MicroRNAs in domestic livestock

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Fatima A, Morris DG. MicroRNAs in domestic livestock. Physiol Genomics 45: 685–696, 2013. First published June 18, 2013; doi:10.1152/physiolgenomics.00009.2013.—MicroRNAs (miRNAs) are a class of small noncoding RNA that bind to complementary sequences in the untranslated regions of multiple target mRNAs resulting in posttranscriptional regulation of gene expression. The recent discovery and expression-profiling studies of miRNAs in domestic livestock have revealed both their tissue-specific and temporal expression pattern. In addition, breed-dependent expression patterns as well as single nucleotide polymorphisms in either the miRNA or in the target mRNA binding site have revealed associations with traits of economic importance and highlight the potential use of miRNAs in future genomic selection programs.

microRNA; livestock; genomics

FOR MANY YEARS IT WAS THOUGHT that only ~2% of the genome was functional or coding, while 98% of the genome or non-coding region was considered as nonfunctional. Recently published findings, however, from the Encyclopedia of DNA Elements project now indicate that up to 80% of this noncoding region has a functional role in gene expression (40). As the noncoding regions of genomes are being characterized and annotated their function is becoming clearer. It is apparent that the noncoding part of a genome makes a large contribution to the regulation of coding gene expression.

Gene expression regulation can occur at the levels of transcription, mRNA processing, and mRNA decay and translation. One such layer of gene regulation is by noncoding RNA (ncRNA) and this mechanism is named RNA interference (RNAi) (120). RNAi is a normal regulatory mechanism of eukaryotic cells whereby small RNA molecules bind to their target mRNAs to silence specific genes (2, 145). Four main types of interfering RNAs have been documented in animals, small interfering RNAs, repeat associated small interfering RNAs, Piwi-interacting RNAs, and microRNAs (miRNAs) (6, 131, 135). miRNAs are the most extensively studied small ncRNAs across all species (66, 88) and are now known to be associated with many traits and various biological networks (22, 67, 128, 146, 176, 178). This review will focus on recent miRNA studies in domestic animals particularly those relating to economically important traits in livestock species.

miRNA DISCOVERY, BIOGENESIS, AND MECHANISM OF ACTION

miRNAs belong to a class of small RNAs ~19–25 nucleotides in length. They regulate gene expression in cellular processes such as signal transduction, cell cycle, differentiation, and transformation, and it is estimated that >60% of genes undergo direct miRNA regulation (44). miRNAs are usually encoded in intergenic regions, but miRNAs can also exist within the introns of pre-mRNAs or be transcribed from ncRNA genes (106). Various computational as well as laboratory-based methods are used for miRNA identification and characterization. Computational methods for the identification of miRNAs consider features such as sequence composition, secondary structure (hairpin loop), thermodynamics, and degree of conservation across species (8). miRNA expression profiling techniques include microarray hybridization (24), quantitative reverse transcription polymerase chain reaction (75), and high-throughput sequencing (RNA-Seq) (60).

A general miRNA data analysis pipeline has four main steps including quality assessment, normalization, annotation, and differential expression analysis. Commonly used bioinformatics analysis packages based on the R statistical language (137) are available from Bioconductor (http://www.bioconductor.org) (48). For putative target searches, miRNA binding sites are predicted generally in the 3′-untranslated regions (UTR) of genes using one or more target prediction algorithms including miRanda (http://www.microrna.org) (15) and TargetScan (http://www.targetscan.org) (93). Before the classification of miRNAs as regulators of gene expression, the first two members of this class of RNA named lin-4 and let-7 were discovered in the roundworm (Caenorhabditis elegans) (91, 138), where they were found to regulate developmental timing. At present the naming convention consists of the prefix “miR” followed by a unique identifying number (e.g., miR-31) to differentiate between miRNAs, while the genes encoding miRNAs use the same prefix but italicized (7, 56). Today the miRNA-mediated regulation of gene expression in various biological pathways is being studied extensively. Because of their stability in body fluids like serum, plasma, urine, and milk (18, 33, 111), miRNAs are also being considered as candidate biomarkers of...
various autoimmune, metabolic, cardiovascular diseases, and cancer (4, 111, 140, 156). In addition, miRNAs are being utilized in drug discovery and developmental studies (107, 162, 167). miRNA regulation also has implications in the developmental reprogramming of the cell and in stem cells research (47) with miRNA expression perturbation techniques being applied to study their regulatory roles in mouse models and various cell lines (136, 173).

The latest release of miRBase (release 19, http://www.mirbase.org) (56), the database of published miRNA sequences and annotation, has a record of mature miRNAs for 193 species (55–57, 84). In the last 10 yr the number of publications on miRNAs in all species has increased significantly (Fig. 1A). The discovery and characterization of miRNAs in domestic livestock are more recent, however (117), but are on the increase (Fig. 1B). Whereas >2,000 miRNAs have been identified in human alone in miRBase, the number of mature miRNAs for cattle, sheep, pig, chicken, goat, and horse discovered to-date is much less (Table 1). Table 2 outlines some studies where miRNAs were computationally predicted or identified in various domestic livestock studies. As more miRNA discovery studies are completed the number of miRNA annotations in domestic livestock is likely to rise substantially.

miRNAs STUDIES IN DOMESTIC LIVESTOCK

Studies relating to miRNAs in livestock are concerned mainly with investigating their association with traits of eco-

<table>
<thead>
<tr>
<th>Table 1. Number of mature miRNAs annotated in miRBase (release 19) for human and livestock species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Human (Homo sapiens)</td>
</tr>
<tr>
<td>Cattle (Bos taurus)</td>
</tr>
<tr>
<td>Chicken (Gallus gallus)</td>
</tr>
<tr>
<td>Horse (Equus caballus)</td>
</tr>
<tr>
<td>Pig (Sus scrofa)</td>
</tr>
<tr>
<td>Sheep (Ovis aries)</td>
</tr>
<tr>
<td>Goat (Capra hircus)</td>
</tr>
</tbody>
</table>

Table 2. Number of miRNAs identified/predicted in various livestock miRNAs studies

<table>
<thead>
<tr>
<th>Species</th>
<th>Study</th>
<th>Tissue</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Gu et al., 2007 (59)</td>
<td>various</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Strozzi et al., 2009 (150)</td>
<td>various</td>
<td>249</td>
</tr>
<tr>
<td></td>
<td>Long and Chen, 2009 (108)</td>
<td>various</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Glazov et al., 2009 (51)</td>
<td>kidney</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Coutinho et al., 2007 (34)</td>
<td>embryo</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Tripurani et al., 2010 (160)</td>
<td>ovary</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Huang et al., 2011 (70)</td>
<td>testes</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>Huang et al., 2011 (70)</td>
<td>ovary</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>Hossain et al., 2009 (68)</td>
<td>ovary</td>
<td>38</td>
</tr>
<tr>
<td>Chicken</td>
<td>Xu et al., 2011 (171)</td>
<td>embryo/adult</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Glazov et al., 2008 (50)</td>
<td>embryo (day 5, 7, 9)</td>
<td>349</td>
</tr>
<tr>
<td></td>
<td>Darnell et al., 2006 (38)</td>
<td>embryo (day 0.5–5)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Hicks et al., 2008 (64)</td>
<td>embryo (day 11)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Yao et al., 2011 (177)</td>
<td>preadipocytes</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Hicks et al., 2010 (65)</td>
<td>liver</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Shao et al., 2008 (147)</td>
<td>various</td>
<td>29</td>
</tr>
<tr>
<td>Horse</td>
<td>Zhou et al., 2009 (181)</td>
<td>various</td>
<td>407</td>
</tr>
<tr>
<td></td>
<td>Lian et al., 2012 (104)</td>
<td>testes</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Chen et al., 2012 (27)</td>
<td>adipose</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Wernersson et al., 2005 (166)</td>
<td>various</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Kim et al., 2008 (80)</td>
<td>heart, liver</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Zhou et al., 2010 (180)</td>
<td>muscle</td>
<td>16</td>
</tr>
<tr>
<td>Sheep</td>
<td>Barozai et al., 2012 (12)</td>
<td>various</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>Torley et al., 2011 (158)</td>
<td>ovary</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Sheng et al., 2011 (148)</td>
<td>various</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Caiment et al., 2010 (22)</td>
<td>skeletal muscle</td>
<td>55</td>
</tr>
<tr>
<td>Goat</td>
<td>Li et al., 2012 (103)</td>
<td>mammary tissue</td>
<td>441</td>
</tr>
</tbody>
</table>

MiRNA, microRNA. n.a., Not available.
onomic importance, especially traits associated with milk, meat, and egg production and traits influencing animal productivity, fertility, embryo survival, and resistance to diseases. In addition miRNAs can also be used as diagnostic tests or biomarkers for certain livestock diseases. Information on functional polymorphisms at miRNA binding sites of target genes can help improve phenotypes of interest during the process of genomic selection in various breeding programs.

**POLYMORPHIC miRNA-TARGET INTERACTIONS, miRNAs ISOFORMS, AND miRNA CLUSTERS**

Sequence variations like single-nucleotide polymorphisms (SNPs) can occur at miRNA binding sites on the target genes that, if functional, can alter the miRNA-mediated regulation of these variant genes (52). Functional SNPs in target sites of miRNAs associated with economically important traits have been reported for livestock, and some of these are listed in Table 3. SNPs in the primary RNAs miR-206/miR-133b cluster were reported to be associated with muscle fiber characteristics, meat quality, and lean meat production in Berkshire, Landrace, and Yorkshire pig breeds. SNPs in miR-206 were associated with muscle fiber percentage, drip loss, lightness, and backfat thickness, while miR-133b SNPs were found to be associated with loin eye area, muscle pH, and total muscle fiber number (89). miRNAs themselves can exist as variants differing in one to three nucleotides, called isomirs. These sequence variants are most often found at the 3′-end of the miRNA and mostly do not affect their functionality (30); however, heterogeneity at the 5′-end of miRNA has also been reported (73, 86, 144). Isomirs have been reported in cattle (59), and most isomirs were found to be 3′-end variants with only a small number showing 5′-end variation. While some cattle miRNAs exist as both type of isomirs, miR-125-p has two 5′-end and two 3′-end variants. Isomirs have also been found in the 3′-end of miR-423 in a porcine muscle tissue study (132), while 5′-end variants of miR-388 and 3′-end variants of miR-96 were reported in the longissimus muscle of sheep (178). In addition, two SNPs were identified within the primary miR-1 sequence in pigs and were found to be associated with genes involved in muscle fiber composition (67).

A further characteristic feature of miRNAs is that they tend to exist as clusters at various genomic loci, and these clusters are mostly conserved among species (3). One study identified 60 miRNA clusters on the cattle genome (14, 41), while clusters have also been reported within specific loci on chromosome 1, 8, 12, 16, and 21 (108). In addition miR-23a and miR-24 were reported to exist in the same cluster and to be coexpressed in various bovine tissues (59). In the case of the porcine genome, 33 miRNAs were found to exist in 14 different clusters on nine different porcine chromosomes (31). So far, little is known about the functional implications of the clustering of miRNAs in livestock genomes.

**EXPRESSION PROFILES OF LIVESTOCK miRNAs**

miRNA expression has now been profiled across a variety of tissues from livestock species. miRNAs have also been characterized in body fluids like milk and blood where they exist in microvesicles or exosomes. miRNAs regulate a large number of genes, and certain miRNAs show ubiquitous expression across various tissues, suggesting that these miRNAs may play a regulatory role in biological processes vital to all cells across all tissues (34, 79). It is evident from the literature, however, that some miRNAs exhibit tissue-specific or stage-specific patterns of expression. Table 4 provides a list of studies that have investigated miRNAs associated with tissue-specific patterns of expression in livestock species. The expression patterns of miRNAs associated with important traits of livestock species are discussed below.

**miRNAs AND MUSCLE TRAITS**

Meat quality can vary with breed and age (10), and certain skeletal muscle traits like those associated with metabolism and contractility are important factors in this regard (11, 16). In a breed-specific study of miRNA expression in muscle a significantly higher expression of miR-206 was recorded in female Piedmontese cattle compared with Friesian cattle (123). In a miRNA expression profile study of slow and fast muscle types of Japanese black cattle, miR-885 and miR-196a were reported to be expressed only in semitendinosus muscle and not in masseter muscle (128). miRNAs have also been reported to play a role in normal myogenesis as well as development of muscle hypertrophy, which is an abnormal increase of muscle size in livestock species (32).

Myostatin is an important muscle gene that functions as a negative regulator of skeletal muscle development and growth in mammals (119, 155). Functional SNPs at binding sites for miR-1 and miR-26 on the myostatin gene have been linked with muscle hypertrophy in sheep (32). The Callipygian (CLPG) phenotype is an inherited muscular hypertrophy in Texel sheep and is found as a paternally inherited trait in animals heterozygous for a point mutation at the CLPG locus (26, 43). This locus comprises four imprinted genes (DLK1, GTL2, RTL1, and MEG8) that have skeletal muscle-specific expression (21, 26). The miRNAs on this locus were found to be maternal in origin and were associated with the CLPG phenotype (22).

**Table 3. Polymorphisms in miRNA binding sites and their gene targets**

<table>
<thead>
<tr>
<th>Species</th>
<th>Study</th>
<th>Gene</th>
<th>Function</th>
<th>miRNAs Binding Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Mullen et al., 2010 (126)</td>
<td>GH21</td>
<td>growth hormone</td>
<td>miR-1306, miR-17-5p, miR-220a, c, d, miR-92-b</td>
</tr>
<tr>
<td>Li et al., 2011 (101)</td>
<td>GH24</td>
<td>miR-671</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hou et al., 2011 (69)</td>
<td>HSF1</td>
<td>miR-671, miR-484</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>Xu et al., 2011 (170)</td>
<td>BOLA-DQA2</td>
<td>immune response</td>
<td>miR-2318</td>
</tr>
<tr>
<td>Sheep</td>
<td>Clop et al., 2006 (32)</td>
<td>DRD2L, DRD2S</td>
<td>dopamine receptor</td>
<td>miR-744, miR-339-5p, miR-1307, miR-1271, miR-328</td>
</tr>
<tr>
<td>Meng et al., 2008 (121)</td>
<td>GDF8</td>
<td>miR-1, miR-206</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>Zidi et al., 2010 (182)</td>
<td>MSTN</td>
<td>protein</td>
<td>miR-1, miR-206</td>
</tr>
</tbody>
</table>

Species: Cattle, Pig, Sheep, Goat

Gene: GH21, GH24, HSF1, BOLA-DQA2, DRD2L, DRD2S, GDF8, MSTN, CSN1S1

Function: Growth hormone, Heat shock protein, Immune response, Dopamine receptor, Muscle mass, Protein, Milk protein

miRNAs Binding Site: miR-1306, miR-17-5p, miR-220a, c, d, miR-92-b, miR-671, miR-484, miR-744, miR-339-5p, miR-1307, miR-1271, miR-328, miR-1, miR-206, miR-1, miR-206, miR-101
In a comparison of miRNA expression between Ujumqin and Texel lamb longissimus muscle, miR-1 and miR-214 were reported to be overexpressed in Ujumqin lambs (178). In a miRNA profile study of porcine skeletal muscle at different developmental stages and adult skeletal muscle, miR-1 was found to be moderately abundant during developmental stages while highly abundant in adult skeletal muscle (118). In contrast, miR-133 was moderately expressed in adult muscle and lowly expressed or absent during early developmental stages. In a deep sequencing study of porcine skeletal muscle miR-1 expression profile was found to be the most differentially expressed and to have a strong regulatory role in adipogenesis (78). Differences in the expression of miR-1 from breed to breed and can be manipulated to some extent with diet (80, 141). In addition, miRNAs also play a role in regulating the adipose tissue formation (81). Adipocytes of subcutaneous and perirenal adipose tissues of subcutaneous and perirenal adipose tissues varied in their composition (81, 143, 168). The amount of adipose can vary by breed and can be manipulated to some extent with diet (80, 141). In addition, miRNAs also play a role in regulating the adipose tissue formation (81). In a comparison of miRNA expression profiles of three groups of beef cattle with different back fat (subcutaneous adipose) thicknesses (78), miR-378 was found to have target sites on mitogen-activated protein kinase 1 that influences the expression of a transcription factor, peroxisome proliferator-activated receptor-γ, which in turn has a regulatory role in adipogenesis (78). Differences in the expression profile of miRNAs were observed in Hereford and Aberdeen Angus crossbred cattle depending upon the fat content of the diet and the location of the adipose depot in the body (139). In that study, adipocytes of subcutaneous and perirenal adipose tissues were found to share 176 common miRNAs, of which 30 showed tissue-specific expression patterns. In a study where miRNA expression in the back fat of Large White (lean type) and Meishan (Chinese indigenous) pigs was compared, miR-215, miR-135, miR-224, and miR-146b were more highly expressed in Large White pigs, while miR-1a, miR-133a, miR-122, miR-204, and miR-183 were more highly expressed in Meishan pigs (27). The authors speculated that two of the differentially expressed miRNAs, miR-135 and miR-183, may have a genetic link (115, 116).

### miRNAs and Adipose Tissue Traits

The fat content of meat greatly influences its flavor and quality (168). Adipocytes are cells that form and store fat globules in the body (62). The adipose depots located in different regions of the body, perirenally, subcutaneously, and intramuscularly, vary in their composition (81, 143, 168). The amount of adipose can vary from breed to breed and can be manipulated to some extent with diet (80, 141). In addition, miRNAs also play a role in regulating the adipose tissue formation (81). In a comparison of miRNA expression profiles of three groups of beef cattle with different back fat (subcutaneous adipose) thicknesses (78), miR-378 was found to be the most differentially expressed and to have a strong association with back fat thickness. In that study also, miR-378 was also reported to have target sites on mitogen-activated protein kinase 1 that influences the expression of a transcription factor, peroxisome proliferator-activated receptor-γ, which in turn has a regulatory role in adipogenesis (78). Differences in the expression profile of miRNAs were observed in Hereford and Aberdeen Angus crossbred cattle depending upon the fat content of the diet and the location of the adipose depot in the body (139). In that study, adipocytes of subcutaneous and perirenal adipose tissues were found to share 176 common miRNAs, of which 30 showed tissue-specific expression patterns.

### Table 4. Tissue-dependent expression of miRNAs in livestock species

<table>
<thead>
<tr>
<th>Species</th>
<th>Study</th>
<th>Tissue</th>
<th>miRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Coutinho et al., 2007 (34)</td>
<td>embryo</td>
<td>miR-199a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thymus</td>
<td>miR-150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>small intestine</td>
<td>miR-140</td>
</tr>
<tr>
<td>Jin et al., 2009 (79)</td>
<td>brain</td>
<td>miR-129</td>
<td></td>
</tr>
<tr>
<td></td>
<td>liver</td>
<td>miR-122</td>
<td></td>
</tr>
<tr>
<td></td>
<td>muscles</td>
<td>miR-1, miR-206</td>
<td></td>
</tr>
<tr>
<td>Hossain et al., 2009 (68)</td>
<td>cumulus cells of ovary</td>
<td>miR-29a, miR-125b, miR-409, miR-503</td>
<td></td>
</tr>
<tr>
<td>Tesfaye et al., 2009 (153)</td>
<td>mature oocytes</td>
<td>miR-496, miR-297, miR-292-3P, miR-99a, miR-410, miR-145, miR-515-5p, miR-512-5p, miR-214</td>
<td></td>
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<tr>
<td>Tripurani et al., 2010 (159)</td>
<td>immature oocytes</td>
<td>miR-424, miR-101</td>
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<tr>
<td>Miles et al., 2012 (122)</td>
<td>germinal vesicle</td>
<td>let-7b, let-7i, miR-106a</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>Cutting et al., 2012 (36)</td>
<td>embryonic chicken</td>
<td>miR-101, miR-202-5p, miR-31</td>
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<td></td>
<td>Wang et al., 2012 (165)</td>
<td>muscle</td>
<td>miR-133a, miR-1</td>
</tr>
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<td>Pig</td>
<td>Huang et al., 2008 (71)</td>
<td>embryo</td>
<td>miR-140</td>
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<td>Stowe et al., 2012 (149)</td>
<td>longissimus muscle (post gestational fetus)</td>
<td>miR-495</td>
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<tr>
<td></td>
<td></td>
<td>longissimus muscle (adult)</td>
<td>miR-193</td>
</tr>
<tr>
<td>Sheep</td>
<td>Luense et al., 2011 (109)</td>
<td>fetal ovary</td>
<td>miR-21, miR-205, miR-195, miR-16, miR-125b, miR-205, miR-128, miR-17, miR-210, miR-92a, miR-302a, miR-129-5p</td>
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<tr>
<td></td>
<td></td>
<td>metaphase II oocytes, 8-cell embryo, blastocyst</td>
<td>miR-497, miR-15b</td>
</tr>
</tbody>
</table>
miRNA IN ANIMALS

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mRNAs AND MAMMARY TISSUE TRAITS

Mammary tissue traits are important for dairy milk production, with udder health traits and mastitis (133) being a major concern in dairy cattle farming. The role of miRNAs in the regulation of mammary tissue genes was reported in a cattle study in which 359 putative target sites for miRNAs on a set of candidate gene and genetic markers for mastitis and milk production in mammary gland were identified (134). In another cattle adipose and mammary tissue miRNA profile study, the mammary tissue-specific expression of miR-21, miR-23a, and miR-24 was reported (59). Mammary tissue of cattle undergoes various changes with age and with physiological conditions like pregnancy or lactation status (9). In one study, expression of 13 miRNAs was profiled in mammary tissues of cattle from three lactation stages: dry, fresh, and early lactation periods (164). Out of these miRNAs, 12 showed higher expression in the fresh period compared with the dry period, while expression of one miRNA (miR-31) was increased in the early lactation period compared with the dry period. The predicted targets of these 13 miRNAs were associated with immune response, cell proliferation, and lipid metabolism in mammary tissues.

The immune response to mastitis in cattle was investigated through expression profiling of a major histocompatibility complex leukocyte antigen gene BOLA-DQA2 (bovine leukocyte antigen DQ alpha2) alongside five miRNAs with target sites on this gene from healthy mammary tissues and tissues affected by mastitis (69). It was reported that DQA2-SV1 as well as miR-296, miR-2430, and miR-671 had a higher expression, while miR-2318 was reported to have a lower expression in tissue affected by mastitis compared with healthy mammary tissues. In another study in which mammary tissue infected with the gram-positive bacteria Streptococcus uberis was compared with normal tissue, miR-181, miR-16, and miR-31 had a lower expression in infected tissues, while miR-223 had a higher expression (129). Putative targets of miR-31 and miR-223 were involved in inhibition of lipid metabolism, whereas miR-181a was reported to target genes that play major roles in phagocytosis and antigen processing and presentation during an immune response to infection in mammary tissue (129). Alteration in miRNAs expression of mammary tissue in response to S. uberis was studied through miRNA profiling at 2, 4, and 6 h postinfection (HPI) of primary bovine mammary epithelial cells indicated temporal alterations in miRNA expression during infection. In that study, miR-708 and miR-29e, were upregulated at 2 HPI, while at 4 HPI let-7b and miR-98 were upregulated and miR-29b-2, miR-193a, and miR-130a downregulated. At 6 HPI miR-29b-2, miR-29c, miR-29e, miR-100, and miR-130a were downregulated and 12 miRNAs were upregulated including let-7b (87).

High-mobility group box protein 1 (HMGB1) plays a major role in the innate immune response against infection during mastitis (95). miR-223 has been reported to have binding sites in the 3’-UTR of HMGB1, and the expression of miR-223 is increased during mastitis in cattle, resulting in downregulation of HMGB1 (99). That study also reported a SNP (g. +2776 A>G) in the 3'-UTR of HMGB1 that alters the binding site for miR-223. Mammary tissue from genotypes with this SNP had higher expression of HMGB1 and hence were less likely to be susceptible to mastitis.

miRNAs were profiled from the mammary gland of primiparous ewes of the Prealpes-du-Sud breed from early, mid-, and late pregnancy and during lactation (46). miR-21 and miR-205 were expressed in early and midpregnancy with miR-21 being more highly expressed in epithelial cells in early pregnancy and miR-205 more highly expressed during late pregnancy. In addition, members of the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) showed increased expression in late pregnancy and lactation (46).

In dairy goats a total of 346 conserved and 95 novel miRNAs were identified in the mammary gland at peak lactation as well as during the dry period (103). In another study, miRNAs were also profiled in the mammary tissue of Laoshan Dairy goats (Capra hircus) from peak (90 days postpartum) and late lactation periods (210 days postpartum) (77). That study reported higher expression of 272 and lower expression of 425 conserved miRNAs during peak lactation. In addition miR-17a* had the highest (19-fold) expression in late lactation, and miR-883 had the lowest expression (17-fold) in the late lactation period. These miRNAs were associated with mammary gland physiology, signal transduction, and cell communications. Overall the most abundant miRNAs coexpressed in both periods were miR-143, miR-143-3p, miR-148a-3p, let-7-5p, and let-7b.

miRNAs AND MILK TRAITS

A key feature of miRNAs is their high stability in a variety of body fluids including milk and blood (72). This feature makes them potential biomarkers for various metabolic and physiological conditions (28). Milk miRNAs are isolated from microvesicles, which are small bodies surrounded by a plasma
miRNAs were screened from cattle milk at different lactation stages, and it was found that 108 miRNAs exhibited increased expression in the colostrum compared with mature milk, while consistent levels of expression were recorded for a set of seven miRNAs during all stages of lactation (29). It was suggested that these miRNAs could be used as quality control markers to test milk purity, as formula milk showed very low amount of these miRNAs compared with pure raw milk.

miRNA abundance was also compared in exosomes isolated from porcine milk in early lactation (0 and 3 days) and later lactation (7, 14, 21, and 28 days) periods (58). Thirteen miRNAs were more abundant in the early lactation periods and found to target immune-related genes. Similar findings were reported in another study where miRNA expression profiles were compared between colostrum and mature milk (74). It was found that miR-15b, miR-27b, miR-34a, miR-106b, miR-130a, miR-155, and miR-223 exhibited higher expression in colostrum. The latter two miRNAs have anti-inflammatory roles with miR-155 associated with regulation of T immune cells differentiation and miR-223 associated with neutrophil activation.

Casein, a phosphoprotein, makes up a major portion of milk protein used for cheese production (63, 113). A study of polymorphism in the 3'-UTR of casein genes (CSN1S1, CSN1S2, CSN2, CSN3) in five breeds of goat including Murciano-Granadina, Cashmere, Canarian, Saanen, and Sahe- lian breeds reported a SNP in the 3'-UTR of CSN1S1 in Murciano-Granadina goats that disrupts the binding site for miR-101 (182).

miRNAs AND FERTILITY TRAITS

miRNAs of livestock gonadal tissues, oocytes, and sperm have been implicated in regulation of many fertility traits in livestock (23). In a cattle miRNA study expression of seven miRNAs was higher in ovarian cortex compared with other compartments of the ovary, while miR-29a was more abundant in cumulus cells (68). Similarly, in another study where miRNA expression in beef cattle ovaries was profiled, expression of miR-106 was higher in oocytes compared with cumulus oocyte complexes (COCs) and granulosa cells (122). In addition, the expression of the predicted target genes of miR-106 were decreased in oocytes compared with COCs. Higher expression of miR-205, miR-150, miR-122, miR-96, miR-146a, and miR-146b-5p was reported in immature cattle oocytes compared with mature oocyte from 0 h to 22 h of oocyte maturation (1). The same set of miRNAs also showed higher expression in early (8-cell) stage embryo compared with the blastocyst stage embryo (1). In addition, a set of 51 miRNAs was reported to be more highly expressed in mature oocytes compared with surrounding cumulus cells with miR-205 being the most highly expressed. It was also reported that 47 miRNAs were more highly expressed in immature oocytes compared with cumulus cells with miR-122 being the most highly expressed (1). Oocytes undergo various morphological changes, and oocyte miRNAs expression varies with the stages of oogenesis. In one study, miR-424 and miR-10b were reported to be highly expressed in the germinal vesicle stage of cattle oocytes (160). In another study, where bovine oocytes at different stages of maturation were compared, seven miRNAs (miR-496, miR-297, miR-292-3p, miR-99a, miR-410, miR-145, and miR-515-5p) were reported to be more abundant in mature oocytes, while two miRNAs (miR-512-5p and miR-214) were more abundant in immature oocytes (153).

Ovarian hormones play many essential roles in reproduction. The aromatase enzyme converts androgen to ovarian estrogens, and the regulation of the aromatase gene CYP19 can have major implications in ovarian development and function (85). In a porcine ovarian study CYP19 was reported to be down-regulated by miR-378 in granulosa cells during follicular development (171). In another study, testosterone was reported to alter the expression of ovine fetal ovarian miR-497 and miR-15b at day 90 of gestation (109). In a chicken study, miR-499 and miR-1709 were found to be associated with the regulation of pleiotrophin (90), a growth hormone with a role in the development of the oviduct and formation of the egg (127).

miRNAs can also have a role in the regulation of sexual differentiation and pathways leading to formation of the male or female gonads. The expression of gonadal miRNAs at the time of sexual differentiation was profiled in the chicken embryo study (36) and reported sexually dimorphic expression patterns for miR-101, miR-31, and miR-202-5p. Target genes of these miRNAs are involved in important gonadal differentiation pathways associated with transforming growth factor-β. Expression of sheep gonadial miRNAs at gestational day (GD) 42 considered as early gestation and the midgestational (GD75) stage of development were profiled from ovaries and testis (158). In that study 24 miRNAs were reported to be differentially expressed between the two sexes at GD42 and another 43 at GD75, targeting genes important in gonadal development pathways. The X chromosome also has a secondary role to play in reproduction with deregulation of certain X linked genes leading to premature ovarian failure (157). One study reported an increased expression of 24 X-linked miRNAs in porcine ovaries compared with testis, which targeted genes involved in various ovarian functions (100).

miRNAs have also been found in testicular tissue and sperm (5). A deep sequencing study of conserved and pig-specific miRNAs from the sexually immature (30 days) and mature (180 days) porcine testis reported higher expression of 96 conserved miRNAs and lower expression of conserved 26 miRNAs in mature testes with predicted targets of these miRNAs having potential roles in the regulation of spermatogenesis (104). In addition 10 miRNAs (including miR-153 and miR-205) were only expressed in the mature testis, whereas three miRNAs (miR-196, miR-485-3p, and miR-149*) were only expressed in the immature testis.

In a cattle study, expression of seven miRNAs was reported to be different in the spermatozoon of high- and low-fertility Holstein bulls (54), suggesting a role for miRNAs in cattle fertility. The let-7 family of miRNA is functionally conserved across species and has been reported to play important role in various biological processes (142) including cell differentiation and cancer (19) and in metabolism (45). In a comparison of...
normal porcine sperm with those from pigs with morphological abnormalities (35) the expression of the let-7 family members, let-7a, let-7d, let-7e, and miR-22 was higher in the morphologically abnormal group, whereas miR-15b was expressed at a lower level. miRNAs expression of chicken embryonic gonads was profiled at days 5.5, 6.5, and 9.5 (10). At each time point a higher expression of miR-202* was reported in the developing testis compared with that in ovaries, suggesting a role for miR-202* in sexual differentiation of testis. In addition, female gonads treated with aromatase inhibitor at day 3.5 had increased expression of miR-202*. miR-202* was found to be associated with DMRT1 (doublesex and mab-3 related transcription factor 1) and SOX9 (sex determining region Y-box 9), both of which have a role in testicular development (82, 163).

A role for miRNAs in bovine ovarian luteal cell apoptosis has also been suggested (112). miR-378 was reported to have a higher expression in regressed corpus luteum (CL) compared with nonregressed CL and was able to decrease interferon gamma receptor 1 protein level in early, mid-, late and regressed cattle CL (112). Sheep miRNAs were profiled in follicular-luteal transitional stages, including growing and preovulatory follicles, as well as from day 3 and day 9 postestrous CL (114). Nine miRNAs had lower expression during the process of follicular-luteal transition, while eight other miRNAs had higher expression. The putative gene targets of these miRNAs were associated with processes involved in follicular differentiation. A significant increase in expression of miR-26b was reported in porcine atretic follicles which in turn targets the ATM (ataxia telangiectasia mutated) gene involved in DNA fragmentation in granulosa cells (105). AHCYL1 (S-adenosylhomocysteine hydrolase-like 1) is expressed in the oviduct and plays a role in embryonic development (20). In chick oviduct AHCYL1 was reported to have putative target sites in its 3′-UTR of miR-218 (76). In addition, miR-1244, miR-1669, miR-1710, and miR-1782 were reported to influence the expression of AHCYL1 in vitro. Furthermore, miR-1244 and miR-1669 were found to have binding sites on genes involved in the development and differentiation of the oviduct.

In a study of equine follicular fluid, miRNAs that were present in follicle fluid were also detected in surrounding granulosa and cumulus cells, suggesting a role for microvesicles in miRNAs transfer (37). In addition the miRNA profile in ovarian follicular fluid was reported to vary among young and old mares (37).

The placenta is essential for the transfer of nutrients and oxygen to the fetus in utero during pregnancy. In a porcine placental study, differential expression of miR-125b, miR-92b, miR-106a, miR-24, and miR-20 was reported between day 30 and 90 of gestation (151).

miRNAs AND EMBRYONIC DEVELOPMENT AND SURVIVAL

Early embryo survival is essential for efficient livestock production (39). The miRNA expression pattern in elongated bovine E17 embryos following transfer as blastocysts on day 7 after in vitro fertilization (IVP) or cloning has also been reported (25). In that study miR-103, miR-203, miR-107, miR-19b, miR-21, and miR-106 were more highly expressed in cloned embryos, while miR-140, miR-106b, miR-342, miR-200c, let-7b, miR-24, miR-30d, and miR-26a were more highly expressed in IVP embryos. In addition, 29 miRNA present in the cloned embryos were absent in the somatic cells used for nuclear transfer, suggesting that a reprogramming of miRNA expression occurs in bovine cloned embryos after somatic cell nuclear transfer. In another study, in which miRNA expression was profiled from bovine oocytes at germinal vesicle and metaphase II stages and from early embryos at two-cell, four-cell, eight-cell, and blastocyst stages, miR-21 and miR-130a showed an increase in expression from the one-cell up to eight-cell stage (124). Putative targets of these miRNAs were implicated in various development processes and suggested a role for miRNAs during the maternal-to-embryonic transition. In a developmental network study of miRNAs from early and hatched-stage cattle blastocysts, miR-127, miR-130a, miR-155, miR-196a, miR-203, miR-28, miR-29c, and miR-376a were reported to be more highly expressed in hatched blastocysts, while miR-218, miR-335, miR-135a, and miR-449b were reported to show a lower expression (53). In addition, a direct regulation of NOTCH1 (notch homolog 1 translocation-associated), a transmembrane protein involved in cell signaling during development (169), by miR-449B and of NANOG (nanog homeobox), a transcription factor involved in development (110), by miR-28 was confirmed. Furthermore, miR-28 was shown to regulate NANOG expression in response to fibroblast growth factor (FGF) signaling. miRNAs are also reportedly to play a role in the germ cell differentiation in chicken (92). In that study miR-181*a was reported to be more highly expressed in primordial germ cells (PGCS), resulting in inhibition of HOXA1 (homeobox A1) expression and repression of NR6A1 (nuclear receptor subfamily 6 group A member 1) and as a consequence inhibition of PGCS differentiation to somatic cells. FGF plays an important role in the regulation of embryonic development (130, 154).

Transcriptional regulation of miR-206 by FGF signaling was demonstrated in chicken embryo from 1.5 days to 5 days (152). FGF can regulate the pluripotency of embryonic stem cells through LIN28B (lin-28 homolog B dependent-pathways (42). LIN28B in turn has been reported to have a regulatory role in miRNA expression (125). It was reported that LIN28B under the influence of FGF can regulate the expression of let-7b, miR-9, miR-19b, miR-107, miR-130b, and miR-218 during porcine gastrulation; however, FGF was reported to regulate miR-9, miR-107, and miR-218 expression through pathways independent of LIN28B also (17). In that study miR-130b and miR-218 were reported to have target sites in the 3′-UTR of platelet-derived growth factor receptor, alpha polypeptide, which encodes a receptor for a growth factor that plays important roles in cell migration and cell lineage decision.

In a porcine study different number of miRNAs were found to be expressed in metaphase II oocytes, eight-cell embryos, and blastocysts, with 63 miRNAs expressed at the eight-cell stage compared with 88 in oocytes and 84 in blastocysts (149). Of these, miR-18a, miR-21, and miR-24 were implicated in important cellular pathways targeting genes involved in cell differentiation, cell signaling, and transcription regulation along with other important cellular processes. In addition, miR-24 expression was lower in the in vivo-produced embryo from artificially inseminated postpubertal gilts relative to in vitro-fertilized embryo from oocytes of prepubertal gilts and cycling sows and was suggested as a biomarker for embryo quality. In a recent study seven miRNA were identified in pig
COCs and early embryos including miR-574, miR-24, let-7e, miR-23B, miR-30d, miR-320, and miR-30c (175).

SUMMARY AND CONCLUSION

This review highlights the potential role of miRNAs in regulating economically important traits in livestock. The breed-dependence variation in expression of miRNAs associated with economically important traits as well as the existence of polymorphism in miRNA target sites on economically important genes could form the basis for a selection programs in cattle, sheep, chickens, pigs, goats, and horses. The potential use of circulating miRNAs as biomarkers for different physiological conditions and diseases could also help to improve the health and fertility of livestock and of milk quality.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.F. performed experiments; A.F. analyzed data; A.F. interpreted results of experiments; A.F. and D.G.M. prepared figures; A.F. drafted manuscript; A.F. and D.G.M. edited and revised manuscript; A.F. and D.G.M. conceived the study and designed the research.

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miRNA IN ANIMALS

Review


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