Postprint: Molecular Mechanism of miRNA Regulation in Heat-Stressed Livestock and Poultry

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Abstract

Heat stress alters animal behavior and physiological functions. miRNAs, as non-coding RNAs of approximately 22 nucleotides, participate in the regulation of heat stress in organisms at the post-transcriptional level by inhibiting target genes. This article reviews the roles and mechanisms of miRNAs in heat stress-induced cell growth and apoptosis, as well as their effects and mechanisms on immunity, stress resistance, organ damage, growth performance, and reproductive performance in heat-stressed livestock and poultry.

Full Text

Preamble

Molecular Mechanisms of miRNA Regulation in Heat-Stressed Livestock and Poultry

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Abstract: Heat stress alters animal behavior and physiological functions. MicroRNAs (miRNAs), as non-coding RNAs of approximately 22 nucleotides, participate in heat stress regulation by inhibiting target genes at the post-transcriptional level. This review summarizes the roles and mechanisms of miRNAs in regulating cell growth and apoptosis under heat stress, as well as their

effects on immunity, anti-stress responses, organ damage, growth performance, and reproductive performance in heat-stressed livestock and poultry.

Keywords: miRNAs; heat stress; livestock and poultry; regulation

Introduction

Global climate change has intensified the impact of heat stress on animal husbandry. In China, vast regions experience heat stress during summer months each year, causing metabolic disorders in livestock and poultry, reducing production and reproductive performance, and resulting in significant economic losses [**?**]. The heat stress response represents a self-protective mechanism in animals, characterized by altered expression of related genes and proteins. As post-transcriptional regulators, miRNAs encode only 1-5% of mammalian genes yet regulate over 60% of the genome [**?**]. Accumulating evidence demonstrates that miRNAs play crucial roles in protecting livestock and poultry from heat stress by regulating heat shock proteins (HSPs), redox genes, immune genes, apoptosis-related genes, and metabolic genes [**?**]. This review aims to summarize recent research on miRNAs under heat stress conditions to provide insights for future studies on heat stress in livestock and poultry.

1. Molecular Mechanism of miRNA Biogenesis

miRNAs function as important post-transcriptional regulators in animal systems [**?**]. The first miRNA family member, lin-4, was discovered through genetic screening for developmental timing defects in *C. elegans* embryos [**?**], sparking widespread interest in miRNA research. The formation of mature miRNAs involves several steps: miRNA genes are transcribed into long primary miRNAs (pri-miRNAs), which are cleaved by Drosha to form precursor miRNAs (pre-miRNAs). These pre-miRNAs are transported to the cytoplasm by Exportin-5 and subsequently processed by Dicer to generate mature miRNAs [**?**]. miRNAs regulate gene expression through the RNA-induced silencing complex (RISC) by mediating mRNA cleavage or translational repression [**?**]. Notably, a single miRNA may target multiple genes, while individual genes can be regulated by multiple miRNAs.

2. Molecular Mechanisms of miRNA Regulation in Heat-Stressed Livestock and Poultry

2.1 Regulation of Cell Growth and Apoptosis Under Heat Stress

Cellular exposure to high temperatures causes damage and inhibits DNA, RNA, and protein synthesis [**?**]. In vitro cultured cells exhibit growth arrest and apoptosis induction under heat stress conditions [**?**]. In heat-stressed bovine mammary epithelial cells, genes related to heat response, DNA repair, and protein

repair are initially upregulated, followed by upregulation of apoptosis-related genes [?]. As single-stranded non-coding small molecules, miRNAs regulate cell proliferation, differentiation, and apoptosis [?]. For instance, miR-17-5p inhibits autophagy by targeting the autophagy-related gene ULK1 [?], while miR-71 directly targets pdk-1 and cdc-25.1, affecting DNA damage repair signaling pathways [?].

Wilmink et al. [?] observed that heat induction in epidermal fibroblasts resulted in differential expression of 83 downregulated and 40 upregulated miRNAs. Target gene analysis revealed that differentially expressed miR-138 and miR-196a were associated with HSP family members HSPA4L and HSPH1. Under heat stress conditions, three categories of miRNAs exhibit regulatory patterns: (1) miRNAs expressed during most stress responses, such as miR-125b, miR-222, miR-22, and let-7c; (2) miRNAs expressed only under high-temperature induction, such as miR-452, miR-382, and miR-378; and (3) miRNAs downregulated under stress conditions. Heat stress inhibits miRNA-24 expression, which reduces apoptosis incidence and promotes growth in bovine mammary epithelial cells [?].

Heat stress stimulates the CYP2e gene to produce excessive reactive oxygen species [?], leading to increased expression of the rate-limiting glycolytic enzyme muscle phosphofructokinase (PFKm), accelerated glycolysis, and elevated lactate concentrations. Chronic stress causes lactate accumulation, resulting in microenvironmental acidosis and subsequent apoptosis. miR-320a targets PFKm to inhibit glycolysis [?]. Heat stress also affects HSP expression for acute homeostatic responses [?]. While HSP70 and HSP90 interact with Ago2 (a key RISC component that modifies miRNAs), suggesting HSP involvement in miRNA-mediated gene silencing [?], Fukuoka et al. [?] found that heat stress enhanced gene silencing activity without altering miRNA expression levels. Their work demonstrated that HSP90 does not affect miRNA-mediated silencing and HSP1 does not participate in this process, suggesting that endogenous miRNA silencing may only play an auxiliary role in inhibiting heat-denatured proteins.

2.2 Regulation of Immunity and Anti-Stress Responses

The heat stress response represents a non-specific defense mechanism that protects organisms from damage. However, excessive or prolonged stress can lead to pathology, organ failure, and even death [?]. Heat stress upregulates the ubiquitin-proteasome pathway to eliminate severely damaged proteins [?] and activates the hypothalamic-pituitary-adrenal axis to enhance stress resistance. Zheng et al. [?] identified seven miRNAs in heat-stressed dairy cow serum associated with stress and immune responses. Upregulated miR-19a and miR-19b participate in heat stress regulation by targeting HSP family genes. Under acute stress, rat circulating HSP72 concentrations increase while miR-142-5p and miR-203 decrease [?]. miR-142-5p regulates immune responses by targeting the interleukin (IL)-6 receptor, while miR-203 suppresses cytokine signaling suppressor protein-3 (SOCS3) to modulate pro-inflammatory cytokines [?].

Cai et al. [?] found 118 upregulated and 197 downregulated miRNAs in heat-stressed Angus cattle peripheral blood. Target gene prediction for ten significantly differentially expressed miRNAs revealed that upregulated miRNAs primarily target genes involved in cell proliferation and apoptosis regulation, while downregulated miRNAs target genes mainly participating in immune function regulation. Specifically, miR-98 targets IL-6 [?], and miR-31 targets NF- B-inducing kinase (NIK) to regulate inflammatory responses [?]. These findings demonstrate that livestock and poultry utilize miRNAs to regulate target gene expression, thereby participating in immune and anti-stress responses under heat stress conditions.

2.3 Organ Damage

2.3.1 Intestinal Damage Heat stress causes intestinal ischemia and hypoxia, leading to intestinal mucosal damage [?]. miRNAs play crucial roles in this process. McKenna et al. [?] detected 1,094 mature miRNAs in mouse jejunal mucosa using high-throughput sequencing, including 65 intestine-specific miRNAs. Dicer1 enzyme (required for miRNA synthesis) deficiency caused intestinal epithelial disorganization, compromised barrier function, and inflammatory responses. Yu et al. [?] identified 18 upregulated miRNAs (including miR-34a, miR-34b, miR-140, miR-375, miR-500) and 11 downregulated miRNAs (including miR-31) in heat-stressed rat small intestine. miR-500 targets hexokinase 2 (HK2) mRNA at the 3 UTR region, inhibiting small intestinal glucose absorption by reducing HK2 activity [?]. miR-375 regulates IL-13 to control thymic stromal lymphopoietin (TSLP) expression in T helper 2 cells, reducing resistin-like molecule β (RELMβ) expression and compromising intestinal mucosal immunity [?]. miR-34a and miR-34b target the apoptosis gene p53 to regulate DNA damage repair, cell cycle arrest, and apoptosis [?], while miR-140 modulates inflammatory responses [?]. miR-31 expression decreases during inflammation and is associated with genes related to cell growth, death, and communication [?].

Heat stress increases intestinal lipopolysaccharide (LPS) levels and damages the mucosal barrier, allowing LPS to enter the liver and systemic circulation, triggering systemic inflammatory responses [?]. LPS activates tumor necrosis factor-α (TNF-α) and IL-6 through the NF- B signaling pathway and induces hepatic insulin resistance and hyperinsulinemia via SOCS3, with cross-talk between insulin and inflammatory signaling pathways [?]. During LPS stimulation, IL-6 secretion negatively correlates with miR-181b expression [?]. Upregulated miR-146a in intestinal mucosa protects against damage by inhibiting IL-1 receptor-associated kinase 1 (IRAK1) in the Toll-like receptor (TLR) signaling pathway [?]. miR-140 targets SOCS3 to regulate LPS-induced inflammatory responses [?]. Collectively, heat stress generates excessive LPS in the intestine, stimulating pro-inflammatory cytokine release through TLR and NF- B pathways, while miRNAs protect intestinal health by regulating genes in these signaling pathways.

2.3.2 Liver Damage Heat stress readily causes hepatic oxidative damage. Glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) in the liver scavenge oxygen free radicals to reduce heat stress-induced oxidative injury [**?**]. miR-214 targets microsomal glutathione S-transferase 1 (MGST1) to modulate antioxidant capacity [**?**]. Additionally, as mitochondria in hepatocytes are primary sites of reactive oxygen species generation in the respiratory chain, miR-93 and miR-214 target ubiquinol-cytochrome c reductase core protein 1 (UQCRC1) to protect liver function [**?**].

2.4 Regulation of Production Performance

Under heat stress, livestock and poultry increase sweating, heart rate, and respiratory frequency to maintain homeostasis, which negatively affects production performance [**?**]. Heat-stressed dairy cows exhibit reduced feed intake, insufficient nutrient intake, negative energy balance, and decreased milk yield [**?**]. Baumgard et al. [**?**] reported that heat-stressed cows in negative energy balance prioritize glucose metabolism in tissues over milk synthesis. miR-143 regulates glucose homeostasis by inhibiting insulin and downstream protein kinase B (AKT) activity [**?**]. Fatima et al. [**?**] identified five upregulated miRNAs (miR-17-5p, miR-31, miR-140, miR-1281, miR-2885) in the liver of cows experiencing negative energy balance. miR-31 targets hepatocyte nuclear factor 3-γ (HNF3-γ) and transcription factors involved in insulin-like growth factor-1 (IGF-1) expression regulation, which is associated with glucose metabolism homeostasis. Under nutritional deficiency, downregulated miR-80 directly targets CRBE-1 and regulates insulin signaling to modulate energy metabolism [**?**]. These findings indicate that heat-stressed animals regulate energy metabolism through miRNAs, thereby affecting feed intake.

Heat stress also compromises product quality. It reduces milk protein and casein concentrations, causing "heat stress-induced milk protein depression" [**?**]. Zhang et al. [**?**] demonstrated that varying heat stress severity differentially affects milk composition; moderately heat-stressed cows showed significantly lower milk protein content than mildly stressed cows, while severely stressed cows exhibited significantly lower milk fat content. miR-199a-3p regulates protein synthesis through the AKT/mTOR signaling pathway [**?**]. miR-15a targets the growth hormone receptor, inhibiting mammary epithelial cell proliferation and casein secretion [**?**]. miR-200a targets signal transducer and activator of transcription 4 (STAT4), a key gene in milk fat synthesis [**?**]. miR-142-3p targets the prolactin receptor to regulate casein and triglyceride secretion in mammary epithelial cells [**?**]. Heat stress reduces skeletal muscle fat content while increasing abdominal fat, causing oxidation of unsaturated fatty acids in muscle tissue and producing off-flavors that compromise meat quality [**?**]. miR-130 targets peroxisome proliferator-activated receptor γ (PPARγ) to regulate adipocyte differentiation and fat deposition [**?**]. miR-27b inhibits intramuscular fat deposition in beef cattle by targeting lipoprotein lipase (LPL) and regulating MAPK and Wnt signaling pathways [**?**].

2.5 Regulation of Reproductive Performance

Heat stress affects sperm motility, reduces conception rates, and increases early embryonic mortality and abortion rates [**?**]. Ji et al. [**?**] found that high temperature and oxidative stress decrease miR-15 expression in sperm, which mitigates stress-induced damage by targeting the 3 UTR region of HSPA1B. miRNAs play essential roles in reproductive development, undergoing an initial decrease followed by increase during progression from single-cell division to the 4-cell stage. The miR-290 cluster is upregulated 15-fold, activating the Wnt signaling pathway by targeting Dickkopf-related protein 1 (Dkk-1) to facilitate embryogenesis, development, and stem cell differentiation [**?**]. Nehammer et al. [**?**] reported that heat stress reduces egg production; miR-80 mutant *C. elegans* showed significantly decreased egg production at 28°C compared to wild-type, while miR-71, miR-80, and miR-239 mutants almost ceased egg production at 30°C. The researchers suggested that reduced egg production in miRNA-deficient nematodes under high temperature may result from either increased gonadal sensitivity or inability of embryos lacking specific miRNAs to survive. These studies confirm that livestock and poultry can mitigate heat stress damage to reproductive performance through miRNA-mediated mechanisms.

Conclusion

As a post-transcriptional regulatory mechanism, miRNAs play crucial roles in multiple aspects of heat stress responses, including cell growth and apoptosis, immune and anti-stress reactions, growth performance, and reproductive performance. Investigating the regulatory functions of miRNAs under heat stress conditions, including differentially expressed miRNAs, coordinated actions of miRNA families, and their target gene regulatory networks and signaling pathways, will facilitate the development of molecular strategies to alleviate heat stress-induced damage in animals.

References

[**?**] Huang Ruhua, Zhang Qiao' e, Su Jiayu, et al. Comparison of milk composition in different seasons in Ningxia region [J]. Heilongjiang Animal Science and Veterinary Medicine: Science and Technology Edition, 2013(12): 77-79.

[**?**] Song Daijun, He Qin, Yao Yanchu. Effects of heat stress on production performance and serum hormone concentrations of dairy cows at different lactation stages [J]. Chinese Journal of Animal Nutrition, 2013, 25(10): 2294-2302.

[**?**] Friedman J R, Kaestner K H. The foxa family of transcription factors in development and metabolism [J]. Cellular and Molecular Life Sciences CMLS, 2006, 63(19/20): 2317-2328.

[**?**] Friedman R C, Farh K K H, Burge C B, et al. Most mammalian mRNAs are conserved targets of microRNAs [J]. Genome Research, 2009, 19(1): 92-105.

[**?**] Sun W, Li Y S J, Huang H D, et al. MicroRNA: a master regulator of

cellular processes for bioengineering systems [J]. Annual Review of Biomedical Engineering, 2010, 12: 1-27.
[?] Yu J, Yin P, Liu F, et al. Effect of heat stress on the porcine small intestine: a morphological and gene expression study [J]. Comparative Biochemistry and Physiology-Part A: Molecular and Integrative Physiology, 2010, 156(1): 199-128.
[?] Yu J, Liu FH, Yin P, et al. Integrating miRNA and mRNA expression profiles in response to heat stress-induced injury in rat small intestine [J]. Functional & Integrative Genomics, 2011, 11(2): 203-213.
[?] Filipowicz W, Bhattacharyya S N, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight [J]. Nature Reviews Genetics, 2008, 9(2): 102-114.
[?] Ambros V. MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing [J]. Cell, 2003, 113(6): 673-676.
[?] Lee R C, Feinbaum R L, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14 [J]. Cell, 1993, 75(5): 843-854.
[?] Han J J, Lee Y, Yeom K H, et al. The Drosha-DGCR8 complex in primary microRNA processing [J]. Genes & Development, 2004, 18(24): 3016-3027.
[?] Kim V N. MicroRNA precursors in motion: exportin-5 mediates their nuclear export [J]. Trends in Cell Biology, 2004, 14(4): 156-159.
[?] Zeng Y. Principles of microRNA production and maturation [J]. Oncogene, 2006, 25(46): 6156-6162.
[?] Baek D, Villén J, Shin C, et al. The impact of microRNAs on protein output [J]. Nature, 2008, 455(7209): 64-71.
[?] Rhoads R P, Baumgard L H, Suagee J K, et al. Nutritional interventions to alleviate the negative consequences of heat stress [J]. Advances in Nutrition, 2013, 4(3): 267-276.
[?] Li X R, Cai M J. Recovery of the yeast cell cycle from heat shock-induced G1 arrest involves a positive regulation of G1 cyclin expression by the S phase cyclin Clb5 [J]. Journal of Biological Chemistry, 1999, 274(34): 24220-24231.
[?] Collier R J, Stiening C M, Pollard B C, et al. Use of gene expression microarrays for evaluating environmental stress tolerance at the cellular level in cattle [J]. Journal of Animal Science, 2006, 84: 1-13.
[?] Tang Jianzhong, Zhang Tao, Ding Shuqin, et al. pSicoR/miR-17-5p targeting ULK1 and its effect on autophagy [J]. Journal of Clinical Laboratory Science, 2014, 3(3): 698-704.
[?] Boulias K, Horvitz H R. The C. elegans microRNA mir-71 acts in neurons to promote DAF-16/FOXO-dependent longevity through germline-mediated signals [J]. Cell Metabolism, 2012, 15(4): 439-450.
[?] Wilmink G J, Roth C L, Ibey B L, et al. Identification of microRNAs associated with hyperthermia-induced cellular stress response [J]. Cell Stress and Chaperones, 2010, 15(6): 1027-1038.
[?] Li Huixia, Wang Zhenyun, Zhang Zhen, et al. Effects of miRNA-24 on proliferation and apoptosis of bovine mammary epithelial cells under high temperature conditions [J]. Scientia Agricultura Sinica, 2010, 43(22): 4732-

4738.
[?] Murakami A E, Sakamoto M I, Natali M R M, et al. Supplementation of glutamine and vitamin E on the morphometry of the intestinal mucosa in broiler chickens [J]. Poultry Science, 2007, 86(3): 488–495.
[?] Tang H B, Lee M, Sharpe O, et al. Oxidative stress-responsive microRNA-320 regulates glycolysis in diverse biological systems [J]. The FASEB Journal, 2012, 26(11): 4710–4721.
[?] Iwasaki S, Kobayshi M, Yoda M, et al. Hsc70/Hsp90 chaperone machinery mediates ATP-dependent RISC loading of small RNA duplexes [J]. Molecular Cell, 2010, 39(2): 292–299.
[?] Fukuoka M, Yoshida M, Eda A, et al. Gene silencing mediated by endogenous microRNAs under heat stress conditions in mammalian cells [J]. PLoS One, 2014, 9(7): e103130.
[?] Liu Lili, Xie Hongbing, Yang Yongsheng, et al. Research progress on gene chip screening of differentially expressed genes under heat stress in livestock and poultry [J]. Chinese Journal of Animal Nutrition, 2012, 24(12): 2287–2294.
[?] Koll i V, Upadhyay R C, Singh D. Peripheral blood leukocytes transcriptomic signature highlights the altered metabolic pathways by heat stress in zebu cattle [J]. Research in Veterinary Science, 2014, 96(1): 102–110.
[?] Zheng Y, Chen K L, Zheng X M, et al. Identification and bioinformatics analysis of microRNAs associated with stress and immune response in serum of heat-stressed and normal Holstein cows [J]. Cell Stress and Chaperones, 2014, 19(6): 973–981.
[?] Beninson L A, Brown P N, Loughridge A B, et al. Acute stressor exposure modifies plasma exosome-associated heat shock protein 72 (Hsp72) and microRNA (miR-142-5p and miR-203) [J]. PLoS One, 2014, 9(9): e108748.
[?] Ru P, Steele R, Hsueh E C, et al. Anti-miR-203 upregulates SOCS3 expression in breast cancer cells and enhances cisplatin chemosensitivity [J]. Genes & Cancer, 2011, 2(7): 720–727.
[?] Cai Mingcheng. Effects of heat stress on physiological and biochemical indices and peripheral blood microRNA expression levels in beef cattle [D]. Master' s thesis. Chongqing: Southwest University, 2014.
[?] Wong P K K, Campbell I K, Egan P J, et al. The role of the interleukin-6 family of cytokines in inflammatory arthritis and bone turnover [J]. Arthritis & Rheumatism, 2003, 48(5): 1177–1189.
[?] Thu Y M, Richmond A. NF- B inducing kinase: a key regulator in the immune system and in cancer [J]. Cytokine & Growth Factor Reviews, 2010, 21(4): 213–226.
[?] McKenna L B, Schug J, Vourekas S A, et al. MicroRNAs control intestinal epithelial differentiation, architecture, and barrier function [J]. Gastroenterology, 2010, 139(5): 1654–1664.
[?] Jin Xiaolu, Yang Jianxiang, Li Zhen, et al. Research progress on miRNAs related to mammary gland development and lactation [J]. Hereditas, 2013, 35(6): 695–706.
[?] Biton M, Levin A, Slyper M, et al. Epithelial microRNAs regulate gut mucosal immunity via epithelium–T cell crosstalk [J]. Nature Immunology,

2011, 12(3): 239-246.
[?] Antonini D, Russo M T, De Rosa L, et al. Transcriptional repression of miR-34 family contributes to p63-mediated cell cycle progression in epidermal cells [J]. Journal of Investigative Dermatology, 2010, 130(5): 1249-1257.
[?] Qin H W, Niyongere S A, Lee S J, et al. Expression and functional significance of SOCS-1 and SOCS-3 in astrocytes [J]. The Journal of Immunology, 2008, 181(5): 3167-3176.
[?] Naeem A, Zhong K, Moisá S J, et al. Bioinformatics analysis of microRNA and putative target genes in bovine mammary tissue infected with *Streptococcus uberis* [J]. Journal of Dairy Science, 2012, 95(11): 6397-6408.
[?] Plaizier J C, Khafipour E, Li S, et al. Subacute ruminal acidosis (SARA), endotoxins and health consequences [J]. Animal Feed Science and Technology, 2012, 172(1/2): 9-21.
[?] Khafipour E, Krause D O, Plaizier J C. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation [J]. Journal of Dairy Science, 2009, 92(3): 1060-1070.
[?] Babon J J, Varghese L N, Nicola N A. Inhibition of IL-6 family cytokines by SOCS3 [J]. Seminars in Immunology, 2014, 26(1): 13-19.
[?] You Qiaoying, Li Chenjiang. Some inflammatory factors and insulin resistance [J]. Foreign Medical Sciences (Section of Endocrinology), 2004, 24(3): 183-186.
[?] Zhang W J, Shen X J, Xie L Y, et al. MicroRNA-181b regulates endotoxin tolerance by targeting IL-6 in macrophage RAW264.7 cells [J]. Journal of Inflammation, 2015, 12: 18.
[?] Chassin C, Kocur M, Pott J, et al. MiR-146a mediates protective innate immune tolerance in the neonate intestine [J]. Cell Host & Microbe, 2010, 8(4): 358-368.
[?] Qin H W, Niyongere S A, Lee S J, et al. Expression and functional significance of SOCS-1 and SOCS-3 in astrocytes [J]. The Journal of Immunology, 2008, 181(5): 3167-3176.
[?] Wang Fei, Huang Yi, Li Yansen, et al. Expression of Keap1/Nrf2/ARE pathway-related genes in heat-induced oxidative stress mouse liver [J]. Chinese Journal of Biochemistry and Molecular Biology, 2014, 30(3): 284-290.
[?] Van Balkom B W M, De Jong O G, Smits M, et al. Endothelial cells require miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells [J]. Blood, 2013, 121(19): 3997-4006.
[?] Maes O C, An J, Sarojini H, et al. Murine microRNAs implicated in liver functions and aging process [J]. Mechanisms of Ageing and Development, 2008, 129(9): 534-541.
[?] Kadzere C T, Murphy M R, Silanikove N, et al. Heat stress in lactating dairy cows: a review [J]. Livestock Production Science, 2002, 77(1): 59-91.
[?] Whelock J B, Rhoads R P, VanBaale M J, et al. Effects of heat stress on energetic metabolism in lactating Holstein cows [J]. Journal of Dairy Science, 2010, 93(2): 644-655.
[?] Baumgard L H, Abuajamieh M K, Stoakes S K, et al. Feeding and managing cows to minimize heat stress [C]//Proceedings of the 23rd Tri-State Dairy

Nutrition Conference, Fort Wayne, Indiana, USA, 14-16 April 2014. Columbo: Ohio State University, 2014: 61–74.
[?] Jordans D, Krüger M, Willmes D M, et al. Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism [J]. Nature Cell Biology, 2011, 13(4): 434–446.
[?] Fatima A, Waters S, O' Boyle P, et al. Alterations in hepatic miRNA expression during negative energy balance in postpartum dairy cattle [J]. BMC Genomics, 2014, 15: 28.
[?] Jiang Y, Langley B, Lubin F D, et al. Epigenetics in the nervous system [J]. The Journal of Neuroscience, 2008, 28(46): 11753–11759.
[?] Bernabucci U, Basiricò L, Morera P, et al. Effect of summer season on milk protein fractions in Holstein cows [J]. Journal of Dairy Science, 2015, 98(3): 1815–1827.
[?] Cowley F C, Barber D G, Houlihan A V, et al. Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism [J]. Journal of Dairy Science, 2015, 98(4): 2356–2368.
[?] Cheng Jianbo, Wang Weiyu, Zheng Nan, et al. Natural production conditions reveal heat stress cycle changes indicating "heat stress milk protein depression syndrome" in mid-lactation dairy cows [J]. China Animal Husbandry & Veterinary Medicine, 2014, 41(10): 73–84.
[?] Zhang Fanjian, Xu Cong, Weng Xiaogang, et al. Effects of different heat stress levels on milk yield and composition in mid-lactation dairy cows [J]. Chinese Journal of Veterinary Science, 2014, 34(10): 1686–1688.
[?] Shen L, Sun C M, Li Y Y, et al. MicroRNA-199a-3p suppresses glioma cell proliferation by regulating the AKT/mTOR signaling pathway [J]. Tumor Biology, 2015, 36(9): 6929–6938.
[?] Li H M, Wang C M, Li Q Z, et al. MiR-15a decreases bovine mammary epithelial cell viability and regulates growth hormone receptor expression during lactation [J]. Molecules, 2012, 17(10): 12037–12048.
[?] Zhang Liping, Luo Jun, Lin Xianzi, et al. Expression analysis of miR-200 family in mammary tissue of Xinong Saanen dairy goats [J]. Acta Veterinaria et Zootechnica Sinica, 2013, 44(6): 944–951.
[?] Li Huiming. Regulation of miR-142-3p on the expression of Prlr, an important functional gene for mouse mammary development and lactation [D]. PhD dissertation. Harbin: Northeast Agricultural University, 2013.
[?] Feng Yuejin, Gu Xianhong. Research progress on effects and mechanisms of heat stress on pork quality [J]. China Animal Husbandry & Veterinary Medicine, 2013, 40(2): 96–99.
[?] Li Hongyi, Xi Qianyun, Zhang Yongliang. Regulatory role of miR-130a in porcine subcutaneous adipocyte differentiation [J]. Chinese Journal of Biochemistry and Molecular Biology, 2014, 30(12): 1216–1222.
[?] Wang Haiyang, Zheng Yue, Li Huixia, et al. Analysis of miRNA expression profiles and miR-27b target genes in intramuscular and subcutaneous fat of Simmental cattle [J]. Scientia Agricultura Sinica, 2013, 46(18): 3894–3900.
[?] De Rensis F, Garcia-Ispierto I, López-Gatius F. Seasonal heat stress: clinical implications and hormone treatments for dairy cow fertility [J]. Theriogenology,

2015, 84(5): 659-666.
[?] Ji Z L, Lu R J, Mou L S, et al. Expressions of miR-15a and its target gene HSPA1B in the spermatozoa of patients with varicocele [J]. Reproduction, 2014, 147(5): 693-701.
[?] Tang F C, Kaneda M, Carroll D O, et al. Maternal microRNAs are essential for mouse zygotic development [J]. Genes & Development, 2007, 21(6): 644-648.
[?] Zovoilis A, Smorag L, Pantazi A, et al. Members of the miR-290 cluster modulate in vitro differentiation of mouse embryonic stem cells [J]. Differentiation, 2009, 78(2/3): 69-78.
[?] Nehammer C, Podolska A, Mackowiak S D, et al. Specific microRNAs regulate heat stress responses in *Caenorhabditis elegans* [J]. Scientific Reports, 2015, 5: 8866.

Note: Figure translations are in progress. See original paper for figures.