähler, C. J. Lang, B. Meder, T. Bartfai, E. Meese, and A. Keller. 2013. A blood based 12-miRNA signature of Alzheimer disease patients. Genome Biology 2013 14:7. 14:1–16. doi:10.1186/GB-2013-14-7-R78. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/articles/10.1186/gb-2013-14-7-r78

Lekchnov, E. A., E. v. Amelina, O. E. Bryzgunova, I. A. Zaporozhchenko, M. Yu. Konoshenko, S. v. Yarmoschuk, I. S. Murashov, O. A. Pashkovskaya, A. M. Gorizkii, A. A. Zheravin, and P. P. Laktionov. 2018. Searching for the Novel Specific Predictors of Prostate Cancer in Urine: The Analysis of 84 miRNA Expression. International Journal of Molecular Sciences 2018, Vol. 19, Page 4088. 19:4088. doi:10.3390/IJMS19124088. Available from: https://www.mdpi.com/1422-0067/19/12/4088/htm

Li, J., L. Mao, W. Li, F. Hao, C. Zhong, X. Zhu, X. Ji, L. Yang, W. Zhang, M. Liu, and J. Jiang. 2018. Analysis of microRNAs expression profiles in madin-darby bovine kidney cells infected with caprine parainfluenza virus type 3. Frontiers in Cellular and Infection Microbiology. 8:93. doi:10.3389/FCIMB.2018.00093/BIBTEX.

Li, Q., C. Yang, J. Du, B. Zhang, Y. He, Q. Hu, M. Li, Y. Zhang, C. Wang, and J. Zhong. 2018a. Characterization of miRNA profiles in the mammary tissue of dairy cattle in response to heat stress. BMC Genomics. 19:1–11. doi:10.1186/S12864-018-5298-1/TABLES/5. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-018-5298-1

Li, Q., C. Yang, J. Du, B. Zhang, Y. He, Q. Hu, M. Li, Y. Zhang, C. Wang, and J. Zhong. 2018b. Characterization of miRNA profiles in the mammary tissue of dairy cattle in response to heat stress. BMC Genomics 2018 19:1. 19:1–11. doi:10.1186/S12864-018-5298-1. Available from: https://link.springer.com/articles/10.1186/s12864-018-5298-1

Li, R., C. L. Zhang, X. X. Liao, D. Chen, W. Q. Wang, Y. H. Zhu, X. H. Geng, D. J. Ji, Y. J. Mao, Y. C. Gong, and Z. P. Yang. 2015a. Transcriptome MicroRNA Profiling of Bovine Mammary Glands Infected with Staphylococcus aureus. International Journal of Molecular Sciences 2015, Vol. 16, Pages 4997-5013. 16:4997–5013. doi:10.3390/IJMS16034997. Available from: https://www.mdpi.com/1422-0067/16/3/4997/htm

Li, R., C. L. Zhang, X. X. Liao, D. Chen, W. Q. Wang, Y. H. Zhu, X. H. Geng, D. J. Ji, Y. J. Mao, Y. C. Gong, and Z. P. Yang. 2015b. Transcriptome MicroRNA Profiling of Bovine Mammary Glands Infected with Staphylococcus aureus. International Journal of Molecular Sciences 2015, Vol. 16, Pages 4997-5013. 16:4997–5013. doi:10.3390/IJMS16034997. Available from: https://www.mdpi.com/1422-0067/16/3/4997/htm

Li, W., E. O'hara, H. Sun, K. S. Schwartzkopf-Genswein, L. L. Guan, L. Li, X. Liu, L. Jiang, H. Yu, S. Nie, M. Xie, and J. Gong. 57 President Oral Presentation Pick: The exploration of blood microRNA profiling in feedlot cattle with different lameness phenotypes. J. Anim. Sci. 98. Available from: https://academic.oup.com/jas/article/98/Supplement_4/40/6011538

Liang, Y., D. Ridzon, L. Wong, and C. Chen. 2007. Characterization of microRNA expression

profiles in normal human tissues. BMC Genomics 2007 8:1. 8:1–20. doi:10.1186/1471-2164-8-166. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/articles/10.1186/1471-2164-8-166

Lin, X., K. C. Pavani, K. Smits, D. Deforce, B. Heindryckx, A. van Soom, and L. Peelman. 2019. Bta-miR-10b Secreted by Bovine Embryos Negatively Impacts Preimplantation Embryo Quality. Frontiers in Genetics. 10:757. doi:10.3389/FGENE.2019.00757/BIBTEX.

Lodewijk, L., A. M. Prins, J. W. Kist, G. D. Valk, O. Kranenburg, I. H. M. B. Rinkes, and M. R. Vriens. 2012. The value of miRNA in diagnosing thyroid cancer: A systematic review. Cancer Biomarkers. 11:229–238. doi:10.3233/CBM-2012-0273.

Logue, D. N., T. Gibert, T. Parkin, S. Thomson, and D. J. Taylor. 2012. A field evaluation of a footbathing solution for the control of digital dermatitis in cattle. The Veterinary Journal. 193:664–668. doi:10.1016/J.TVJL.2012.06.050.

Love, M. I., W. Huber, and S. Anders. 2014a. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology. 15:1–21. doi:10.1186/S13059-014-0550-8/FIGURES/9. Available from: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-014-0550-8

Love, M. I., W. Huber, and S. Anders. 2014b. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology. 15:1–21. doi:10.1186/S13059-014-0550-8/FIGURES/9. Available from: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-014-0550-8

Love, M. I., W. Huber, and S. Anders. 2014c. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology. 15:1–21. doi:10.1186/S13059-014-0550-8/FIGURES/9. Available from: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-014-0550-8

Luoreng, Z. M., X. P. Wang, C. G. Mei, and L. sen Zan. 2018a. Expression profiling of peripheral blood miRNA using RNAseq technology in dairy cows with Escherichia coli-induced mastitis. Scientific Reports 2018 8:1. 8:1–10. doi:10.1038/S41598-018-30518-2. Available from:

https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-30518-2

Luoreng, Z. M., X. P. Wang, C. G. Mei, and L. sen Zan. 2018b. Comparison of microRNA Profiles between Bovine Mammary Glands Infected with Staphylococcus aureus and Escherichia coli. International Journal of Biological Sciences. 14:87. doi:10.7150/IJBS.22498. Available from: /pmc/articles/PMC5821052/

Luoreng, Z.-M., X.-P. Wang, C.-G. Mei, and L.-S. Zan. 2018. Expression profiling of peripheral blood miRNA using RNAseq technology in dairy cows with Escherichia coli-induced mastitis. Scientific Reports 2018 8:1. 8:1–10. doi:10.1038/S41598-018-30518-2. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-30518-2

Ma, S., C. Tong, E. M. Ibeagha-Awemu, and X. Zhao. 2019. Identification and characterization of differentially expressed exosomal microRNAs in bovine milk infected with Staphylococcus aureus. BMC Genomics. 20:1–13. doi:10.1186/S12864-019-6338-1/FIGURES/5. Available from: https://bmcgenomics-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12864-019-6338-1

MacFarlane, L.-A., and P. R. Murphy. 2010a. MicroRNA: Biogenesis, Function and Role in Cancer. Current Genomics. 11:537. doi:10.2174/138920210793175895. Available from: /pmc/articles/PMC3048316/

MacFarlane, L.-A., and P. R. Murphy. 2010b. MicroRNA: Biogenesis, Function and Role in Cancer. Current Genomics. 11:537. doi:10.2174/138920210793175895. Available from: /pmc/articles/PMC3048316/

Maertens, W., J. Vangeyte, J. Baert, A. Jantuan, K. C. Mertens, S. de Campeneere, A. Pluk, G. Opsomer, S. van Weyenberg, and A. van Nuffel. 2011. Development of a real time cow gait tracking and analysing tool to assess lameness using a pressure sensitive walkway: The GAITWISE system. Biosystems Engineering. 110:29–39. doi:10.1016/J.BIOSYSTEMSENG.2011.06.003.

Manson, F. J., and J. D. Leaver. 1988. The influence of concentrate amount on locomotion and clinical lameness in dairy cattle. Animal Science. 47:185–190. doi:10.1017/S0003356100003251.

Available from: https://www.cambridge.org/core/journals/animal-science/article/abs/influence-of-concentrate-amount-on-locomotion-and-clinical-lameness-in-dairy-cattle/983225C537C45191FD0EDBA758972761

Marini, F., E. Luzi, and M. L. Brandi. 2011. MicroRNA role in thyroid cancer development. Journal of Thyroid Research. 2011. doi:10.4061/2011/407123.

Marti, S., M. D. Jelinski, E. D. Janzen, M. J. Jelinski, C. L. Dorin, K. Orsel, E. A. Pajor, J. Shearer, S. T. Millman, and K. S. Schwartzkopf-Genswein. 2021. A prospective longitudinal study of risk factors associated with cattle lameness in southern alberta feedlots. Canadian Journal of Animal Science. 101:647–654. doi:10.1139/CJAS-2020-0128/ASSET/IMAGES/CJAS-2020-0128TAB3.GIF. Available from: https://cdnsciencepub-com.login.ezproxy.library.ualberta.ca/doi/abs/10.1139/cjas-2020-0128

Masaki, T., S. Chiba, H. Tatsukawa, T. Yasuda, H. Noguchi, M. Seike, and H. Yoshimatsu. 2004. Adiponectin protects LPS-induced liver injury through modulation of TNF-α in KK-Ay obese mice. Hepatology. 40:177–184. doi:10.1002/HEP.20282. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/hep.20282

Mayes, C., R. Houston, S. Seashols-Williams, B. LaRue, and S. Hughes-Stamm. 2019. The stability and persistence of blood and semen mRNA and miRNA targets for body fluid identification in environmentally challenged and laundered samples. Legal Medicine. 38:45–50. doi:10.1016/J.LEGALMED.2019.03.007.

McDaneld, T. G. 2009. MicroRNA: Mechanism of gene regulation and application to livestock. Journal of Animal Science. 87:E21–E28. doi:10.2527/JAS.2008-1303. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/jas/article/87/suppl_14/E21/4731144

McGuire, A., J. A. L. Brown, and M. J. Kerin. 2015a. Metastatic breast cancer: the potential of miRNA for diagnosis and treatment monitoring. Cancer and Metastasis Reviews 2015 34:1. 34:145–155. doi:10.1007/S10555-015-9551-7. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s10555-015-9551-7

McGuire, A., J. A. L. Brown, and M. J. Kerin. 2015b. Metastatic breast cancer: the potential of

miRNA for diagnosis and treatment monitoring. Cancer and Metastasis Reviews 2015 34:1. 34:145–155. doi:10.1007/S10555-015-9551-7. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s10555-015-9551-7

McIver, S. C., S. D. Roman, B. Nixon, and E. A. McLaughlin. 2012. miRNA and mammalian male germ cells. Human Reproduction Update. 18:44–59. doi:10.1093/HUMUPD/DMR041. Available from: https://academic-oup-com.login.ezproxy.library.ualberta.ca/humupd/article/18/1/44/855033

McMillan, S., G. Penner, J. McKinnon, K. Larson, F. Añez-Osuna, D. Damiran, and H. Lardner. 2018. Use of extensive winter feeding systems for backgrounding beef calves and the effect on finishing. doi:10.15232/pas.2017-01614. Available from: https://doi.org/10.15232/pas.2017-01614

Meister, G., M. Landthaler, A. Patkaniowska, Y. Dorsett, G. Teng, and T. Tuschl. 2004. Human Argonaute2 Mediates RNA Cleavage Targeted by miRNAs and siRNAs. Molecular Cell. 15:185–197. doi:10.1016/J.MOLCEL.2004.07.007.

Menjivar, N. G., S. Gebremedhn, D. Tesfaye, N. G. Menjivar, S. Gebremedhn, and D. Tesfaye. 2021. 108 Preferential loading of thermal stress-associated microRNAs into extracellular vesicles: attempt to mitigate effects of heat stress in bovine granulosa cells. Reproduction, Fertility and Development. 34:291–291. doi:10.1071/RDV34N2AB108. Available from: https://www-publish-csiro-au.login.ezproxy.library.ualberta.ca/rd/RDv34n2Ab108

Mercadal, M., C. Herrero, O. López-Rodrigo, M. Castells, A. de la Fuente, F. Vigués, L. Bassas, and S. Larriba. 2020. Impact of Extracellular Vesicle Isolation Methods on Downstream miRNA Analysis in Semen: A Comparative Study. International Journal of Molecular Sciences 2020, Vol. 21, Page 5949. 21:5949. doi:10.3390/IJMS21175949. Available from: https://www.mdpi.com/1422-0067/21/17/5949/htm

van Metre, D. C. 2017. Pathogenesis and Treatment of Bovine Foot Rot. Veterinary Clinics of North America: Food Animal Practice. 33:183–194. doi:10.1016/J.CVFA.2017.02.003.

Meyer, S. U., M. W. Pfaffl, and S. E. Ulbrich. 2010. Normalization strategies for microRNA profiling experiments: a ‘normal’ way to a hidden layer of complexity? Biotechnology Letters

2010 32:12. 32:1777–1788. doi:10.1007/S10529-010-0380-Z. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s10529-010-0380-z

Miretti, S., C. Lecchi, F. Ceciliani, and M. Baratta. 2020. MicroRNAs as Biomarkers for Animal Health and Welfare in Livestock. Frontiers in Veterinary Science. 7:985. doi:10.3389/FVETS.2020.578193/BIBTEX.

Mitchelson, K. R., and W.-Y. Qin. 2015. Roles of the canonical myomiRs miR-1, -133 and -206 in cell development and disease. World Journal of Biological Chemistry. 6:162. doi:10.4331/WJBC.V6.I3.162. Available from: /pmc/articles/PMC4549760/

Montani, F., and F. Bianchi. 2016. Circulating Cancer Biomarkers: The Macro-revolution of the Micro-RNA. EBioMedicine. 5:4–6. doi:10.1016/J.EBIOM.2016.02.038.

Mulrane, L., S. F. McGee, W. M. Gallagher, and D. P. O'Connor. 2013a. miRNA Dysregulation in Breast Cancer. Cancer Research. 73:6554–6562. doi:10.1158/0008-5472.CAN-13-1841. Available from: https://cancerres-aacrjournals-org.login.ezproxy.library.ualberta.ca/content/73/22/6554

Mulrane, L., S. F. McGee, W. M. Gallagher, and D. P. O'Connor. 2013b. miRNA Dysregulation in Breast Cancer. Cancer Research. 73:6554–6562. doi:10.1158/0008-5472.CAN-13-1841. Available from: https://cancerres-aacrjournals-org.login.ezproxy.library.ualberta.ca/content/73/22/6554

Murakami, Y., H. Toyoda, T. Tanahashi, J. Tanaka, T. Kumada, Y. Yoshioka, N. Kosaka, T. Ochiya, and Y. Taguchi. 2012. Comprehensive miRNA Expression Analysis in Peripheral Blood Can Diagnose Liver Disease. PLOS ONE. 7:e48366. doi:10.1371/JOURNAL.PONE.0048366. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0048366

Nagandla, H., S. Lopez, W. Yu, T. L. Rasmussen, H. O. Tucker, R. J. Schwartz, and M. D. Stewart. 2016. Defective myogenesis in the absence of the muscle-specific lysine methyltransferase SMYD1. Developmental Biology. 410:86–97. doi:10.1016/J.YDBIO.2015.12.005.

Nagaraja, T. G., and K. F. Lechtenberg. 2007. Acidosis in Feedlot Cattle. Veterinary Clinics of North America: Food Animal Practice. 23:333–350. doi:10.1016/J.CVFA.2007.04.002.

Nagl, S., M. Schaeferling, and O. S. Wolfbeis. 2005. Fluorescence Analysis in Microarray

Technology. Microchimica Acta 2005 151:1. 151:1–21. doi:10.1007/S00604-005-0393-9. Available from: https://link.springer.com/article/10.1007/s00604-005-0393-9

Nordlund, K. v., N. B. Cook, and G. R. Oetzel. 2004. Investigation Strategies for Laminitis Problem Herds. Journal of Dairy Science. 87:E27–E35. doi:10.3168/JDS.S0022-0302(04)70058-2.

Norring, M., J. Häggman, H. Simojoki, P. Tamminen, C. Winckler, and M. Pastell. 2014a. Short communication: Lameness impairs feeding behavior of dairy cows. Journal of Dairy Science. 97:4317–4321. doi:10.3168/JDS.2013-7512.

Norring, M., J. Häggman, H. Simojoki, P. Tamminen, C. Winckler, and M. Pastell. 2014b. Short communication: Lameness impairs feeding behavior of dairy cows. Journal of Dairy Science. 97:4317–4321. doi:10.3168/JDS.2013-7512.

Norring, M., J. Häggman, H. Simojoki, P. Tamminen, C. Winckler, and M. Pastell. 2014c. Short communication: Lameness impairs feeding behavior of dairy cows. Journal of Dairy Science. 97:4317–4321. doi:10.3168/JDS.2013-7512.

Norsworthy, P. J., A. G. B. Thompson, T. H. Mok, F. Guntoro, L. C. Dabin, A. Nihat, R. W. Paterson, J. M. Schott, J. Collinge, S. Mead, and E. A. Viré. 2020. A blood miRNA signature associates with sporadic Creutzfeldt-Jakob disease diagnosis. Nature Communications 2020 11:1. 11:1–11. doi:10.1038/S41467-020-17655-X. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41467-020-17655-x

van Nuffel, A., W. Saeys, B. Sonck, J. Vangeyte, K. C. Mertens, B. de Ketelaere, and S. van Weyenberg. 2015. Variables of gait inconsistency outperform basic gait variables in detecting mildly lame cows. Livestock Science. 177:125–131. doi:10.1016/J.LIVSCI.2015.04.008.

van Nuffel, A., J. Vangeyte, K. C. Mertens, L. Pluym, S. de Campeneere, W. Saeys, G. Opsomer, and S. van Weyenberg. 2013. Exploration of measurement variation of gait variables for early lameness detection in cattle using the GAITWISE. Livestock Science. 156:88–95. doi:10.1016/J.LIVSCI.2013.06.013.

Nuffel, A. van, I. Zwertvaegher, L. Pluym, S. van Weyenberg, V. M. Thorup, M. Pastell, B. Sonck,

and W. Saeys. 2015. Lameness Detection in Dairy Cows: Part 1. How to Distinguish between Non-Lame and Lame Cows Based on Differences in Locomotion or Behavior. Animals 2015, Vol. 5, Pages 838-860. 5:838–860. doi:10.3390/ANI5030387. Available from: https://www.mdpi.com/2076-2615/5/3/387/htm

O'Brien, J., H. Hayder, Y. Zayed, and C. Peng. 2018. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Frontiers in Endocrinology. 9:402. doi:10.3389/FENDO.2018.00402/BIBTEX.

of Alberta – Agriculture, G., F. Economics, and C. Branch. 2020. July 1 2020 livestock inventory estimates : Alberta/Canada highlights.

Okamura, K., A. Ishizuka, H. Siomi, and M. C. Siomi. 2004. Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways. Genes & Development. 18:1655–1666. doi:10.1101/GAD.1210204. Available from: http://genesdev.cshlp.org.login.ezproxy.library.ualberta.ca/content/18/14/1655.full

O'Leary, N. W., D. T. Byrne, A. H. O'Connor, and L. Shalloo. 2020a. Invited review: Cattle lameness detection with accelerometers. Journal of Dairy Science. 103:3895–3911. doi:10.3168/JDS.2019-17123.

O'Leary, N. W., D. T. Byrne, A. H. O'Connor, and L. Shalloo. 2020b. Invited review: Cattle lameness detection with accelerometers. Journal of Dairy Science. 103:3895–3911. doi:10.3168/JDS.2019-17123.

de Oliveira, P. S. N., L. L. Coutinho, P. C. Tizioto, A. S. M. Cesar, G. B. de Oliveira, W. J. da S. Diniz, A. O. de Lima, J. M. Reecy, G. B. Mourão, A. Zerlotini, and L. C. A. Regitano. 2018a. An integrative transcriptome analysis indicates regulatory mRNA-miRNA networks for residual feed intake in Nelore cattle. Scientific Reports 2018 8:1. 8:1–12. doi:10.1038/S41598-018-35315-5. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-35315-5

de Oliveira, P. S. N., L. L. Coutinho, P. C. Tizioto, A. S. M. Cesar, G. B. de Oliveira, W. J. da S. Diniz, A. O. de Lima, J. M. Reecy, G. B. Mourão, A. Zerlotini, and L. C. A. Regitano. 2018b. An

integrative transcriptome analysis indicates regulatory mRNA-miRNA networks for residual feed intake in Nelore cattle. Scientific Reports 2018 8:1. 8:1–12. doi:10.1038/S41598-018-35315-5. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-35315-5

Özdemir, S. 2020. Identification and comparison of exosomal microRNAs in the milk and colostrum of two different cow breeds. Gene. 743:144609. doi:10.1016/J.GENE.2020.144609.

Paetsch, C., K. Fenton, T. Perrett, E. Janzen, T. Clark, J. Shearer, and M. Jelinski. 2017. Prospective case-control study of toe tip necrosis syndrome (TTNS) in western Canadian feedlot cattle. The Canadian Veterinary Journal. 58:247. Available from: /pmc/articles/PMC5302198/

Pedersen, I. M., G. Cheng, S. Wieland, S. Volinia, C. M. Croce, F. v. Chisari, and M. David. 2007. Interferon modulation of cellular microRNAs as an antiviral mechanism. Nature 2007 449:7164. 449:919–922. doi:10.1038/nature06205. Available from: https://www.nature.com/articles/nature06205

Penny, C., S. Bradley, and D. Wilson. 2017. Lameness due to toe-tip necrosis syndrome in beef calves. Veterinary Record. 180:154–154. doi:10.1136/VR.J678. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1136/vr.j678

Peterson, E. B., D. R. Strohbehn, G. W. Ladd, and R. L. Willham. 1989. Effects of Preconditioning on Performance of Beef Calves before and after Entering the Feedlot. Journal of Animal Science. 67:1678–1686. doi:10.2527/JAS1989.6771678X. Available from: https://academic.oup.com/jas/article/67/7/1678/4696962

Phillips, C. 2007. Cattle Behaviour and Welfare: Second Edition. Cattle Behaviour and Welfare: Second Edition. 1–264. doi:10.1002/9780470752418. Available from: https://onlinelibrary.wiley.com/doi/book/10.1002/9780470752418

Plummer, P. J., and A. Krull. 2017. Clinical Perspectives of Digital Dermatitis in Dairy and Beef Cattle. Veterinary Clinics of North America: Food Animal Practice. 33:165–181. doi:10.1016/J.CVFA.2017.02.002.

Pogue, S. J., R. Kröbel, H. H. Janzen, K. A. Beauchemin, G. Legesse, D. M. de Souza, M. Iravani,

C. Selin, J. Byrne, and T. A. McAllister. 2018. Beef production and ecosystem services in Canada's prairie provinces: A review. Agricultural Systems. 166:152–172. doi:10.1016/J.AGSY.2018.06.011.

Poursaberi, A., C. Bahr, A. Pluk, D. Berckmans, I. Veermäe, E. Kokin, and V. Pokalainen. 2011. Online lameness detection in dairy cattle using Body Movement Pattern (BMP). International Conference on Intelligent Systems Design and Applications, ISDA. 732–736. doi:10.1109/ISDA.2011.6121743.

Pradervand, S., J. Weber, F. Lemoine, F. Consales, A. Paillusson, M. Dupasquier, J. Thomas, H. Richter, H. Kaessmann, E. Beaudoing, O. Hagenbüchle, and K. Harshman. 2018. Concordance among digital gene expression, microarrays, and qPCR when measuring differential expression of microRNAs. https://doi.org/10.2144/000113367. 48:219–222. doi:10.2144/000113367. Available from: https://www.future-science.com/doi/abs/10.2144/000113367

Prastiwi, A., Z. Okumuş, A. Hayirli, D. Çelebi, L. E. Yanmaz, E. Doğan, M. G. Şenocak, and U. Ersoz. 2019. Effectiveness of Copper Sulfate for Footbath Solutions in Dairy Cattle. Atatürk University Journal of Veterinary Sciences. 14:238–245. doi:10.17094/ATAUNIVBD.538342. Available from: https://dergipark.org.tr/en/pub/ataunivbd/538342

Qadir, M. I., and A. Faheem. 2017. miRNA: A Diagnostic and Therapeutic Tool for Pancreatic Cancer. Critical Reviews™ in Eukaryotic Gene Expression. 27:197–204. doi:10.1615/CRITREVEUKARYOTGENEEXPR.2017019494. Available from: https://www-dl-begellhouse-com.login.ezproxy.library.ualberta.ca/journals/6dbf508d3b17c437,3e1db9ff199ae23c,4c2b35b40e0bd753.html

Qiao, Y., H. Kong, C. Clark, S. Lomax, D. Su, S. Eiffert, and S. Sukkarieh. 2021a. Intelligent Perception-Based Cattle Lameness Detection and Behaviour Recognition: A Review. Animals 2021, Vol. 11, Page 3033. 11:3033. doi:10.3390/ANI11113033. Available from: https://www.mdpi.com/2076-2615/11/11/3033/htm

Qiao, Y., H. Kong, C. Clark, S. Lomax, D. Su, S. Eiffert, and S. Sukkarieh. 2021b. Intelligent

Perception-Based Cattle Lameness Detection and Behaviour Recognition: A Review. Animals 2021, Vol. 11, Page 3033. 11:3033. doi:10.3390/ANI11113033. Available from: https://www.mdpi.com/2076-2615/11/11/3033/htm

Qiu, J., W. Zhang, C. Zang, X. Liu, F. Liu, R. Ge, Y. Sun, Q. Xia, J. Qiu, W. Zhang, C. Zang, X. Liu, F. Liu, R. Ge, Y. Sun, and Q. Xia. 2018. Identification of key genes and miRNAs markers of papillary thyroid cancer. Biological Research. 51:45. doi:10.1186/S40659-018-0188-1. Available from: http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0716-97602018000100237&lng=es&nrm=iso&tlng=en

Quan, S., X. Nan, K. Wang, L. Jiang, J. Yao, and B. Xiong. 2020. Characterization of Sheep Milk Extracellular Vesicle-miRNA by Sequencing and Comparison with Cow Milk. Animals 2020, Vol. 10, Page 331. 10:331. doi:10.3390/ANI10020331. Available from: https://www.mdpi.com/2076-2615/10/2/331/htm

RA, L., and L. DN. 2006. Treatment strategies for digital dermatitis for the UK. Vet J. 171:79–88. doi:10.1016/J.TVJL.2004.08.009. Available from: https://pubmed.ncbi.nlm.nih.gov/16427584/

Rapado-González, Ó., B. Majem, A. Álvarez-Castro, R. Díaz-Peña, A. Abalo, L. Suárez-Cabrera, A. Gil-Moreno, A. Santamaría, R. López-López, L. Muinelo-Romay, and M. M. Suarez-Cunqueiro. 2019. A Novel Saliva-Based miRNA Signature for Colorectal Cancer Diagnosis. Journal of Clinical Medicine 2019, Vol. 8, Page 2029. 8:2029. doi:10.3390/JCM8122029. Available from: https://www.mdpi.com/2077-0383/8/12/2029/htm

Read, D. H., and R. L. Walker. 2016. Papillomatous Digital Dermatitis (Footwarts) in California Dairy Cattle: Clinical and Gross Pathologic Findings: http://dx.doi.org/10.1177/104063879801000112. 10:67–76. doi:10.1177/104063879801000112. Available from: https://journals.sagepub.com/doi/abs/10.1177/104063879801000112

Reddy, K. B. 2015. MicroRNA (miRNA) in cancer. Cancer Cell International 2015 15:1. 15:1–6. doi:10.1186/S12935-015-0185-1. Available from: https://cancerci-biomedcentral-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12935-015-0185-1

Ren, L., Q. Li, X. Hu, Q. Yang, M. Du, Y. Xing, Y. Wang, J. Li, and L. Zhang. 2020. A Novel

Mechanism of bta-miR-210 in Bovine Early Intramuscular Adipogenesis. Genes (Basel). 11. doi:10.3390/GENES11060601. Available from: /pmc/articles/PMC7349823/

Rhoades, M. W., B. J. Reinhart, L. P. Lim, C. B. Burge, B. Bartel, and D. P. Bartel. 2002. Prediction of Plant MicroRNA Targets. Cell. 110:513–520. doi:10.1016/S0092-8674(02)00863-2.

Rosenberg, M. I., S. A. Georges, A. Asawachaicharn, E. Analau, and S. J. Tapscott. 2006. MyoD inhibits Fstl1 and Utrn expression by inducing transcription of miR-206. Journal of Cell Biology. 175:77–85. doi:10.1083/JCB.200603039. Available from: http://www.jcb.org/cgi/doi/10.1083/jcb.200603039

RW, B., and S. MW. 1988a. Digital dermatitis in dairy cattle. The Veterinary Record. 122:505–508. doi:10.1136/VR.122.21.505. Available from: https://europepmc.org/article/med/3407109

RW, B., and S. MW. 1988b. Digital dermatitis in dairy cattle. The Veterinary Record. 122:505–508. doi:10.1136/VR.122.21.505. Available from: https://europepmc.org/article/med/3407109

SA, H., W. WR, and M. RD. 1993. Effects of lameness on the behaviour of cows during the summer. The Veterinary Record. 132:578–580. doi:10.1136/VR.132.23.578. Available from: https://europepmc.org/article/med/8337801

Sahu, T. K., A. K. S. Gurjar, P. K. Meher, C. Varghese, S. Marwaha, G. P. Rao, A. Rai, N. Guleria, S. H. Basagoudanavar, A. Sanyal, and A. R. Rao. 2020. Computational insights into RNAi-based therapeutics for foot and mouth disease of Bos taurus. Scientific Reports 2020 10:1. 10:1–13. doi:10.1038/S41598-020-78541-6. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-020-78541-6

Salehi, S., O. D. Tavabie, S. Verma, M. J. W. McPhail, F. Farzaneh, W. Bernal, K. Menon, K. Agarwal, and V. R. Aluvihare. 2020. Serum MicroRNA Signatures in Recovery From Acute and Chronic Liver Injury and Selection for Liver Transplantation. Liver Transplantation. 26:811–822. doi:10.1002/LT.25781. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/lt.25781

Satake, E., M. G. Pezzolesi, Z. I. M. Dom, A. M. Smiles, M. A. Niewczas, and A. S. Krolewski. 2018. Circulating miRNA Profiles Associated With Hyperglycemia in Patients With Type 1

Diabetes. Diabetes. 67:1013–1023. doi:10.2337/DB17-1207. Available from: https://diabetes-diabetesjournals-org.login.ezproxy.library.ualberta.ca/content/67/5/1013

Schepeler, T., J. T. Reinert, M. S. Ostenfeld, L. L. Christensen, A. N. Silahtaroglu, L. Dyrskjøt, C. Wiuf, F. J. Sørensen, M. Kruhøffer, S. Laurberg, S. Kauppinen, T. F. Ørntoft, and C. L. Andersen. 2008. Diagnostic and Prognostic MicroRNAs in Stage II Colon Cancer. Cancer Research. 68:6416–6424. doi:10.1158/0008-5472.CAN-07-6110. Available from: https://cancerres-aacrjournals-org.login.ezproxy.library.ualberta.ca/content/68/15/6416

Scheuer, C., E. Boot, N. Carse, A. Clardy, J. Gallagher, S. Heck, S. Marron, L. Martinez-Alvarez, D. Masarykova, P. Mcmillan, F. Murphy, E. Steel, H. van Ekdom, and H. Vecchione. 2010. The origins and consequences of the American feedlot system. G. Balint, B. Antala, C. Carty, J.-M. A. Mabieme, I. B. Amar, and A. Kaplanova, editors. Physical Education and Sport for Children and Youth with Special Needs Researches – Best Practices – Situation. 343–354. doi:10.2/JQUERY.MIN.JS. Available from: https://baylor-ir-tdl-org.login.ezproxy.library.ualberta.ca/handle/2104/8032

Schrank, K., B.-K. Choi, S. Grund, A. Moter, K. Heuner, H. Nattermann, and U. B. Göbel. 1999. Treponema brennaborense sp. nov., a novel spirochaete isolated from a dairy cow suffering from digital dermatitis. International Journal of Systematic and Evolutionary Microbiology. 49:43–50. doi:10.1099/00207713-49-1-43. Available from: https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/00207713-49-1-43

Scott, G. B. 1989. Changes in limb loading with lameness for a number of friesian cattle. British Veterinary Journal. 145:28–38. doi:10.1016/0007-1935(89)90005-5.

Sengar, G. S., R. Deb, U. Singh, T. v. Raja, R. Kant, B. Sajjanar, R. Alex, R. R. Alyethodi, A. Kumar, S. Kumar, R. Singh, S. J. Jakhesara, and C. G. Joshi. 2018. Differential expression of microRNAs associated with thermal stress in Frieswal (Bos taurus x Bos indicus) crossbred dairy cattle. Cell Stress and Chaperones. 23:155–170. doi:10.1007/S12192-017-0833-6/TABLES/5. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s12192-017-0833-6

Seong, J., • Hyojeong Yoon, and H. S. Kong. 2016. Identification of microRNA and target gene associated with marbling score in Korean cattle (Hanwoo). Genes & Genomics. 38. doi:10.1007/s13258-016-0401-y. Available from: www.targetscan.org

Shearer, J. K. 1998. Lameness of dairy cattle: The Bovine Practitioner. 79–85. doi:10.21423/BOVINE-VOL1998NO32.1P79-85. Available from: https://bovine-ojs-tamu-tdl-org.login.ezproxy.library.ualberta.ca/bovine/index.php/bovine/article/view/1563

Shearer, J. K., S. R. van Amstel, and B. W. Brodersen. 2012a. Clinical Diagnosis of Foot and Leg Lameness in Cattle. Veterinary Clinics: Food Animal Practice. 28:535–556. doi:10.1016/J.CVFA.2012.07.003. Available from: http://www.vetfood.theclinics.com/article/S0749072012000540/fulltext

Shearer, J. K., S. R. van Amstel, and B. W. Brodersen. 2012b. Clinical Diagnosis of Foot and Leg Lameness in Cattle. Veterinary Clinics of North America: Food Animal Practice. 28:535–556. doi:10.1016/J.CVFA.2012.07.003.

Shearer, J. K., M. L. Stock, S. R. van Amstel, and J. F. Coetzee. 2013a. Assessment and Management of Pain Associated with Lameness in Cattle. Veterinary Clinics: Food Animal Practice. 29:135–156. doi:10.1016/J.CVFA.2012.11.012. Available from: http://www.vetfood.theclinics.com/article/S0749072012001004/fulltext

Shearer, J. K., M. L. Stock, S. R. van Amstel, and J. F. Coetzee. 2013b. Assessment and Management of Pain Associated with Lameness in Cattle. Veterinary Clinics: Food Animal Practice. 29:135–156. doi:10.1016/J.CVFA.2012.11.012. Available from: http://www.vetfood.theclinics.com/article/S0749072012001004/fulltext

Sheppard, S. C., S. Bittman, G. Donohoe, D. Flaten, K. M. Wittenberg, J. A. Small, R. Berthiaume, T. A. McAllister, K. A. Beauchemin, J. McKinnon, B. D. Amiro, D. Macdonald, F. Mattos, and K. H. Ominski. 2015. Beef cattle husbandry practices across ecoregions of canada in 2011. Canadian Journal of Animal Science. 95:305–321. doi:10.4141/CJAS-2014-158/SUPPL_FILE/CJAS-2014-158-SUPPLTAB1.DOC. Available from: https://cdnsciencepub-com.login.ezproxy.library.ualberta.ca/doi/abs/10.4141/cjas-2014-158

Shiino, S., J. Matsuzaki, A. Shimomura, J. Kawauchi, S. Takizawa, H. Sakamoto, Y. Aoki, M. Yoshida, K. Tamura, K. Kato, T. Kinoshita, Y. Kitagawa, and T. Ochiya. 2019. Serum miRNA–based Prediction of Axillary Lymph Node Metastasis in Breast Cancer. Clinical Cancer Research. 25:1817–1827. doi:10.1158/1078-0432.CCR-18-1414. Available from: https://clincancerres-aacrjournals-org.login.ezproxy.library.ualberta.ca/content/25/6/1817

Shin, V. Y., and K.-M. Chu. 2014. MiRNA as potential biomarkers and therapeutic targets for gastric cancer. World Journal of Gastroenterology : WJG. 20:10432. doi:10.3748/WJG.V20.I30.10432. Available from: /pmc/articles/PMC4130850/

Siracusa, J., N. Koulmann, A. Sourdrille, C. Chapus, C. Verret, S. Bourdon, M. E. Goriot, and S. Banzet. 2018. Phenotype-specific response of circulating miRNAs provides new biomarkers of slow or fast muscle damage. Frontiers in Physiology. 9:684. doi:10.3389/FPHYS.2018.00684/BIBTEX.

Smart, M. E. 1985. Nutritional Factors of Lameness and Metabolic Bone Disease in Cattle. Veterinary Clinics of North America: Food Animal Practice. 1:13–24. doi:10.1016/S0749-0720(15)31347-5.

Smith, G. R., D. Wallace, and D. E. Noakes. 1989. Enhancement of the infectivity of Fusobacterium necrophorum by other bacteria. Epidemiology & Infection. 102:447–458. doi:10.1017/S0950268800030168. Available from: https://www-cambridge-org.login.ezproxy.library.ualberta.ca/core/journals/epidemiology-and-infection/article/enhancement-of-the-infectivity-of-fusobacterium-necrophorum-by-other-bacteria/24DB4FC8DF9B6DCDFD516F2046FBABA9

Sprecher, D. J., D. E. Hostetler, and J. B. Kaneene. 1997a. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology. 47:1179–1187. doi:10.1016/S0093-691X(97)00098-8.

Sprecher, D. J., D. E. Hostetler, and J. B. Kaneene. 1997b. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology. 47:1179–1187. doi:10.1016/S0093-691X(97)00098-8.

Stokka, G. L., K. Lechtenberg, T. Edwards, S. MacGregor, K. Voss, D. Griffin, D. M. Grotelueschen, R. A. Smith, and L. J. Perino. 2001a. Lameness in Feedlot Cattle. Veterinary Clinics of North America: Food Animal Practice. 17:189–207. doi:10.1016/S0749-0720(15)30062-1.

Stokka, G. L., K. Lechtenberg, T. Edwards, S. MacGregor, K. Voss, D. Griffin, D. M. Grotelueschen, R. A. Smith, and L. J. Perino. 2001b. Lameness in feedlot cattle. Vet Clin North Am Food Anim Pract. 17. doi:10.1016/S0749-0720(15)30062-1. Available from: https://pubmed.ncbi.nlm.nih.gov/11320695/

Strotbek, M., L. Florin, J. Koenitzer, A. Tolstrup, H. Kaufmann, A. Hausser, and M. A. Olayioye. 2013. Stable microRNA expression enhances therapeutic antibody productivity of Chinese hamster ovary cells. Metabolic Engineering. 20:157–166. doi:10.1016/J.YMBEN.2013.10.005.

Sun, H. Z., Y. Chen, and L. L. Guan. 2019a. MicroRNA expression profiles across blood and different tissues in cattle. Scientific Data 2019 6:1. 6:1–8. doi:10.1038/sdata.2019.13. Available from: https://www.nature.com/articles/sdata201913

Sun, H. Z., Y. Chen, and L. L. Guan. 2019b. MicroRNA expression profiles across blood and different tissues in cattle. Scientific Data 2019 6:1. 6:1–8. doi:10.1038/sdata.2019.13. Available from: https://www.nature.com/articles/sdata201913

Sun, H. Z., Y. Chen, and L. L. Guan. 2019c. MicroRNA expression profiles across blood and different tissues in cattle. Scientific Data 2019 6:1. 6:1–8. doi:10.1038/sdata.2019.13. Available from: https://www.nature.com/articles/sdata201913

Sun, J., T. S. Sonstegard, C. Li, Y. Huang, Z. Li, X. Lan, C. Zhang, C. Lei, X. Zhao, and H. Chen. 2015a. Altered microRNA expression in bovine skeletal muscle with age. Animal Genetics. 46:227–238. doi:10.1111/AGE.12272. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/age.12272

Sun, J., T. S. Sonstegard, C. Li, Y. Huang, Z. Li, X. Lan, C. Zhang, C. Lei, X. Zhao, and H. Chen. 2015b. Altered microRNA expression in bovine skeletal muscle with age. Animal Genetics. 46:227–238. doi:10.1111/AGE.12272. Available from: https://onlinelibrary-wiley-

com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/age.12272

Sun, J., T. S. Sonstegard, C. Li, Y. Huang, Z. Li, X. Lan, C. Zhang, C. Lei, X. Zhao, and H. Chen. 2015c. Altered microRNA expression in bovine skeletal muscle with age. Animal Genetics. 46:227–238. doi:10.1111/AGE.12272. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/age.12272

Sun, J., B. Zhang, X. Lan, C. Zhang, C. Lei, and H. Chen. 2014. Comparative Transcriptome Analysis Reveals Significant Differences in MicroRNA Expression and Their Target Genes between Adipose and Muscular Tissues in Cattle. PLOS ONE. 9:e102142. doi:10.1371/JOURNAL.PONE.0102142. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0102142

Sun, Y., X. Zhao, Y. Zhou, and Y. Hu. 2012. MiR-124, miR-137 and miR-340 regulate colorectal cancer growth via inhibition of the Warburg effect. Oncology Reports. 28:1346–1352. doi:10.3892/OR.2012.1958/HTML. Available from: http://www.spandidos-publications.com.login.ezproxy.library.ualberta.ca/10.3892/or.2012.1958/abstract

Sun, Z., K. Shi, S. Yang, J. Liu, Q. Zhou, G. Wang, J. Song, Z. Li, Z. Zhang, and W. Yuan. 2018. Effect of exosomal miRNA on cancer biology and clinical applications. Molecular Cancer 2018 17:1. 17:1–19. doi:10.1186/S12943-018-0897-7. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/articles/10.1186/s12943-018-0897-7

Swarbrick, S., N. Wragg, S. Ghosh, and A. Stolzing. 2019. Systematic Review of miRNA as Biomarkers in Alzheimer's Disease. Molecular Neurobiology 2019 56:9. 56:6156–6167. doi:10.1007/S12035-019-1500-Y. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s12035-019-1500-y

Tadich, N., E. Flor, and L. Green. 2010. Associations between hoof lesions and locomotion score in 1098 unsound dairy cows. The Veterinary Journal. 184:60–65. doi:10.1016/J.TVJL.2009.01.005.

Takeda, Y., Y. Mishima, T. Fujiwara, H. Sakamoto, and K. Inoue. 2009. DAZL Relieves miRNA-Mediated Repression of Germline mRNAs by Controlling Poly(A) Tail Length in Zebrafish. PLOS ONE. 4:e7513. doi:10.1371/JOURNAL.PONE.0007513. Available from: https://journals-plos-

org.login.ezproxy.library.ualberta.ca/plosone/article?id=10.1371/journal.pone.0007513

Taxis, T. M., F. v. Bauermann, J. F. Ridpath, and E. Casas. 2017. Circulating MicroRNAs in serum from cattle challenged with bovine viral diarrhea virus. Frontiers in Genetics. 8:91. doi:10.3389/FGENE.2017.00091/BIBTEX.

Tewari, R. S., U. Ala, P. Accornero, M. Baratta, and S. Miretti. 2021. Circulating skeletal muscle related microRNAs profile in Piedmontese cattle during different age. Scientific Reports 2021 11:1. 11:1–13. doi:10.1038/S41598-021-95137-W. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-021-95137-w

Thomas, L. H., P. D. Wood, and J. M. Longland. 1978. The Influence of Disease on the Performance of Beef Cattle. British Veterinary Journal. 134:152–161. doi:10.1016/S0007-1935(17)33539-X.

Thomsen, P. T., L. Munksgaard, and F. A. Togersen. 2008. Evaluation of a Lameness Scoring System for Dairy Cows. Journal of Dairy Science. 91:119–126. doi:10.3168/JDS.2007-0496.

Tian, F., J. Luo, H. Zhang, S. Chang, and J. Song. 2012. MiRNA expression signatures induced by Marek's disease virus infection in chickens. Genomics. 99:152–159. doi:10.1016/J.YGENO.2011.11.004.

Tong, A. W., and J. Nemunaitis. 2008. Modulation of miRNA activity in human cancer: a new paradigm for cancer gene therapy? Cancer Gene Therapy 2008 15:6. 15:341–355. doi:10.1038/CGT.2008.8. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/cgt20088

Trott, D. J., M. R. Moeller, R. L. Zuerner, J. P. Goff, W. R. Waters, D. P. Alt, R. L. Walker, and M. J. Wannemuehler. 2003. Characterization of Treponema phagedenis-like spirochetes isolated from papillomatous digital dermatitis lesions in dairy cattle. Journal of Clinical Microbiology. 41:2522–2529. doi:10.1128/JCM.41.6.2522-2529.2003. Available from: https://journals.asm.org/journal/jcm

Tunstall, J., K. Mueller, D. Grove-White, J. W. H. Oultram, and H. M. Higgins. 2021. Lameness in Beef Cattle: A Cross-Sectional Descriptive Survey of On-Farm Practices and Approaches.

Frontiers in Veterinary Science. 0:490. doi:10.3389/FVETS.2021.657299.

Tunstall, J., K. Mueller, D. G. White, J. W. H. Oultram, and H. M. Higgins. 2019. Lameness in beef cattle: UK farmers' perceptions, knowledge, barriers, and approaches to treatment and control. Frontiers in Veterinary Science. 6:94. doi:10.3389/FVETS.2019.00094/BIBTEX.

Turri, F., E. Capra, B. Lazzari, P. Cremonesi, A. Stella, and F. Pizzi. 2021. A Combined Flow Cytometric Semen Analysis and miRNA Profiling as a Tool to Discriminate Between High- and Low-Fertility Bulls. Frontiers in Veterinary Science. 8. doi:10.3389/FVETS.2021.703101. Available from: /pmc/articles/PMC8329915/

Uddin, A., and S. Chakraborty. 2018. Role of miRNAs in lung cancer. Journal of Cellular Physiology. doi:10.1002/JCP.26607. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/jcp.26607

Ufkin, M. L., S. Peterson, X. Yang, H. Driscoll, C. Duarte, and P. Sathyanarayana. 2014. miR-125a regulates cell cycle, proliferation, and apoptosis by targeting the ErbB pathway in acute myeloid leukemia. Leukemia Research. 38:402–410. doi:10.1016/J.LEUKRES.2013.12.021.

Umezu, T., K. Tsuneyama, K. Kanekura, M. Hayakawa, T. Tanahashi, M. Kawano, Y. Taguchi, H. Toyoda, A. Tamori, M. Kuroda, and Y. Murakami. 2020. Comprehensive analysis of liver and blood miRNA in precancerous conditions. Scientific Reports 2020 10:1. 10:1–11. doi:10.1038/S41598-020-78500-1. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-020-78500-1

Unger, L., V. Jagannathan, A. Pacholewska, T. Leeb, and V. Gerber. 2019. Differences in miRNA differential expression in whole blood between horses with sarcoid regression and progression. Journal of Veterinary Internal Medicine. 33:241–250. doi:10.1111/JVIM.15375. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1111/jvim.15375

Valadi, H., K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee, and J. O. Lötvall. 2007. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nature Cell Biology 2007 9:6. 9:654–659. doi:10.1038/NCB1596. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/ncb1596

Vegh, P., D. A. Magee, N. C. Nalpas, K. Bryan, M. S. McCabe, J. A. Browne, K. M. Conlon, S. v. Gordon, D. G. Bradley, D. E. Machugh, and D. J. Lynn. 2015. MicroRNA profiling of the bovine alveolar macrophage response to Mycobacterium bovis infection suggests pathogen survival is enhanced by microRNA regulation of endocytosis and lysosome trafficking. Tuberculosis. 95:60–67. doi:10.1016/J.TUBE.2014.10.011.

Vickers, K. C., B. T. Palmisano, B. M. Shoucri, R. D. Shamburek, and A. T. Remaley. 2011. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nature Cell Biology 2011 13:4. 13:423–433. doi:10.1038/NCB2210. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/ncb2210

Võsa, U., T. Vooder, R. Kolde, J. Vilo, A. Metspalu, and T. Annilo. 2013. Meta-analysis of microRNA expression in lung cancer. International Journal of Cancer. 132:2884–2893. doi:10.1002/IJC.27981. Available from: https://onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1002/ijc.27981

Wagner, J. J., S. L. Archibeque, and D. M. Feuz. 2014. The Modern Feedlot for Finishing Cattle. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.1146/annurev-animal-022513-114239. 2:535–554. doi:10.1146/ANNUREV-ANIMAL-022513-114239. Available from: https://www-annualreviews-org.login.ezproxy.library.ualberta.ca/doi/abs/10.1146/annurev-animal-022513-114239

Walker, R. L., D. H. Read, K. J. Loretz, and R. W. Nordhausen. 1995a. Spirochetes isolated from dairy cattle with papillomatous digital dermatitis and interdigital dermatitis. Veterinary Microbiology. 47:343–355. doi:10.1016/0378-1135(95)00114-X.

Walker, R. L., D. H. Read, K. J. Loretz, and R. W. Nordhausen. 1995b. Spirochetes isolated from dairy cattle with papillomatous digital dermatitis and interdigital dermatitis. Veterinary Microbiology. 47:343–355. doi:10.1016/0378-1135(95)00114-X.

Wang, L., Z.-H. You, X. Chen, Y.-M. Li, Y.-N. Dong, L.-P. Li, and K. Zheng. 2019. LMTRDA: Using logistic model tree to predict MiRNA-disease associations by fusing multi-source information of sequences and similarities. PLOS Computational Biology. 15:e1006865.

doi:10.1371/JOURNAL.PCBI.1006865. Available from: https://journals-plos-org.login.ezproxy.library.ualberta.ca/ploscompbiol/article?id=10.1371/journal.pcbi.1006865

Wang, O., M. Zhou, Y. Chen, T. A. McAllister, G. Plastow, K. Stanford, B. Selinger, and L. L. Guan. 2021a. MicroRNAomes of Cattle Intestinal Tissues Revealed Possible miRNA Regulated Mechanisms Involved in Escherichia coli O157 Fecal Shedding. Frontiers in Cellular and Infection Microbiology. 11. doi:10.3389/FCIMB.2021.634505/FULL. Available from: /pmc/articles/PMC7959717/

Wang, O., M. Zhou, Y. Chen, T. A. McAllister, G. Plastow, K. Stanford, B. Selinger, and L. L. Guan. 2021b. MicroRNAomes of Cattle Intestinal Tissues Revealed Possible miRNA Regulated Mechanisms Involved in Escherichia coli O157 Fecal Shedding. Frontiers in Cellular and Infection Microbiology. 11. doi:10.3389/FCIMB.2021.634505/FULL. Available from: /pmc/articles/PMC7959717/

Wang, O., M. Zhou, Y. Chen, T. A. McAllister, G. Plastow, K. Stanford, B. Selinger, and L. L. Guan. 2021c. MicroRNAomes of Cattle Intestinal Tissues Revealed Possible miRNA Regulated Mechanisms Involved in Escherichia coli O157 Fecal Shedding. Frontiers in Cellular and Infection Microbiology. 11. doi:10.3389/FCIMB.2021.634505/FULL. Available from: /pmc/articles/PMC7959717/

Wang, S., Z. Li, Q. Chen, L. Wang, J. Zheng, Z. Lin, and W. Li. 2018. NF-κB-Induced MicroRNA-211 Inhibits Interleukin-10 in Macrophages of Rats with Lipopolysaccharide-Induced Acute Respiratory Distress Syndrome. Cellular Physiology and Biochemistry. 45:332–342. doi:10.1159/000486814. Available from: https://www-karger-com.login.ezproxy.library.ualberta.ca/Article/FullText/486814

Wang, X. C., L. L. Tian, H. L. Wu, X. Y. Jiang, L. Q. Du, H. Zhang, Y. Y. Wang, H. Y. Wu, D. G. Li, Y. She, Q. F. Liu, F. Y. Fan, and A. M. Meng. 2010. Expression of miRNA-130a in Nonsmall Cell Lung Cancer. The American Journal of the Medical Sciences. 340:385–388. doi:10.1097/MAJ.0B013E3181E892A0.

Wang, X., P. Zhang, L. Li, D. Che, T. Li, H. Li, Q. Li, H. Jia, S. Tao, J. Hua, W. Zeng, and M. Liao.

2018. miRNA editing landscape reveals miR-34c regulated spermatogenesis through structure and target change in pig and mouse. Biochemical and Biophysical Research Communications. 502:486–492. doi:10.1016/J.BBRC.2018.05.197.

Wang, Y., X. Zhang, H. Li, J. Yu, and X. Ren. 2013. The role of miRNA-29 family in cancer. European Journal of Cell Biology. 92:123–128. doi:10.1016/J.EJCB.2012.11.004.

Wang, Z., L. Cong Kong, B. Y. Jia, J. R. Chen, Y. Dong, X. Y. Jiang, and H. X. Ma. 2019. Analysis of the microRNA Expression Profile of Bovine Monocyte-derived Macrophages Infected with Mycobacterium avium subsp. Paratuberculosis Reveals that miR-150 Suppresses Cell Apoptosis by Targeting PDCD4. International Journal of Molecular Sciences 2019, Vol. 20, Page 2708. 20:2708. doi:10.3390/IJMS20112708. Available from: https://www.mdpi.com/1422-0067/20/11/2708/htm

Wara, A. B., S. Chhotaray, B. U. D. Shafi, S. Panda, M. Tarang, S. Yosuf, N. A. Baba, and A. Kumar. 2019. Role of miRNA Signatures in Health and Productivity of Livestock. International Journal of Current Microbiology and Applied Sciences. 8:727–738. doi:10.20546/IJCMAS.2019.804.079.

Warnick, L. D., D. Janssen, C. L. Guard, and Y. T. Gröhn. 2001. The Effect of Lameness on Milk Production in Dairy Cows. Journal of Dairy Science. 84:1988–1997. doi:10.3168/JDS.S0022-0302(01)74642-5.

Whay, H. 2002a. Locomotion scoring and lameness detection in dairy cattle. In Practice. 24:444–449. doi:10.1136/INPRACT.24.8.444. Available from: https://bvajournals-onlinelibrary-wiley-com.login.ezproxy.library.ualberta.ca/doi/full/10.1136/inpract.24.8.444

Whay, H. 2002b. Locomotion scoring and lameness detection in diary cattle. In Practice. 24:444–449. doi:10.1136/INPRACT.24.8.444.

Whay, H. 2002c. Locomotion scoring and lameness detection in dairy cattle. In Practice. 24:444–449. doi:10.1136/INPRACT.24.8.444. Available from: https://onlinelibrary.wiley.com/doi/full/10.1136/inpract.24.8.444

Whay, H. R., A. E. Waterman, A. J. F. Webster, and J. K. O'Brien. 1998. The influence of lesion

type on the duration ofhyperalgesia associated with hindlimb lameness in dairy cattle. The Veterinary Journal. 156:23–29. doi:10.1016/S1090-0233(98)80058-0.

White, B. J., D. E. Anderson, D. G. Renter, R. L. Larson, D. A. Mosier, L. L. Kelly, M. E. Theurer, B. D. Robért, and M. L. Walz. 2012. Clinical, behavioral, and pulmonary changes in calves following inoculation with Mycoplasma bovis. American Journal of Veterinary Research. 73:490–497. doi:10.2460/AJVR.73.4.490. Available from: https://avmajournals-avma-org.login.ezproxy.library.ualberta.ca/view/journals/ajvr/73/4/ajvr.73.4.490.xml

Wilczynska, A., and M. Bushell. 2014a. The complexity of miRNA-mediated repression. Cell Death & Differentiation 2014 22:1. 22:22–33. doi:10.1038/CDD.2014.112. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/cdd2014112

Wilczynska, A., and M. Bushell. 2014b. The complexity of miRNA-mediated repression. Cell Death & Differentiation 2014 22:1. 22:22–33. doi:10.1038/CDD.2014.112. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/cdd2014112

Wilson-Welder, J. H., D. P. Alt, and J. E. Nally. 2015. The etiology of digital dermatitis in ruminants: recent perspectives. Veterinary Medicine : Research and Reports. 6:155. doi:10.2147/VMRR.S62072. Available from: /pmc/articles/PMC6070020/

Winckler, C., and S. Willen. 2010. The Reliability and Repeatability of a Lameness Scoring System for Use as an Indicator of Welfare in Dairy Cattle. http://dx.doi.org/10.1080/090647001316923162. 51:103–107. doi:10.1080/090647001316923162. Available from: https://www.tandfonline.com/doi/abs/10.1080/090647001316923162

Wu, H.-H., W. Lin, and K.-W. Tsai. 2014. Advances in molecular biomarkers for gastric cancer: miRNAs as emerging novel cancer markers. Expert Reviews in Molecular Medicine. 16. doi:10.1017/ERM.2013.16. Available from: https://www-cambridge-org.login.ezproxy.library.ualberta.ca/core/journals/expert-reviews-in-molecular-medicine/article/advances-in-molecular-biomarkers-for-gastric-cancer-mirnas-as-emerging-novel-cancer-markers/8BC834935B87146C58D23AF7606E60D4

Xu, K., J. Lin, R. Zandi, J. A. Roth, and L. Ji. 2016. MicroRNA-mediated target mRNA cleavage

and 3′-uridylation in human cells. Scientific Reports 2016 6:1. 6:1–14. doi:10.1038/srep30242. Available from: https://www.nature.com/articles/srep30242

Ye, J., M. Xu, X. Tian, S. Cai, and S. Zeng. 2019. Research advances in the detection of miRNA. Journal of Pharmaceutical Analysis. 9:217–226. doi:10.1016/J.JPHA.2019.05.004.

Yerukala Sathipati, S., and S. Y. Ho. 2018a. Identifying a miRNA signature for predicting the stage of breast cancer. Scientific Reports 2018 8:1. 8:1–11. doi:10.1038/S41598-018-34604-3. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-34604-3

Yerukala Sathipati, S., and S.-Y. Ho. 2018b. Identifying a miRNA signature for predicting the stage of breast cancer. Scientific Reports 2018 8:1. 8:1–11. doi:10.1038/S41598-018-34604-3. Available from: https://www-nature-com.login.ezproxy.library.ualberta.ca/articles/s41598-018-34604-3

Yu, X., M. Odenthal, and J. W. U. Fries. 2016. Exosomes as miRNA Carriers: Formation–Function–Future. International Journal of Molecular Sciences 2016, Vol. 17, Page 2028. 17:2028. doi:10.3390/IJMS17122028. Available from: https://www.mdpi.com/1422-0067/17/12/2028/htm

Zanolari, P., S. Dürr, J. Jores, A. Steiner, and P. Kuhnert. 2021. Ovine footrot: A review of current knowledge. The Veterinary Journal. 271:105647. doi:10.1016/J.TVJL.2021.105647.

Zhang, C. 2009. MicroRNA-145 in vascular smooth muscle cell biology: A new therapeutic target for vascular disease. http://dx.doi.org.login.ezproxy.library.ualberta.ca/10.4161/cc.8.21.9837. 8:3469–3473. doi:10.4161/CC.8.21.9837. Available from: https://www-tandfonline-com.login.ezproxy.library.ualberta.ca/doi/abs/10.4161/cc.8.21.9837

Zhang, C. 2010. MicroRNAs in Vascular Biology and Vascular Disease. Journal of Cardiovascular Translational Research 2010 3:3. 3:235–240. doi:10.1007/S12265-010-9164-Z. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s12265-010-9164-z

Zhang, J. X., W. Song, Z. H. Chen, J. H. Wei, Y. J. Liao, J. Lei, M. Hu, G. Z. Chen, B. Liao, J. Lu, H. W. Zhao, W. Chen, Y. L. He, H. Y. Wang, D. Xie, and J. H. Luo. 2013. Prognostic and predictive value of a microRNA signature in stage II colon cancer: a microRNA expression analysis. The Lancet Oncology. 14:1295–1306. doi:10.1016/S1470-2045(13)70491-1.

Zhao, C., F. Tian, Y. Yu, G. Liu, L. Zan, M. S. Updike, and J. Song. 2012. MiRNA-dysregulation

associated with tenderness variation induced by acute stress in Angus cattle. Journal of Animal Science and Biotechnology. 3:1–8. doi:10.1186/2049-1891-3-12/FIGURES/5. Available from: https://jasbsci.biomedcentral.com/articles/10.1186/2049-1891-3-12

Zhao, K., G. Liang, X. Sun, and L. L. Guan. 2016a. Comparative miRNAome analysis revealed different miRNA expression profiles in bovine sera and exosomes. BMC Genomics. 17:1–12. doi:10.1186/S12864-016-2962-1/TABLES/6. Available from: https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-016-2962-1

Zhao, K., G. Liang, X. Sun, and L. L. Guan. 2016b. Comparative miRNAome analysis revealed different miRNA expression profiles in bovine sera and exosomes. BMC Genomics. 17:1–12. doi:10.1186/S12864-016-2962-1/TABLES/6. Available from: https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-016-2962-1

Zhao, K., G. Liang, X. Sun, and L. L. Guan. 2016c. Comparative miRNAome analysis revealed different miRNA expression profiles in bovine sera and exosomes. BMC Genomics. 17:1–12. doi:10.1186/S12864-016-2962-1/TABLES/6. Available from: https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-016-2962-1

Zhao, X. J., X. Y. Wang, J. H. Wang, Z. Y. Wang, L. Wang, and Z. H. Wang. 2015. Oxidative Stress and Imbalance of Mineral Metabolism Contribute to Lameness in Dairy Cows. Biological Trace Element Research. 164:43–49. doi:10.1007/S12011-014-0207-1/FIGURES/1. Available from: https://link-springer-com.login.ezproxy.library.ualberta.ca/article/10.1007/s12011-014-0207-1

Zhou, J., and J. Zhang. 2014. Identification of miRNA-21 and miRNA-24 in plasma as potential early stage markers of acute cerebral infarction. Molecular Medicine Reports. 10:971–976. doi:10.3892/MMR.2014.2245. Available from: http://www.spandidos-publications.com.login.ezproxy.library.ualberta.ca/10.3892/mmr.2014.2245/abstract

Appendix

Table A1. Sample information for recovered (RE) and unrecovered cattle definition

Date	Sample ID	Final Diagnosis	Animal ID	Week	Gait	Sex	Breed
	9	DDM2	DD-3	0	2	heifer	Black Angus
	12	DDM4.1	DD-4	0	2	heifer	Charolais/Black Angus
	21	DD	DD-1	0	1	steer	Black Angus / Red Angus
	379	DDM4	DD-12	0	2	steer	Red Angus
	407	DDM4.1	DD-15	0	2	Steer	Red Angus
DDREW0	409	DDM4.1	DD-17	0	3	Steer	Hereford
	410	DDM4.1	DD-18	0	1	Steer	Red Angus
	420	DDM2	DD-19	0	3	steer	Charolais
	422	DDM2	DD-21	0	3	steer	Black Angus
	445	DDM4.1	DD-24	0	3	steer	Black Angus
	446	DDM2	DD-25	0	3	steer	Red Angus
	17	DDM4	DD-4	1	0	heifer	Charolais/Black Angus
	44	DD	DD-1	1	3	steer	Black Angus / Red Angus
	397	DD	DD-12	1	1	Steer	Red Angus
	415	DDM4.1	DD-15	1	1	steer	Red Angus
DDREW1	416	DDM4.1	DD-17	1	1	steer	Hereford
	417	DDM4.1	DD-18	1	2	steer	Red Angus
	431	DDM2	DD-20	1	2	steer	Red Angus
	432	DDM0	DD-21	1	0	steer	Black Angus
	433	DDM4	DD-19	1	2	steer	Charolais
DDREW2	30	DDM0	DD-4	2	1	heifer	Black Angus/Charolais

	59	DDM0	DD-1	2	1	steer	Black Angus / Red Angus
	405	DDM0	DD-12	2	0	Steer	Red Angus
	428	DDM4.1	DD-15	2	1	steer	Red Angus
	429	DDM4.1	DD-17	2	1	steer	Hereford
	434	DDM0	DD-18	2	0	steer	Red Angus
	436	DDM0	DD-19	2	0	steer	Charolais
	437	DDM2	DD-20	2	1	steer	Red Angus
DDUNRW0	242	DDM4.1	DD-6	0	N/A	heifer	Black Angus
	349	DDM2	DD-10	0	1	steer	Simmental
	396	DDM4.1	DD-13	0	2	Steer	Red Angus
	423	DDM4.1	DD-22	0	2	steer	Black Angus
	443	DDM2	DD-23	0	1	steer	Black Angus
DDUNRW1	256	DD	DD-6	1	0	heifer	Black Angus
	363	DDM4.1	DD-10	1	1	steer	Simmental
	406	DDM4.1	DD-13	1	1	Steer	Red Angus
	430	DDM4.1	DD-22	1	1	steer	Black Angus
DDUNRW2	259	DDM4.1	DD-6	2	1	heifer	Black Angus
	375	DDM2	DD-10	2	2	steer	Simmental
	418	DDM4.1	DD-13	2	2	steer	Red Angus
	435	DDM4.1	DD-22	2	2	steer	Black Angus
TTNREW0	24	TTN	TTN-1	0	3	steer	Black Angus
	28	TTN	TTN-2	0	3	steer	Black Angus
	37	TTN	TTN-3	0	2	steer	Black Angus
	353	TTN	TTNS-8	0	2	steer	Black Angus
	355	TTN	TTN-9	0	1	steer	Black Angus
	369	TTN	TTN-10	0	N/A	steer	Black Angus
	371	TTN	TTN-11	0	2	steer	Black Angus
	390	TTN	TTN-12	0	3	Steer	Red Angus

TTNREW1	45	TTN	TTN-1	1	3	steer	Black Angus
	47	TTN	TTN-2	1	3	steer	Black Angus
	58	TTN	TTN-3	1	0	steer	Black Angus
	368	TTN	TTN-9	1	1	steer	Black Angus
	374	TTN	TTN-8	1	1	steer	Black Angus
	386	TTN	TTN-11	1	1	Steer	Black Angus
TTNREW2	60	TTN	TTN-2	2	2	steer	Black Angus
	61	TTN	TTN-1	2	3	steer	Black Angus
	75	TTN	TTN-3	2	1	steer	Black Angus
	383	TTN	TTN-8	2	1	Steer	Black Angus
	384	TTN	TTN-9	2	2	Steer	Black Angus
	399	TTN	TTN-10	2	1	Steer	Black Angus
	400	TTN	TTN-11	2	1	Steer	Black Angus
	414	TTN	TTN-12	2	1	Steer	Red Angus
TTNUNRW0	40	TTN	TTN-4	0	3	steer	Black Angus
	82	TTN	TTN-5	0	1	steer	Black Angus
	394	TTN	TTN-13	0	1	Steer	Black Angus cross
TTNUNRW1	62	TTN	TTN-4	1	3	steer	Black Angus
	97	TTN	TTN-5	1	3	steer	Black Angus
TTNUNRW2	109	TTN	TTN-5	2	1	steer	Black Angus
	413	TTN	TTN-13	2	1	Steer	Black Angus cross
FRDDREW0	170	FR/DDM4	FRDD-4	0	2	heifer	Black Angus/Hereford
	203	FR/DDM4.1	FRDD-12	0	2	heifer	Black Angus/Hereford
	226	FR/DDM2	FRDD-15	0	2	heifer	Charolais
	231	FR/DDM2	FRDD-17	0	2	heifer	Black Angus
	243	FR/DDM4	FRDD-20	0	2	steer	Black Angus
	303	FR/DDM4.1	FRDD-24	0	1	Heifer	Black Angus
	326	FR/DDM2	FRDD-30	0	3	steer	Red Angus

FRDDREW1	199	FR/DD	FRDD-4	1	1	heifer	Black Angus/Hereford
	211	FR/DDM2	FRDD-12	1	1	heifer	Black Angus/Hereford
	239	FR(healed)/DDM4.1	FRDD-15	1	0	heifer	Charolais
	246	FR(healed)/DDM2	FRDD-17	1	1	heifer	Black Angus
	252	FR(healed)/DDM4.1	FRDD-20	1	1	steer	Black Angus
	316	FR/DDM0	FRDD-24	1	N/A	Heifer	Black Angus
	341	FR/DDM2	FRDD-30	1	0	steer	Red Angus
FRDDREW2	210	FR/DDM4.1	FRDD-4	2	1	heifer	Black Angus
	223	FR(healed)/DD4.1	FRDD-12	2	1	heifer	Black Angus/Hereford
	244	FR(healed)/DDM4.1	FRDD-15	2	0	heifer	Charolais
	251	FR(healed)/DDM2	FRDD-17	2	1	heifer	Black Angus
	258	FR(healed)/DDM2	FRDD-20	2	0	steer	Black Angus
	328	FR/DDM0	FRDD-24	2	0	heifer	Black Angus
	357	FR/DDM0	FRDD-30	2	0	steer	Red Angus
FRDDUNRW0	171	FR/DD2 + DDM4	FRDD-5	0	2	steer	Black Angus
	175	FR/DDM2	FRDD-6	0	2	heifer	Black Angus/Hereford
	186	FR/DDM4	FRDD-10	0	2	heifer	Black Angus
	229	FR/DDM4.1	FRDD-16	0	2	heifer	Charolais
	234	FR/DDM4.1	FRDD-19	0	1	steer	Black Angus
	261	FR/DDM2	FRDD-22	0	2	heifer	Simmental
FRDDUNRW1	194	FR/DDM4.1	FRDD-5	1	1	steer	Black Angus
	195	FR/DDM2	FRDD-10	1	1	heifer	Black Angus
	198	FR/DDM4.1	FRDD-6	1	1	heifer	Black Angus/Hereford
	238	FR(healed)/DDM4	FRDD-16	1	2	heifer	Charolais
	245	FR(healed)/DDM4.1	FRDD-19	1	0	heifer	Black Angus
	265	FR/DDM2	FRDD-22	1	2	heifer	Simmental
FRDDUNRW2	204	FR/DDM4.1	FRDD-10	2	1	heifer	Black Angus
	206	FR/DDM4.1	FRDD-5	2	1	steer	Black Angus

207	FR/DDM4.1	FRDD-6	2	1	heifer	Black Angus/Hereford
241	FR/DDM0	FRDD-16	2	2	heifer	Charolais
253	FR(healed)/DDM4	FRDD-19	2	1	heifer	Black Angus
274	FR/DDM0	FRDD-22	2	2	steer	Simmental