In Utero Heat Stress Programs Reduced Performance and Health in Calves

Geoffrey E. Dahl, PhD*, Amy L. Skibiel, PhD, Jimena Laporta, PhD

KEYWORDS

• Mammary development • Methylation • Milk yield • Heat stress

KEY POINTS

- In utero heat stress reduces the birth weight of calves.
- In utero heat stress compromises passive transfer.
- Methylation patterns differ in hepatic and mammary tissues after in utero heat stress.
- Compared with calves born to cooled dams, calves from heat stressed dams have lower milk yields.

INTRODUCTION: EFFECTS OF LATE GESTATION HEAT STRESS ON THE DAM

It is well-recognized that heat stress, characterized by high ambient temperature and relative humidity, is a major factor adversely affecting cattle throughout the world, even in temperate regions.[1] Heat stressed dairy cows are more vulnerable to disease, have reduced fertility, and drastically lower milk production.[2] In fact, milk yield decreases of up to 40% have been reported,[1,3,4] which is partially attributed to the reduced feed intake of heat stressed cows, with the remaining decline caused by physiologic adjustments in an effort to dissipate metabolic heat.[5,6] It is estimated that, in the United States alone, environmental heat stress on lactating cows costs the dairy industry more than $1.5 billion in losses annually owing to decreased milk yield and increased morbidity and mortality.[1,7] For that reason, many farms in the United States have adopted heat abatement systems, such as fans and water sprayers, to actively cool lactating cows.[8] However, this estimation only accounts for lactating cows. Our recent study estimated that heat stress

Disclosure Statement: Work reported in this paper was supported by grants from the National Science Foundation (Award #1247362 to GED), USDA-NIFA (Award #2015–67015–23409 to GED) and the UF/IFAS Climate Change Grant Program (to JL).
Department of Animal Sciences, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, 2250 Shealy Drive, POB 110910, FL 32611, USA
* Corresponding author.
E-mail address: gdahl@ufl.edu

Vet Clin Food Anim 35 (2019) 343–353
https://doi.org/10.1016/j.cvfa.2019.02.005
vetfood.theclinics.com

occurring only during the dry period translates into an additional $810 million in milk losses annually.[9] Dairy industries in hot states such as Florida, Texas, and California suffer the greatest economic impact, but even states such as Michigan and Wisconsin experience hundreds of kilograms of milk losses in a typical lactation owing to dry period heat stress.

Although cows are highly susceptible to heat stress during lactation, heat stress during the dry period, the nonlactating period between successive lactations, also negatively impacts milk production in the subsequent lactation.[10–12] For example, housing late gestation dry cows (from approximately 230 days pregnant to calving) in shaded barns without access to active cooling devices, such as water soakers and fans, induces heat stress that cause systemic and tissue-specific changes that culminate in milk loss (approximately 5 kg per cow per day) in the subsequent lactation.[10,12–14] Moreover, when cows are heat stressed during the dry period, they exhibit altered mammary gland microstructure during early subsequent lactation, featuring fewer alveoli compared with cooled cows.[15] Also, nonlactating, heat-stressed cows have aberrant patterns of hepatic protein expression consistent with oxidative stress, mitochondrial dysfunction, and liver-specific changes in lipid and glucose metabolism early postpartum.[16]

Heat stress, therefore, negatively impacts mature cows during established lactation and during the dry period, wherein it causes recoverable effects on mammary output and metabolic accommodations to reduce heat load. In dry cows, heat stress programs mammary growth and reduces yield in the subsequent lactation. The reduction in yield is associated with decreased autophagy[17] and apoptosis[11] during involution shortly after cessation of milk removal, followed by a delay in epithelial cell proliferation later in the dry period.[11] These programming effects, however, seem to be transient and restricted to that lactation, whereas the impacts of in utero heat stress on the developing fetus are permanent and transgenerational. Those effects are the focus of the remainder of this article.

DEVELOPMENTAL PROGRAMMING AND THE MAMMARY GLAND

During specific developmental windows, conditions experienced in early life can affect gene expression, cells, tissues, and organs with consequences for future physiologic function, health, and disease outcomes later in life,[18,19] a concept known as developmental programming.[20] The dry period of a dairy cow coincides with the last trimester of gestation, a time of maximal fetal development.[21] As a cow is stressed in late gestation, so too is the fetus, which shapes their future performance. It is evident from nutritional studies in ruminants that conditions experienced early in life can program future mammary function. Fetuses of ewes fed a maintenance diet throughout gestation have heavier mammary glands compared with fetuses carried by ewes on an ad libitum diet. Independent of diet, ewe size during pregnancy also influences fetal mammary development. Fetuses of heavier ewes have a larger mammary ductal area.[22] Further, lambs of heavier ewes and those fed a maintenance diet also produce more milk during their first lactation, indicative of in utero programming of mammary function through changes in early gland development.

Early life nutritional manipulation can also impact mammary development. Calves fed a higher plane of nutrition during the preweaning period produce more milk during the first lactation.[23,24] This may occur through an increase in parenchymal and mammary fat pad weight, and parenchymal DNA content (an indicator of cell number).[24] Geiger and colleagues[25] also reported greater mammary epithelial cell (MEC) proliferation after weaning among calves fed a greater energy milk replacer before weaning,

although others have found no effect of nutrition on mammary cell proliferation.[26,27] Postweaning, prepubertal mammary development is also affected by diet and impacts milk production in the subsequent lactation, although in an opposite direction to the effects of preweaning nutrition.[28] For example, dietary manipulation to enhance prepubertal weight gain in dairy heifers impairs mammogenesis and decreases subsequent milk yield,[29] which is at least partially attributed to changes in mammogenic hormone secretion.[30] In addition, greater average daily gains result in heifers attaining puberty at a younger age, which truncates the allometric growth phase of mammogenesis.[27] Together these studies suggest mammary ductal and fat pad development, before the first pregnancy, are critical for secretory tissue development during pregnancy and mammary function postpartum.[31–33] But, how these developmental processes might be influenced by environmental factors such as heat stress remains unknown.

HEAT STRESS AND MAMMARY DEVELOPMENT AND FUNCTION

Factors experienced early in life, such as disease, nutrition, and management interventions, can influence the lactation performance of heifers.[34] Of particular concern is environmental heat stress. Adverse effects of high temperatures on mammary development and function have been demonstrated both in vitro and in vivo. Bovine MEC growth, as estimated by DNA content, branching morphogenesis, and ductal branch extension is completely halted over a 24-hour period of exposure to high temperature in vitro.[35] Subjecting bovine MEC to a high incubation temperature for just 1 hour induces changes in cell ultrastructure characteristic of apoptosis, including chromatin condensation, formation of apoptotic bodies, and the presence of secondary lysosomes.[36] Moreover, increases in the proportion of MEC undergoing apoptosis and necrosis and decreases in cell viability and proliferation are detected after acute heat stress of both bovine and buffalo MEC in vitro.[36–38] In dairy cattle in vivo, dry pregnant cows exposed to heat stress have lower mammary cell proliferation relative to cows provided with active cooling.[11] The capacity of the lactating mammary gland to synthesize milk is a product of the number and metabolic activity of the secretory epithelial cells.[39] Thus, the inhibition of proliferation and increased cell death via apoptosis and necrosis likely contribute to reduced milk yield among heat-stressed cows.

Changes in molecular events in response to heat exposure have also been reported. Bovine MEC incubated briefly at high temperature upregulate expression of genes in the heat shock protein family.[35,37] Collier and colleagues[35] found that heat shock protein-70 gene expression increases up to approximately 4 hours after heat stress initiation followed by a sharp decrease to basal expression level concurrent with an increase in expression of proapoptotic genes. This decreases suggests thermotolerance loss as the duration of heat exposure increases. Similarly, the exposure of cultured buffalo MEC to high temperature induces changes in the expression of genes involved in apoptosis.[38] *BAX*, a proapoptotic gene, was upregulated, whereas, *BCL-2*, an antiapoptotic gene, and *IGFBP-5* were downregulated. IGFBP-5 is involved in tissue turnover by reducing the availability of IGF-1 and by its involvement in apoptosis via promotion of extracellular matrix degradation.[40] Also, IGFBP-5 increases in response to prolactin, which is higher in systemic circulation of heat-stressed, dry cows in vivo.[10,41] Shortly after heat stress initiation, bovine MEC increase expression of genes and proteins involved in the stress response and DNA and protein repair, with a downregulation of genes associated with the cell cycle, differentiation, the cytoskeleton, and milk synthesis.[35,37] Likewise, bovine MEC exposed to acute thermal stress

have a lower expression of fatty acid synthase and several casein proteins, as well as lower concentrations of fatty acid synthase and beta casein in the culture media, indicative of milk synthesis impediment.[42] However, these results were not replicated in an in vivo study of heat-stressed dry cows, which did not detect differences in the expression of several genes involved in milk synthesis.[14] These empirical studies have contributed to our understanding of the cellular and molecular events occurring in mammary tissue after heat stress exposure.

Our recent transcriptomics analysis of mammary glands harvested from heat stressed or cooled dry cows, further support an effect of heat stress on mammary gland development and function.[43] Enrichment analysis of differentially expressed genes revealed that genes impacted by heat stress play a role in key processes in mammary gland development and health, such as ductal branching morphogenesis, extracellular matrix remodeling, cell death and proliferation, immune function, inflammation, and protection from cellular stress. The involvement of epithelial cell cilia in ductal branching morphogenesis is mediated by signaling pathways, such as Wnt and Sonic Hedgehog. Several genes in these pathways, including WIF1, LCA5, and MYO3B, were downregulated in the mammary gland of heat-stressed cows during the initial 14 days of the dry period. Moreover, we found upstream regulators and target genes involved in branching morphogenesis were negatively impacted by heat stress. This study is the first to directly link these genes and physiologic functions to an in vivo heat-stressed bovine model. Several genes associated with extracellular matrix degradation, such as MMP7 and MMP16, apoptosis, and lysosomal activity were downregulated, whereas Hsp40 was upregulated. The latter is congruent with reports of enhanced expression of genes and proteins in the heat shock family in bovine MEC in vitro when exposed to a thermal insult.

IN UTERO HEAT STRESS EFFECTS ON MAMMARY DEVELOPMENT AND FUNCTION

Heat stress also exerts transgenerational effects on the subsequent generation of heifers born to cows that experience heat stress in late gestation. The fetus can be affected by maternal heat stress through the intrauterine environment. Across 5 years of studies by our group, heifers born to dams that were heat stressed during late gestation were smaller through 1 year of age and produced significantly less milk (ie, 5 kg/d) in their first lactation relative to heifers born to dams that were cooled during late gestation, despite their similar age and weight at calving.[44] A more recent metaanalysis of 9 years of heat stress studies using the same experimental design (late gestation cows that experienced cooling or heat stress) confirmed that heat-stressed late gestation cows produce less milk during the subsequent lactation (**Fig. 1**A) and revealed in utero heat-stressed heifers produce significantly less milk during their first and second lactations, approximately 3.5 kg/d less, relative to in utero cooled heifers (**Fig. 1**B, C).[45] Through a preliminary study, we found in utero heat-stressed heifers have smaller mammary alveoli composed of fewer milk-producing cells during their first lactation relative to heifers born to cooled dams, which likely contributes to their poorer lactation performance.[46] Results of these studies point to effects of in utero heat stress on early mammary development that impair future growth, structure, and function.

To assess whether the thermal conditions experienced as a fetus alters tissue structure and cellular processes in the mammary gland, we harvested mammary biopsies from heifers (eg, gestated by heat stressed or cooled dams) at 21 and 42 days into their first lactation (entailing the early rising phase of milk yield and peak lactation, respectively).[46] Using immunohistochemistry techniques, we estimated the proportion

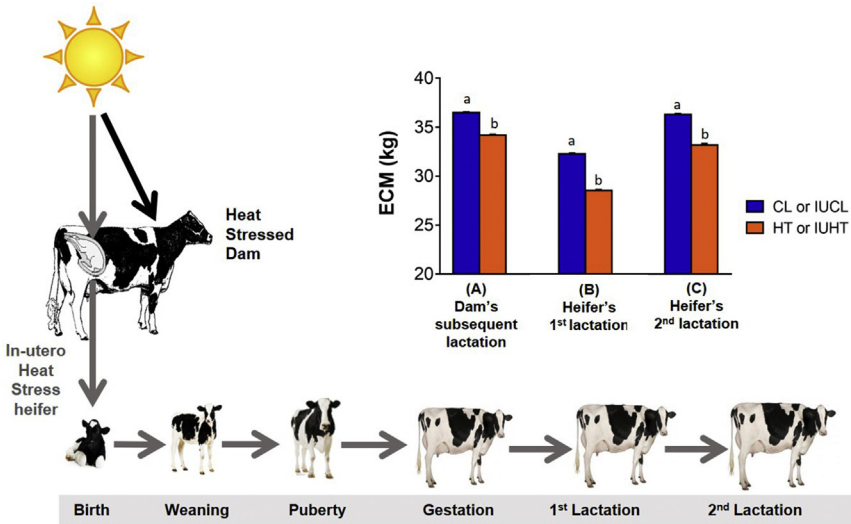

Fig. 1. (*A*) Dam energy-corrected milk yield (ECM) during subsequent lactation if under heat stress (HT, shade only; *orange bars*) or cooling (CL, fans and soakers; *blue bars*) when dry (approximately 46 days before calving). (*B*) First and (*C*) second lactation ECM of heifers born to heat stressed (IUHT, *orange bars*) or cooled dams (IUCL, *blue bars*). Different letters indicate significant differences between groups ($P<.05$).

of cells undergoing apoptosis and proliferation, the 2 main cell processes responsible for cell turnover in the lactating mammary gland.[47] The mammary glands of in utero heat-stressed heifers had alveoli with smaller luminal area compared with in utero cooled heifers, although the mammary alveoli number was similar between groups (**Fig. 2**). In addition, mammary glands of in utero heat-stressed heifers tended to contain a higher proportion of stromal connective tissue.

Alveolar size was associated with the number of secretory cells; smaller alveoli had fewer secretory epithelial cells. Thus, the mammary glands of in utero heat-stressed heifers had lower milk secretory capacity. In utero heat-stressed heifers had a lower percent of proliferating mammary cells, but no effect on the number of cells undergoing apoptosis (see **Fig. 2**).[46] Similarly, Tao and colleagues[11] documented reduced cell proliferation among heat-stressed, late gestation cows. Importantly, the disparity in mammary tissue morphology between in utero heat-stressed and in utero cooled heifers was not attributed to differences in the time available for secretory tissue growth and differentiation during gestation because both in utero heat-stressed and in utero cooled heifers had average gestation durations of 237 days. Likewise, differences in mammary structure were not attributed to disparities in thermal load during lactation or calf birth weight, because rectal temperatures, respiration rates, and calf birth weights were similar between in utero heat-stressed and cooled heifers. Overall, these studies suggest that an unfavorable intrauterine environment initiates aberrant mammary development that persists at least through the second lactation, more than 2 years after the insult.

Influences of the early life environment on offspring phenotype are often mediated by epigenetic modifications, such as DNA methylation, that regulate tissue-specific gene expression. In the mammary gland, the epigenome plays a critical role in the progressive commitment of mammary stem cells to specific progenitors and

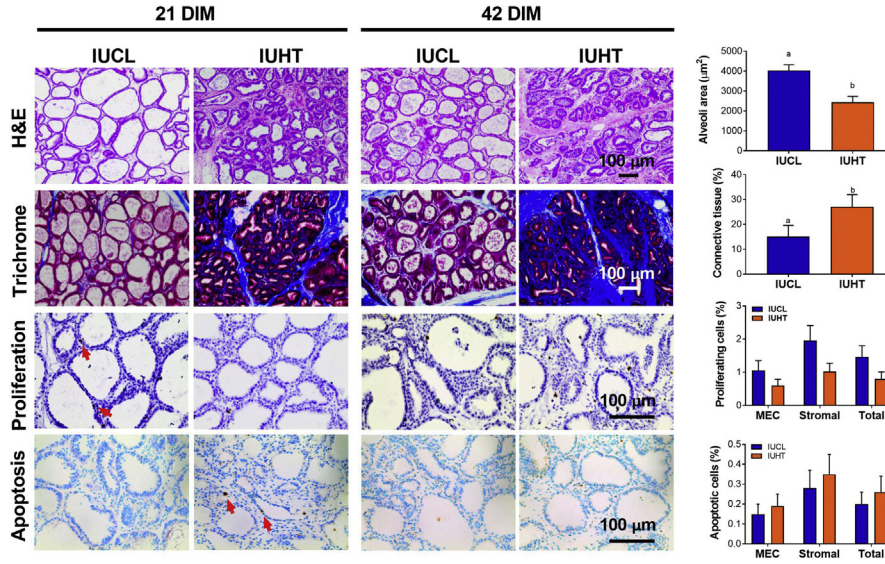

Fig. 2. Histology of mammary glands from first lactation heifers (21 and 42 days in milk [DIM]) born to heat stressed or cooled dams (IUHT vs IUCL). Mammary gland alveoli and connective tissue area (stain: hematoxylin and eosin and Masson's trichrome, respectively), percent of cells proliferating (Ki-67 assay), and percent of apoptotic cells (TUNEL assay). IUHT, *orange bars*; IUCL, *blue bars*. *Red arrows* indicate cells proliferating or undergoing apoptosis. Different letters indicate significant differences between groups ($P<.05$). (*From* Skibiel AL, Dado-Senn BM, Fabris TF, et al. In utero exposure to thermal stress has long-term effects on mammary gland microstructure and function in dairy cattle. PLoS One 2018b;13:e0206046, with permission.)

differentiated cells.[48] In addition, mammary cell maintenance and milk synthesis can be mediated by epigenetic mechanisms.[49] The growth and development of the liver, a key organ supporting the metabolic demands of copious milk secretion, is also modulated by the epigenome.[50] For these reasons, we examined the methylation profiles of mammary tissue harvested from in utero heat-stressed and cooled heifers during their first lactation and the liver of in utero heat-stressed and cooled bull calves at birth.[51] We identified more than 300 genes differentially methylated between the in utero heat-stressed and cooled groups associated with functions such as cell signaling, transcription, enzyme activation, immune function, cell proliferation, apoptosis, and development (**Fig. 3**). Heat stress induced changes in the epigenetic profiles of genes involved in proliferation, apoptosis, and development are particularly interesting in light of our histologic results (see **Fig. 2**) and our observation that many organs, including the liver, are lighter at birth in calves born to heat-stressed dams.[52,53] Notably, 50 of the differentially methylated genes identified were common to both heifer mammary gland and bull calf liver, suggesting that in utero heat stress may epigenetically program organs critical to lactation in a similar manner.

OTHER PHYSIOLOGIC IMPACTS OF IN UTERO HEAT STRESS

One of the clearest phenotypic observations made regarding in utero heat stress is a decrease in early life immune status; specifically, heat-stressed calves have lower circulating immunoglobulin concentrations compared with calves from cooled

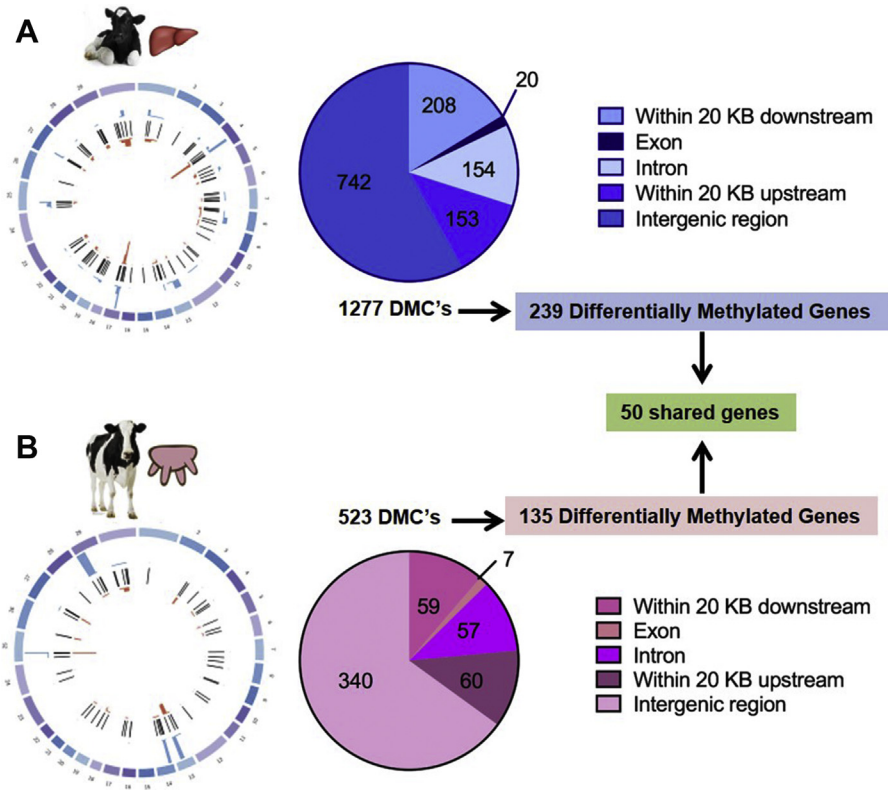

Fig. 3. Genomic and chromosomal locations of differentially methylated cytosines (DMCs) and genes (DMGs). Liver tissue was collected from in utero heat stressed and in utero cooled bull calves at birth. Mammary tissue was collected from in utero heat stressed and in utero cooled heifers at 21 days into their first lactation. Chromosomal and genomic location of DMGs and DMCs (*circular plot*) and genomic locations of DMCs (*pie graph*) for (*A*) bull calf liver and (*B*) heifer mammary gland.

dams.[54] We have shown that this response is not due to altered colostrum quality or IgG content, but rather is related to specific calf factors.[55] Further investigation supports the concept that in utero heat stress accelerates gut closure, thereby decreasing the capacity for IgG uptake regardless of the colostrum source.[53] Reduced IgG uptake owing to accelerated gut closure leads to lower immune status early in life, which is associated with poorer survival and a decreased number of heifers completing the first lactation after in utero heat stress.[44]

In utero heat stress alters endocrine systems with effects evident at birth and through early life that are consistent with metabolic adaptations to accumulate energy in peripheral tissues and reduce lean growth. Specifically, calves born to heat-stressed dams have greater circulating insulin in the first week of life relative to those born to cooled dams.[2] As calves that experience in utero heat stress develop, altered glucoregulatory responses are evident as illustrated by increased glucose clearance rate after a glucose tolerance test and insulin challenge relative to calves that were born to cooled dams. Of interest, circulating concentrations of cortisol at birth are increased in calves born to cooled dams relative to those from heat-stressed dams, but this effect dissipates quickly in early life.[54] Metabolic shifts may account for

differences in adiposity later in life in heat-stressed calves similar to lambs that experience intrauterine growth restriction.[56,57] Indeed, we have noted persistent decreases in body weight in heat-stressed calves as they grow and that is accompanied by reduced stature, a gross indicator of lean mass accretion.[55] Because adiposity around puberty has been related to subsequent milk yield,[58] the potentially greater accumulation of fat in calves from heat-stressed dams may further predispose those calves to reduced milk yield and altered mammary growth.

Heat stress in utero clearly causes adaptive responses to thermoregulation as well, but they seem to vary with age. At birth, in utero heat-stressed calves have a higher rectal temperature relative to calves from cooled dams,[59] which is consistent with the elevated uterine temperature found in heat-stressed dams. However, that elevation of rectal temperature continues at least through 28 days of life, suggesting an extended adaptation beyond the influence of the uterine environment. At maturity, there is no difference in basal rectal temperatures when comparing cows that were heat stressed in utero with herd mates gestated in cooled dams.[52] But responses to acute heat stress do differ, with in utero heat-stressed animals maintaining normal rectal temperatures after 8 hours of acute heat stress, whereas cows born to cooled dams show increases in rectal temperatures under the same conditions. Functionally, the in utero heat-stressed cows have higher skin temperatures but lower sweating rates than in utero cooled cows, which may reflect a greater capacity to rid the body of heat relative to cows from cooled dams. However, this adaptive response seems to be a trade-off with lactation performance.

SUMMARY

Our group has documented numerous adverse effects of in utero heat stress on the postnatal calf (reviewed herein and in Refs.[2,60]). Moreover, heifers born to heat-stressed dams produce less milk during their first and second lactation.[44,45] Evidence from the literature and our preliminary data suggest in utero heat stress derails normal mammary development, which affects mammary growth throughout postnatal life, and ultimately impairs function. These shifts in mammary function are mirrored by altered endocrine, growth, and thermoregulatory responses. Accumulating evidence supports the concept that in utero heat stress programs a phenotype of reduced productivity at maturity in the dairy cow, and epigenetic mechanisms contribute to these morphologic and physiologic adaptations.

REFERENCES

1. Collier RJ, Dahl GE, VanBaale MJ. Major advances associated with environmental effects on dairy cattle. J Dairy Sci 2006;89:1244–53.
2. Tao S, Dahl GE. Heat stress effects during late gestation on dry cows and their calves. J Dairy Sci 2013;96:4079–93.
3. Bernabucci U, Lacetera N, Baumgard LH, et al. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal 2010;4:1167–83.
4. Hansen PJ. Genetic control of heat stress. Proc 49th Florida Dairy Prod Conf. Gainesville, Florida, March 16, 2013. p. 26–32.
5. Rhoads ML, Rhoads RP, VanBaale MJ, et al. Effects of heat stress and plane of nutrition on lactating Holstein cows: production, metabolism, and aspects of circulating somatotropin. J Dairy Sci 2009;92:1986–97.
6. Wheelock JB, Rhoads RP, VanBaale MJ, et al. Effects of heat stress on energetic metabolism in lactating Holstein cows. J Dairy Sci 2010;93:644–55.

7. St-Pierre NR, Cobanov B, Schnitkey G. Economic losses from heat stress by U.S. livestock industries. J Dairy Sci 2003;86(Suppl):E52–77.
8. Flamenbaum I. The beneficial effects of cooling cows. In: Proc. Cow Longevity Conf. Tumba, Sweden, August 28-29, 2013. p. 113–25.
9. Ferriera FC, Gennari RS, Dahl GE, et al. Economic feasibility of cooling dry cows across the United States. J Dairy Sci 2016;99:9931–41.
10. do Amaral BC, Connor EE, Tao S, et al. Heat-stress abatement during the dry period: does cooling improve transition into lactation? J Dairy Sci 2009;92: 5988–99.
11. Tao S, Bubolz JW, do Amaral BC, et al. Effect of heat stress during the dry period on mammary gland development. J Dairy Sci 2011;94:5976–86.
12. Tao S, Thompson IM, Monteiro APA, et al. Effect of cooling heat-stressed dairy cows during the dry period on insulin response. J Dairy Sci 2012;95:5035–46.
13. do Amaral BC, Connor EE, Tao S, et al. Heat stress abatement during the dry period influences metabolic gene expression and improves immune status in the transition period of dairy cows. J Dairy Sci 2011;94:86–96.
14. Tao S, Connor EE, Bubolz JW, et al. Effect of heat stress during the dry period on gene expression in mammary tissue and peripheral blood mononuclear cells. J Dairy Sci 2013;96:378–83.
15. Mejia C, Skibiel AL, Dado-Senn B, et al. Exposure of dairy cows to heat stress during late gestation or while in utero affects mammary gland microstructure. J Dairy Sci 2017;100(Suppl. 2):167 (Abstr.).
16. Skibiel AL, Zachut M, do Amaral BC, et al. Liver proteomic analysis of postpartum Holstein cows exposed to heat stress or cooling conditions during the dry period. J Dairy Sci 2018;101:705–16.
17. Wohlgemuth SE, Ramirez-Lee Y, Tao S, et al. Short Communication: effect of heat stress on mammary gland autophagy during the dry period. J Dairy Sci 2016;99: 4875–80.
18. Barker BJP, Eriksson JG, Forsén T, et al. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol 2002;31:1235–9.
19. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev 2005;85:571–633.
20. Lucas A. Programming by early nutrition in man. Ciba Found Symp 1991;156: 38–55.
21. Mao WH, Albrecht E, Teuscher F, et al. Growth- and breed-related changes of fetal development in cattle. Asian-Australas J Anim Sci 2008;21:640–7.
22. van der Linden DS, Kenyon PR, Blair HT, et al. Effects of ewe size and nutrition on fetal mammary gland development and lactation performance. J Anim Sci 2009; 87:3944–54.
23. Soberon F, Raffrenato E, Everett RW, et al. Preweaning milk replacer intake and effects on long-term productivity of dairy calves. J Dairy Sci 2012;95:783–93.
24. Geiger AJ, Parsons CLM, Akers RM. Feeding a higher plane of nutrition and providing exogenous estrogen increases mammary gland development in Holstein heifer calves. J Dairy Sci 2016;99:7642–53.
25. Geiger AJ, Parsons CLM, Akers RM. Feeding an enhanced diet to Holstein heifers during the preweaning period alters steroid receptor expression and increases cellular proliferation. J Dairy Sci 2017;100:8534–43.
26. Brown EG, VandeHaar MJ, Daniels KM, et al. Effect of increasing energy and protein intake on mammary development in heifer calves. J Dairy Sci 2005;88: 595–603.

27. Meyer MJ, Capuco AV, Ross DA, et al. Developmental and nutritional regulation of the prepubertal bovine mammary gland: II. epithelial cell proliferation, parenchymal accretion rate, and allometric growth. J Dairy Sci 2006;89:4298–304.
28. Akers RM, McFadden TB, Purup S, et al. Local IGF-I axis in peripubertal ruminant mammary development. J Mammary Gland Biol Neoplasia 2000;5:43–51.
29. Sejrsen K, Purup S. Influence of prepubertal feeding level on milk yield potential of dairy heifers: a review. J Anim Sci 1997;75:828–35.
30. Sejrsen K, Huber JT, Tucker HA. Influence of amount fed on hormone concentrations and their relationship to mammary growth in heifers. J Dairy Sci 1983;66:845–55.
31. Knight CH, Sorenson A. Windows in early mammary development: critical or not? Reproduction 2001;122:337–45.
32. Capuco AV, Ellis S, Wood DL, et al. Postnatal mammary ductal growth: three-dimensional imaging of cell proliferation, effects of estrogen treatment, and expression of steroid receptors in prepubertal calves. Tissue Cell 2002;34:143–54.
33. Akers RM. Plasticity of mammary development in the prepubertal bovine mammary gland. J Anim Sci 2017;95:5653–63.
34. Heinrichs AJ, Heinrichs BS. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. J Dairy Sci 2011;94:336–41.
35. Collier RJ, Stiening CM, Pollard BC, et al. Use of gene expression microarrays for evaluating environmental stress tolerance at the cellular level in cattle. J Anim Sci 2006;84(Suppl.):E1–13.
36. Du J, Di H-S, Guo L, et al. Hyperthermia causes bovine mammary epithelial cell death by a mitochondrial-induced pathway. J Therm Biol 2008;33:37–47.
37. Li L, Sun Y, Li X, et al. The global effect of heat on gene expression in cultured bovine mammary epithelial cells. Cell Stress Chaperones 2015;20:381–9.
38. Kapila N, Sharma A, Kishore A, et al. Impact of heat stress on cellular and transcriptional adaptation of mammary epithelial cells in riverine buffalo (*Bubalus bubalis*). PLoS One 2016;11:e0157237.
39. Capuco AV, Wood DL, Baldwin R, et al. Mammary cell number, proliferation, and apoptosis during a bovine lactation: relation to milk production and effect of bST. J Dairy Sci 2001;84:2177–87.
40. Nørgaard JV, Theil PK, Sørensen MT, et al. Cellular mechanisms in regulating mammary cell turnover during lactation and dry period in dairy cows. J Dairy Sci 2008;91:2319–27.
41. Collier RJ, Beede DK, Thatcher WW, et al. Influences of environment and its modification on dairy animal health and production. J Dairy Sci 1982;65:2213–27.
42. Li L, Wang Y, Li C, et al. Proteomic analysis to unravel the effect of heat stress on gene expression and milk synthesis in bovine mammary epithelial cells. Anim Sci J 2017;88:2090–9.
43. Dado-Senn BM, Skibiel AL, Fabris TF, et al. RNA-Seq reveals novel genes and pathways involved in bovine mammary involution during the dry period and under environmental heat stress. Sci Rep 2018;8:11096.
44. Monteiro APA, Tao S, Thompson IMT, et al. In utero heat stress decreases calf survival and performance through the first lactation. J Dairy Sci 2016;99:8443–50.
45. Laporta J, Ferreira FC, Dado-Senn B, et al. Dry period heat stress reduces dam, daughter, and granddaughter productivity. J Dairy Sci 2018;101(Suppl. 2):151 (Abstr.).

46. Skibiel AL, Dado-Senn BM, Fabris TF, et al. In utero exposure to thermal stress has long-term effects on mammary gland microstructure and function in dairy cattle. PLoS One 2018;13:e0206046.
47. Knight CH. The importance of cell division in udder development and lactation. Livest Prod Sci 2000;66:169–76.
48. Visvader JE, Stingl J. Mammary stem cells and the differentiation hierarchy: current status and perspectives. Genes Dev 2014;28:1143–58.
49. Singh K, Molenaar AJ, Swanson KM, et al. Epigenetics: a possible role in acute and transgenerational regulation of dairy cow milk production. Animal 2012;6:375–81.
50. Snykers S, Henkens T, De Rop E, et al. Role of epigenetics in liver-specific gene transcription, hepatocyte differentiation and stem cell reprogrammation. J Hepatol 2009;51:187–211.
51. Skibiel AL, Peñagaricano F, Rocio A, et al. In utero heat stress alters the offspring epigenome. Sci Rep 2018;8:14609.
52. Ahmed BMS, Younas U, Asar TO, et al. Cows exposed to heat stress during fetal life exhibit improved thermal tolerance. J Anim Sci 2017;95:3497–503.
53. Ahmed BMS, Younas U, Asar TO, et al. Maternal heat stress reduces body and organ growth in calves: relationship to immune tissue development. J Dairy Sci 2016;99:606 (Abstr.).
54. Tao S, Monteiro AP, Thompson IM, et al. Effect of late-gestation maternal heat stress on growth and immune function of dairy calves. J Dairy Sci 2012;95:7128–36.
55. Monteiro APA, Tao S, Thompson IMT, et al. Effect of heat stress during late gestation on immune function and growth performance of calves: isolation of altered colostral and calf factors. J Dairy Sci 2014;97:6426–39.
56. Morrison JL. Sheep models of intrauterine growth restriction: fetal adaptations and consequences. Clin Exp Pharmacol Physiol 2008;35:730–43.
57. Yates DT, Green AS, Limesand SW. Catecholamines mediate multiple fetal adaptations during placental insufficiency that contribute to intrauterine growth restriction: lessons from hyperthermic sheep. J Pregnancy 2011;2011:740408.
58. Silva LF, VandeHaar MJ, Whitlock BK, et al. Short communication: relationship between body growth and mammary development in dairy heifers. J Dairy Sci 2002;85:2600–2.
59. Laporta J, Fabris TF, Skibiel AL, et al. In-utero exposure to heat stress during late-gestation has prolonged effects on activity patterns and growth of dairy calves. J Dairy Sci 2017;100:2976–84.
60. Dahl GE, Tao S, Laporta J. Late gestation heat stress of dairy cattle programs dam and daughter milk production. J Anim Sci 2017;95:5701–10.