Skin and hair coat play important functions in maintaining homeostasis and thermoregulation for cattle, which can affect all modes of heat loss. Our objective was to investigate the effect of hyperthermia experienced in utero during late gestation on postnatal hair length, skin properties, and thermoregulation. Pregnant dams were heat stressed (n = 41) or actively cooled (n = 41) for the last ~56 d of gestation and gave birth to heifers that were in utero heat stressed (IUHT) or in utero cooled (IUCL), respectively. Hair samples and skin tissue biopsies were collected from neck and rump locations at birth (d 0), 1 wk after weaning (d 63), and at 12 mo. Hair samples were also obtained at 4 and 8 mo. Skin tissue was stained with hematoxylin and eosin to visualize morphology. Hair length (short and long hairs, undercoat and topcoat, respectively), stratum corneum (SC) area, SC thickness, epidermis thickness, sweat gland (SWT) number, SWT cross-sectional area, SWT average size, sebaceous gland (SEB) number, SEB cross-sectional area, SEB average size, and sweat gland depth were assessed. Respiration rate, skin temperature, sweating rate, and rectal temperature was measured weekly from d 7 to 63. Additionally, thermoregulatory patterns were measured every 4 h over a 36-h interval beginning 4 d after weaning. Data were analyzed using PROC MIXED in SAS with a main effect of in utero treatment with location and time points analyzed separately. No difference in hair parameters were detected at d 0 or 12 mo. At d 63, IUHT heifers had longer average hair length (14.8 vs. 13.8 ± 0.2 mm), shorter undercoats (9.3 vs. 10.4 ± 0.3 mm), longer topcoats (19.6 vs. 17.1 ± 0.3 mm), and a greater difference between topcoat and undercoat (10.1 vs. 7.0 ± 0.4 mm). At 4 mo, IUHT heifers had longer average hair lengths (26.1 vs. 22.2 ± 1.0 mm) and longer topcoats (36.9 vs. 33.9 ± 1.1 mm), and at 8 mo, IUHT had longer average hair lengths (17.9 vs. 16.2 ± 0.6 mm), relative to IUCL. At d 0, IUHT heifers had more (13 vs. 9 ± 2 glands) but smaller average sized SEB (neck: 1,636 vs. 2,238 ± 243 µm²; rump: 2,100 vs. 3,352 ± 379 µm²) and reduced SC area (79,243 vs. 169,419 ± 13,071 µm²). At d 63, IUHT had fewer SEB (11 vs. 15 ± 2 glands), smaller SWT (0.16 vs. 0.23 ± 0.02 mm²), fewer SWT (16 vs. 23 ± 4 glands), and deeper SWT (0.5 vs. 0.4 ± 0.03 mm²). At 12 mo, IUHT had greater distance from the skin surface to the most superficial SWT (0.016 vs. 0.015 ± 0.0004 mm), shorter distance to the deepest SWT (0.031 vs. 0.033 ± 0.001 mm), and smaller SWT (81.1 vs. 108.9 ± 10.8 µm²), relative to IUCL. When measured both weekly and hourly, IUHT heifers had higher rectal temperature and sweating rate. Overall, in utero hyperthermia triggers long-lasting hair and skin adaptations, possibly leading to differences in postnatal thermoregulation.

**Key words:** sweat gland, late gestation, sebaceous gland, heat stress

**INTRODUCTION**

Cattle balance heat loss and heat gain to maintain core body temperature within their physiological range (Hahn, 1999). Although research examining the effects of heat stress on the physiology of lactating and dry cows is abundant, literature reporting negative carry-over effects of late gestation hyperthermia on postnatal thermoregulation is emerging (Tao et al., 2012; Ahmed et al., 2017; Dado-Senn et al., 2020; Davidson et al., 2021). For mature cows, when the temperature-humidity index (THI) is above 68 (Zimbelman et al., 2009), the upper critical temperature of the thermoneutral zone (TNZ) has been surpassed. Recent literature suggests that welfare of dairy calves may be affected at a THI of 78, but significant heat stress may not be felt until a THI over 82 (Kovács et al., 2020). This higher TNZ for calves grants them an improved heat load tolerance compared with adult cows.

Temperatures above the TNZ promote heat dissipation through sensible (convective and thermal radia-
tion) and latent (evaporation) heat transfer routes. As the metabolic and environmental heat load accumulate, cattle become less effective at dissipating heat and begin experiencing increasing levels of heat stress (Bernabucci et al., 2010). Heat stress challenges the animal’s ability to maintain a steady core body temperature, compromising other physiological functions and ultimately productivity. Sensible heat loss becomes less effective at high THI and cattle rely on evaporative heat loss through the skin’s surface as a direct result of sweating or through the respiratory tract through panting (West, 2003; Maia et al., 2005a,b). It has been reported that 70 to 85% of maximal heat loss is lost by evaporative mechanisms through the skin (Finch, 1986). Thus, sweating is key to the thermoregulatory process in cattle, and therefore, skin morphology plays an important role in heat dissipation methods (Jian et al., 2014).

The skin is a multilayered organ comprised of the epidermis, dermis, and hypodermis, all of which participate in protection, immunological defense, and thermoregulation (Dellmann, 1993; Bhattacharya et al., 2003). Various species achieve evaporative cooling from the skin’s surface or respiratory tract in different ways. For example, horses are effective at sweating, dogs utilize panting, and rats wet their skin with saliva (Romanovsky, 2007). Murrah buffalo have thicker skin and more frequent and more superficial blood vessels and capillaries than Gir and Hariana cattle (Hafez et al., 1955). In comparison to buffalo, cattle have a higher efficiency for sweating but relative to horses, maximum sweating in cattle is only 10% of a horses’ ability to dissipate heat through these means (Collier et al., 2008). Moreover, Zebu cattle and Criollo cattle that carry the slick mutation are known for their adaptations (i.e., altered sweat gland distribution and slick, short hair coats, respectively), which grant them improved thermotolerance.

In the bovine, hair follicle units consist of a hair follicle, pili muscle, one sweat gland, and one or more sebaceous glands. In Bos taurus breeds, hair follicle initiation begins at fetal d 77, reaches a maximum follicle density by d 166, and hairs emerge by d 200 of gestation (Lyne and Heideman, 1959). During hair follicle initiation and growth, the epidermis and the dermis increase in thickness (Lyne and Heideman, 1959). Sweat glands reach a maximum length by fetal d 250 and their maximum depth, relative to the skin surface, is reached by birth (Lyne and Heideman, 1959).

Heat dissipation is facilitated by multiple factors, including density of hair follicles and sweat glands, nerve fibers, and capillary surface (Taneja, 1956). The capillary surface is important in the thermoregulation process because an increase in blood flow to the capillary surface is important in the thermoregulation process because an increase in body temperature (Hales et al., 1978). This ultimately activates heat dissipation from the respiratory tract and skin surface (Scharf et al., 2008), which is believed to be dependent on sweat glands (Cunningham, 2002). Yet, linkage between in utero determinants of hair development and postnatal thermoregulation has yet to be made.

Herein, we investigated the effects of prenatal heat stress exposure, specifically the last 2 mo of gestation on postnatal skin, hair characteristics, and thermoregulatory responses. We hypothesized that exposure to in utero hyperthermia would trigger fetal skin and hair adaptations, such as reduced skin thickness and hair length with increased sweat gland size and number. These adaptations would prepare the calf for similar postnatal environments (i.e., heat stress) and aid in effective thermoregulation.

**MATERIALS AND METHODS**

**Animals, Experimental Design, and Treatments**

This study was conducted from August 2020 to October 2021 and approved by the Institutional Animal Care and Use Committees at the University of Florida (protocol #201910599) and the University of Wisconsin-Madison (protocol #A006415-A02).

**In Utero Calf Treatment During Late Gestation.** Experimental design and dam treatments have been previously described in depth by Dado-Semm et al. (2021). Briefly, 82 multiparous Holstein pregnant dams were housed in a compost-bedded freestall barn at a commercial dairy farm in Trenton, Florida. They were bred to sexed semen and blocked by parity, mature-equivalent milk production, and offspring sire. Beginning at dry-off, ~56 d before expected calving date, dams were enrolled in one of 2 treatments: heat stress (HT, n = 41) or active cooling as a heat abatement method (CL, n = 41). The HT cows were provided with only the shade of the open-sided barn, whereas the CL cows were provided the shade of the open-sided barn, plus the addition of water soakers over the feed line, and fans over the freestall beds. Fans (Typhoon Power P-51, Seneca Dairy Systems, NY) ran continuously, and water soakers turned on automatically for 1.5 min at 5-min intervals when ambient temperatures exceeded 21.1°C. Ambient temperature and relative humidity of the barn was recorded and averaged hourly with Hobo Pro series temperature probes (Onset Computer Corp.).

The THI was calculated using the equation established by the NRC and suggested for use in subtropical environments (NRC, 1971; Dikmen and Hansen, 2009):

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)], \quad T = \text{ambient temperature} \quad \text{(°C)} \quad \text{and} \quad \text{RH} =$$
relative humidity (%). The THI remained above 68 and respiration rates (RR, flank movements per minute) and skin temperatures (ST, Raytek MiniTemp MT6 Infrared Thermometer; Instrument, South Burlington, VT) were elevated for the HT dams, relative to CL dams (77.36 vs. 53.51 ± 0.63 breaths/min, P < 0.001 for RR and 35.95 vs. 34.09 ± 0.05°C, P < 0.001 for ST; HT vs. CL, respectively; Dado-Senn et al., 2021). These thermal indices indicate heat stress was achieved, and the heat abatement system was successful in reducing thermoregulatory parameters. Details on diet composition during the late gestation phase are reported by Dado-Senn et al. (2021). Offspring born to these dams that were heat stressed during late gestation were considered in utero heat stressed (IUHT; n = 36) and those born to dams actively cooled during late gestation were considered in utero cooled (IUCCL; n = 37).

A total of 73 heifer calves, born between September 14, 2020, and October 10, 2020 (i.e., 26 d), were successfully enrolled in this study.

**Calf Management from Birth to 1 Year of Age.** Following birth, calves were separated from their dam, weighed, navel dipped with 7% tincture iodine, and fed 3.78 L of good quality colostrum (digital BRIX refractometer reading ≥21%, MA871, Milwaukee Instruments; Quigley et al., 2013) via esophageal tubing within 2 h of birth (0.8 ± 0.5 h). Within 24 h of birth, heifer calves were relocated to a University of Florida on-campus animal facility and managed identically from September to December 2020. They were housed in individual sand-bedded wire hutches under an open-sided barn with oscillating fans for air circulation. Calves were fed 0.45 kg of milk replacer (UF Special 28/15 Bova DFB Medicated, Southeast Milk) mixed in 3.78 L of warm water at 0600 and 1700 h. Starting at 4 d of age, starter grain concentrate (Ampli-Calf Starter 20 Warm Weather, Purina Animal Nutrition LLC) was offered ad libitum. Milk replacer weaning began at 49 d of age and was complete by 56 d of age. During this period calves were fed 2 times daily, but at each feeding were offered 0.23 kg of milk replacer mixed in 1.89 L of warm water. At 60 d of age, calves were reassigned to group-housed pens where they received ad libitum grain and water. Heifers were routinely monitored and vaccinated according to University of Florida’s College of Veterinary Medicine standard operating procedures.

After weaning (December 2020), heifers were relocated to a university-owned calf raising facility in Arlington, Wisconsin, where they were raised identically until 1 yr of age. Briefly, heifers were housed in a shaded, compost-bedded pack barn and fed ad libitum water and TMR to meet NRC requirements for growing, post-weaning heifers (NRC, 2001). Heifers were routinely monitored and vaccinated according to the University of Wisconsin-Madison’s College of Veterinary Medicine standard operating procedures.

### Sample Collection, Processing, and Morphological Measures

Skin and hair samples were collected from 2 locations (i.e., neck and rump) from 3 subsets of heifers from the same cohort described above (n = 8 per in utero treatment and time point). At birth, (d 0, 4.6 ± 2.3 h after calving) a skin tissue biopsy and hair sample were collected. Hair samples were obtained from the right shoulder, approximately 4 in down from the spine, and over the right hip bone. Hair samples were stored individually in resealable plastic bags until further analysis. Skin tissue was harvested, using a sterile biopsy punch (Standard Biopsy Punch, 5 mm, Integra Miltex Life Sciences Corp.) from the right side of the neck and over the right side thurl. This first subset of calves was not fed colostrum before tissue collection, as they were slaughtered for organ weights and other tissue collections (Dado-Senn et al., 2021). A second set of hair and skin tissue biopsies were collected 1 wk after complete weaning (d 63, 62.9 ± 1.5 d). Lastly, hair samples were collected at 4, 8, and 12 mo of age (4, 8, and 12 mo, respectively) in addition to a skin tissue biopsy at 12 mo of age (377.9 ± 1.4 d). Biopsied skin tissues were washed in sterile, cold PBS and immediately placed in 10% formalin for fixation at room temperature for 16 to 24 h. Biopsies were bisected, placed in histology cassettes, and stored in 70% ethanol. Tissues were dehydrated, paraffin embedded, sectioned at 7 μm, and affixed to glass slides. Sectioned tissues were stained with hematoxylin and eosin following the standard manufacturer protocol (Hematoxylin 7211, Clarifier1, Bluing, and Eosin Y Alcoholic; Thermo Fisher Scientific) to visualize skin morphology.

Hair and skin morphological measures were preformed using ImageJ software (version 1.53e, National Institutes of Health) based on methods reported by Sarlo Davila et al. (2019). Hair length was measured utilizing the segmented line tool. Hairs selected for measurement fell into 2 categories: short hairs or long hairs, which were identified as undercoat and topcoat, respectively. Ten hairs of each length were used to measure the average length of all short and long hairs, average of short hairs, average of long hairs, and the difference between short and long hair lengths. Skin tissue biopsy images of the hematoxylin and eosin histological staining (d 0 and d 63) were captured with the EVOS XL Core imaging system (Advanced Microscopy Group). Images from 12 mo skin biopsies were captured with the Keyence BZ-X800 Microscope (Keyence Corp.). Images at 4× magnification were cropped to 1,000 × 1,000 pixels.
and stratum corneum (SC) area, SC thickness, epidermis thickness, sweat gland (SWT) number, SWT cross-sectional area, SWT average size, sebaceous gland (SEB) number, SEB cross-sectional area, SEB average size, and sweat gland depth (2 measurements, from the skin surface to the highest and to the lowest sweat gland) was quantified (Supplemental Figure S1; https://data.mendeley.com/datasets/xn2stfpksf; Davidson et al., 2022). Cross-sectional area of SEB was traced using the freehand tool, whereas SWT cross-sectional area and stratum corneum area was quantified by altering threshold measurements. With the straight-line tool, SC thickness was measured thrice along the tissue sample and averaged, whereas epidermis thickness was measured from the skin surface to the dermis, visually identified as a sudden change in tissue texture. Number of glands (SWT and SEB) were counted, and SWT depth was measured twice, with the straight-line tool, from the closest and furthest glands in relation to the skin surface. The average size of SWT and SEB per tissue sample were calculated by dividing the cross-sectional area by the number of glands counted.

Thermoregulatory Responses

Beginning at 7 d of age, thermal indices, including RR, rectal temperature (RT), skin temperature, and sweating rate (SR), were recorded (n = 24 per in utero treatment). The sweating rate and skin temperature were recorded from a 3-cm² shaved and unshaved area over the right front shoulder blade and the right hip bone. All measurements of interest included RR, RT, skin temperature neck shaved (STNS), skin temperature neck unshaved (STNU), skin temperature rump shaved (STRS), skin temperature rump unshaved (STRU), SR neck shaved (SRNS), SR neck unshaved (SRNU), SR rump shaved (SRRS), and SR rump unshaved (SRRU).

These measurements were repeated weekly on d 14, 21, 28, 35, 42, 49, 56, and 63 between 1200 and 1500 h. Respiration rates were measured by counting flank movements for 1 min (breaths/min) and RT was measured with a digital thermometer (GLA M900, accuracy ± 0.1°C, GLA Agricultural Electronics). To assess the barrier function of the skin, referred to as SR, the transepidermal water loss was measured using a VapoMeter (Delfin Technologies, measurement range: 3–300, accuracy ± 4%). Skin temperature was measured with an infrared thermometer (Raytek MiniTemo MT6 Infrared Thermometer; Instromart; accuracy ± 1.0°C). To assess daily patterns of thermoregulatory responses RT, SR (shaved and unshaved), and skin temperature (shaved and unshaved) were recorded every 4 h during a 36-h period (d 1 = 0700 to 2300 h, d 2 = 0300 to 1900 h) beginning 4 d after complete weaning (n = 10 per in utero treatment, selected calves were born between September 26 and October 2, 2020 (i.e., 6 d).

Statistical Analysis

All statistical analyses were performed in SAS (version 9.4, SAS Institute Inc.) with the in utero calf as the experimental unit. Data were tested using Levene’s test and residual normality was assessed using the Shapiro-Wilk statistic (UNIVARIATE procedure). To meet the homogeneity of variance criteria, raw data were transformed when deemed appropriate and back transformed for visual representation. Using a generalized linear mixed model, hair length, SC area, SC thickness, epidermis thickness, SWT number and cross-sectional area, SEB gland number and cross-sectional area, and SWT depth were analyzed. Models included the main effect of in utero treatment with sampling location and time points analyzed separately. All other variables were analyzed using the PROC MIXED procedure. The model included fixed effects of in utero treatment, time (hour or day, as repeated measure), and all possible interactions, and animal identification number nested within treatment was used as the random effect. The model to analyze thermoregulatory responses on a weekly basis included THI at the time of sampling as a covariate. Significance was declared at P ≤ 0.05 and tendency was declared at 0.05 < P ≤ 0.10. Data are presented as least squares means ± standard error, unless otherwise stated.

RESULTS

Hair Length

At d 0 and 12 mo, there were no differences (all P ≥ 0.11, Table 1) in either location (rump or neck) for average hair length, short hair length, long hair length, and the difference between short and long hair length. For hair length measured at d 63, 4 mo, and 8 mo, there were no differences at the rump location (all P ≥ 0.12, Table 1). However, at d 63 in the neck location, IUHT calves had longer average hair length (P = 0.0007; Table 1), shorter short hair length (P = 0.03; Table 1), longer long hair length (P < 0.0001; Table 1), and a greater difference between short and long hairs (P = 0.0003; Table 1), relative to IUCL heifers.

In the neck location at 4 mo, there were no differences between treatments for short hair length and the difference between short and long hairs. Yet, IUHT heifers have longer average hair lengths (P = 0.01; Table 1) and tended to have longer long hair lengths (P = 0.07; Table 1), compared with their IUCL counterparts.
At 8 mo of age, there were no differences between treatments in the neck for short hair length, long hair length, and differences between short and long hair lengths. However, at 8 mo of age IUHT heifers tended to have overall longer average hair lengths ($P = 0.08$; Table 1) in the neck, relative to IUCL heifers.

### Skin Morphology

At d 0, there were no differences (all $P \geq 0.14$, Figure 1) in either location for SC thickness, epidermis thickness, SEB cross-sectional area, and SWT number, cross-sectional area, average size, or depth. Yet, in the neck, IUHT heifers had reduced SC area (79.243 vs. 169.419 ± 13.071 µm$^2$, $P = 0.0003$, Figure 1I, IUHT vs. IUCL, respectively) and reduced average SEB size (1,636 vs. 2,238 ± 243 µm$^2$, $P = 0.03$, Figure 1J) relative to IUCL heifers. In the rump, IUHT heifers had smaller averaged SEB (2,100 vs. 3,352 ± 379 µm$^2$, $P = 0.007$, Figure 1H), but more SEB relative to IUCL heifers (13 vs. 9 ± 2 glands, $P = 0.05$, Figure 1F).

On d 63, there were no differences in SC thickness, SC area, epidermis thickness, average size SWT, and SEB cross-sectional area or average size for both neck and rump locations (all $P \geq 0.13$, Figure 2). In the neck, IUHT heifers had fewer SEB (11 vs. 15 ± 2 glands, $P = 0.04$, Figure 2E) and SWT (16 vs. 23 ± 4 glands, $P = 0.10$, Figure 2I), relative to IUCL heifers. Moreover, SWT cross-sectional area was smaller in IUHT heifer calves (0.16 vs. 0.23 ± 0.02 mm$^2$, $P = 0.01$, Figure 2G). In the rump, at D63, IUHT had greater distance from the skin’s surface to the most superficial SWT (0.44 vs. 0.50 ± 0.03 mm, $P = 0.05$, Figure 2L), relative to IUCL heifers.

At 12 mo, there were no differences (all $P \geq 0.14$, Figure 3) in either neck or rump locations for SC area, SC thickness, epidermis thickness, SWT number or average size, and SEB number, cross-sectional area, or average size. In the neck, IUHT heifers had longer distance to the highest SWT (0.016 vs. 0.015 ± 0.0001 mm, $P = 0.04$, Figure 3E) and tended to have shorter distance to the lowest SWT (0.031 vs. 0.033 ± 0.001 mm, $P = 0.07$, Figure 3G), compared with IUCL counterparts. In the rump, IUHT heifers tended to have decreased SWT cross-sectional area (81.10 vs. 108.97 ± 10.83 µm$^2$, $P = 0.09$, Figure 3J) relative to IUCL heifers.

### Weekly Thermoregulatory Outcomes

The THI during the pre-weaning period was 75 ± 6. Heifer calves which were in utero heat stressed during the last ~56 d of gestation had overall higher SR at both the unshaved neck ($P = 0.03$; Table 2) and unshaved rump ($P = 0.03$; Table 2) and higher RT.
Day had a significant effect on SR and RT, whereby SR (both neck and rump) decreased from d 7 to 63 and RT decreased from d 7 to 42 and increased until d 63. Sweating rate at the shaved neck location was not different \((P = 0.34; \text{Table 2})\), whereas the SRRS was higher in the IUHT heifers \((P = 0.03; \text{Table 2})\). Both SRNS and SRRS had a day effect, whereby they overall decreased from d 7 to 63.

There was a tendency for a day by in utero treatment interaction (Supplemental Figure S2; https://data.mendeley.com/datasets/xn2stfpksf; Davidson et al., 2022) for STRU, whereby IUHT heifers have higher skin temperature on d 21 \((P = 0.09, \text{Supplemental Figure S2C})\). Respiration rate \((P = 0.31; \text{Table 2})\) and STRS \((P = 0.15; \text{Table 2})\) was not different between IUCL and IUHT heifers, when measured weekly over the preweaning period. There was a day effect for RR, whereby RR decreased from d 7 to 28, increased from d 28 to 35, and decreased from d 35 to 63 \((P < 0.0001; \text{Table 2})\). Shaved skin temperature also had a day effect, whereby temperatures increased from d 7 to 28, increased from d 28 to 56, and decreased to d 63 \((P = 0.0001; \text{Table 2})\). Both STNS and STNU had significant in utero treatment by day interactions \((P < 0.05, \text{Supplemental Figure S2})\), whereby STNS was lower in the IUHT heifers at d 28 and 42 (Supplemental Figure S2B) whereas STNU was higher in the IUHT heifers at d 21 and was lower at d 28 and 42 (Supplemental Figure S2A). There tended to be an effect by day for both shaved and unshaved skin temperature \((P < 0.10; \text{Table 2})\), by which temperatures decreased from d 7 to 21, increased from d 21 to 35, and decreased from d 35 to 63.

**Daily Pattern of Thermoregulatory Responses**

Responses for SRNS (Figure 4B), SRRS (Figure 4D), STRU (Figure 4G), STRS (Figure 4H), and rectal temperature (Figure 4I) were not different between in utero treatments. However, SRNS, SRRS, STRU, STRS, and RT all had significant day effects. Both SRNS and SRRS increased from 0700 to 1500 h on d 1, decreased from 1500 h on d 1 to 0700 h on d 2, increased from 0700 to 1500 h on d 2, and decreased from 1500 h on d 2 to 1900 h on d 2 of measurement. STRU increased from 0700 to 1100 h on d 1, decreased from 1100 h on d 1 to 0700 h on d 2, increased from 0700 to 1500 h on d 2, and decreased from 1500 to 1900 h on d 2 of measurement. STRS increased from 0700 to 1100 h on d 1, decreased from 1100 h on d 1 to 0300 h on d 2, increased from 0300 to 1500 h on d 2, and decreased from 1500 to 1900 h on d 2. Rectal temperature increased from 0700 to 1500 h on d 1, decreased from 1500 h on d 1, and increased from 0700 to 1500 h on d 2.

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**Figure 1.** Skin tissue morphology at birth. Skin tissue biopsies were harvested at birth (d 0) from the neck and rump locations and stained with hematoxylin and eosin (A–D). Heifers were exposed to in utero heat stress (IUHT, red columns) or in utero cooling (IUCL, blue columns) during the last ~56 d of gestation \((n = 8 \text{ per treatment; E–J})\). Measurements of interest include sebaceous gland number (E, F), average sebaceous gland size (G, H), and stratum corneum area (I, J). Data are presented as LSM \(\pm\) SE. ** indicates significance \((P \leq 0.05)\).
d 1 to 0300 h on d 2, increased from 0300 to 1500 h on d 2, and decreased from 1500 to 1900 h on d 2 of measurement.

Sweating rate (unshaved) in the neck (14.55 vs. 12.78 ± 0.25 g/m² per hour, \( P = 0.03 \), IUCL vs. IUHT, respectively, Figure 4A) was overall higher in the IUHT heifers. There was a day effect for SRNU whereby, sweating increased from 0700 to 1500 h on d 1, decreased from 1500 h on d 1 to 0700 h on d 2, increased from 0700 to 1500 h on d 2, and decreased from 1500 to 1900 h on d 2. In the neck, STNS tended to be higher in the IUHT heifers (32.98 vs. 32.67 ± 0.12°C, \( P = 0.08 \), Figure 4F). There was a day effect for STNS whereby, temperatures increased from 0700 to 1500 h on d 1, decreased from 1500 h on d 1 to 0700 h on d 2, increased from 0700 to 1500 h on d 2, and decreased from 1500 to 1900 h on d 2. For SRRU, there was a significant in utero treatment by hour interaction, whereby IUHT heifers had significantly elevated SR at 1100 h of d 1 and at 1500 h of d 2, relative to IUCL (\( P = 0.03 \), Figure 4C). In the neck, STNU had a tendency for an interaction between hour and in utero treatment. Overall, IUHT heifers had lower skin temperature at 0700 h on d 2, relative to IUCL (\( P = 0.08 \), Figure 4E).

**DISCUSSION**

Intrauterine exposure to hyperthermia during late gestation has notorious carryover effects on the developing calf, including decreased gestation length, compromised fetal growth and organ development, lower BW, and stunted postnatal growth (Collier et al., 1982; Tao et al., 2012; Monteiro et al., 2014; Dado-Senn et al., 2020; 2021). However, the extent to which a hyperthermic intrauterine insult during late gestation affects future postnatal thermoregulatory responses in cattle is less studied.

A variety of phenotypic responses arising from prenatal hyperthermia have been observed in different species, ranging from increased heat susceptibility to improvements in thermotolerance. For instance, birds exposed to embryonic heat stress have an improved ability to maintain core body temperature during a future thermal insult (Tzschentke, 2007; Piestun et al., 2008), and rodents demonstrate heat acclimation when exposed to heat stress after birth (Tetievsky and Horowitz, 2010). Offspring of first parity gilts exposed to hyperthermia for 114 d of gestation compromises their future thermoregulatory response to a thermal insult (Johnson et al., 2013). Limited research in the bovine has shown that exposure to in utero hyperthermia can negatively affect thermoregulatory responses in early postnatal life (Laporta et al., 2017; Davidson et al., 2021) and one study that imposed a thermal challenge at maturity suggests it might confer some advantages with regard to thermoregulation under high THI (Ahmed et al., 2017). However, Ahmed et al. (2017) was limited in that they did not consider the interplay between skin
temperatures and sweating rates and did not report nocturnal thermal indices. In the present study, we investigated the effects of in utero hyperthermia on hair coat length and skin morphology in Holstein heifers at various life stages including birth, weaning, and 1 yr of age. We also explored the potential effect on basal thermal indices across the preweaning period and their diurnal and nocturnal patterns shortly after weaning.

Despite the lack of RR differences between in utero treatments, IUHT heifers had increased RT and SR, and decreased skin temperature, relative to IUCL. The observed increase in rectal temperature and lack of

Figure 3. Skin tissue morphology at 1 yr of age. Skin tissue biopsies were harvested at 12 mo of age from the neck and rump locations and stained with hematoxylin and eosin (A–D). Heifers were exposed to in utero heat stress (IUHT, red columns) or in utero cooling (IUCL, blue columns) during the last ~56 d of gestation (n = 8 per treatment; E–J). Measurements of interest include distance from skin surface to the highest sweat gland (E, F), distance from skin surface to the lowest sweat gland (G, H), and sweat gland cross-sectional area (I, J). Data are presented as LSM ± SE. **, # indicate significance ($P \leq 0.05$) and tendencies ($0.10 \geq P > 0.05$), respectively.

Table 2. Weekly thermal indices were measured from heifer calves exposed to in utero heat stress or in utero cooling during the last ~56 d of gestation (n = 24 per treatment)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>SEM</th>
<th>Trt^3</th>
<th>Day</th>
<th>Trt × Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR, breaths/min</td>
<td>IUCL</td>
<td>52.25</td>
<td>50.50</td>
<td>1.20</td>
<td>0.31</td>
</tr>
<tr>
<td>RT, °C</td>
<td>IUHT</td>
<td>38.64</td>
<td>38.80</td>
<td>0.03</td>
<td>0.0003</td>
</tr>
<tr>
<td>SRNS, g/m^2h</td>
<td>IUCL</td>
<td>15.54</td>
<td>16.39</td>
<td>0.27</td>
<td>0.34</td>
</tr>
<tr>
<td>SRNU, g/m^2h</td>
<td>IUHT</td>
<td>24.80</td>
<td>27.38</td>
<td>0.84</td>
<td>0.03</td>
</tr>
<tr>
<td>SRRS, g/m^2h</td>
<td>IUCL</td>
<td>13.54</td>
<td>15.79</td>
<td>0.70</td>
<td>0.03</td>
</tr>
<tr>
<td>SRRU, g/m^2h</td>
<td>IUHT</td>
<td>20.50</td>
<td>22.94</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>STNS, °C</td>
<td>IUCL</td>
<td>35.96</td>
<td>35.22</td>
<td>0.08</td>
<td>0.21</td>
</tr>
<tr>
<td>STNU, °C</td>
<td>IUHT</td>
<td>31.98</td>
<td>31.77</td>
<td>0.10</td>
<td>0.16</td>
</tr>
<tr>
<td>STRS, °C</td>
<td>IUCL</td>
<td>35.46</td>
<td>35.28</td>
<td>0.09</td>
<td>0.15</td>
</tr>
<tr>
<td>STRU, °C</td>
<td>IUHT</td>
<td>31.84</td>
<td>31.66</td>
<td>0.12</td>
<td>0.27</td>
</tr>
</tbody>
</table>

^1Respiration rate (RR), rectal temperature (RT), sweating rate (g/m^2 per hour) from the shaved neck (SRNS), unshaved neck (SRNU), shaved rump (SRRS), and unshaved rump (SRRU), and skin temperature from the shaved neck (STNS), unshaved neck (STNU), shaved rump (STRS), and unshaved rump (STRU) were obtained on d 7, 14, 21, 28, 35, 42, 49, 56, and 63 after birth.

^2Multiparous dams were either cooled (shade of freestall barn, fans, soakers) or heat stressed (shade of freestall barn) and produced calves which were either in utero cooled (IUCL) or heat stressed (IUHT).

^3Trt = treatment.

*Significance was declared at $P \leq 0.05$; tendency was declared at $0.10 \geq P > 0.05$. 

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Figure 4. Daily patterns of thermoregulation. Sweating rate (SR; A–D; g/m² per hour), skin temperature (ST; E–H), and rectal temperature (I) measurements from in utero cooled (dams provided shade of a freestall barn, fans, and soakers; blue lines with circles) or in utero heat stressed (dams provided only shade; red line with squares) heifers during the last ~56 d of gestation (n = 10 per in utero treatment). Beginning on d 4 after complete weaning, sweating rate, skin temperature, and rectal temperature were recorded every 4 h during a 36-h period (d 1 = 0700 to 2300 h, d 2 = 0300 to 1900 h). Sweating rate and skin temperatures were measured from shaved and unshaved portions in the rump and neck locations. Unshaved results are presented here. Raw data were transformed when deemed appropriate and back transformed for visual representation. **, # indicate significance (P ≤ 0.05) and tendencies (0.10 ≥ P > 0.05), respectively. Data are graphed using the LSM ± SE of the interaction (in utero treatment by hour).
respiration rate differences after exposure to intrauterine hyperthermia is in accordance with observations by Laporta et al. (2017) and Davidson et al. (2021), respectively. Literature reporting skin temperature and SR in dairy calves is scarce. We reported elevated skin temperature and sweating rate in IUHT offspring from nulliparous heifers (Davidson et al., 2021) and multiparous dams (Dado-Senn et al., 2020).

To fully understand the biological importance of skin temperature and SR, in terms of thermoregulation, we must understand how hair coat and skin composition factor into these phenotypic thermal measurements. Hair length, thickness, and sweat gland activity affect thermal tolerance (Collier and Gebremedhin, 2015). Skin is the largest organ in the body, it protects the animal from external factors (Singh et al., 2013) and is composed of many layers including the epidermis, dermis, and hypodermis. Outside of its’ roles in protection from mechanical loads and immune function, skin is also important in controlling body temperature and maintaining homeostasis (Ebling et al., 1992; Singh et al., 2013). Within the skin layers are sweat glands and sebaceous glands. The number and distribution of skin glands play a role in the adaptive ability of the skin to heat stress (Raghav et al., 2021). Sweat glands bring water to the skins surface to aid in the evaporative path of heat loss (i.e., SR) and each hair follicle is associated with a sebaceous gland, which releases sebum to protect against destructive agents and assists in heat tolerance (Saxena et al., 1994; Berman, 2011).

Heat loss through means of radiation from the skin, evaporation of water from the surface, and the interplay of the layers of air at the skin surface and that which surrounds the animal (conduction) are all dependent on the temperature gradient of the environment. Whereas the role of hair coat and skin properties under heat stress conditions has been studied extensively in mature cattle, the influence of in utero hyperthermia on these factors and the potential consequences on postnatal thermoregulation has received less attention. Our data indicate that in utero hyperthermia influences hair coat and skin composition in a way that might explain the altered ability of these animals to thermoregulate in their postnatal life.

Breed and hair coat properties are just some of the multifaceted regulators of thermal tolerance (Turner and Schleger, 1960; Jenkinson and Nay, 1975; Gaughan et al., 1999; Olson et al., 2003; Burrow, 2012; Porto-Neto et al., 2014; Barendse, 2017). Two breeds of cattle that have been extensively studied due to their improved heat tolerance are the Zebu; an Indian breed thought to be derived from the aurochs and Slick cattle; genetically related to the Senepol breed with a mutation in the prolactin receptor gene. An increased capacity for sweating (i.e., larger and more numerous sweat glands) and a short, sleek, and glossy hair coat gives these breeds of cattle the ability to regulate internal body temperatures efficiently (Finch, 1986; Carvalho et al., 1995; Gaughan et al., 1999; Olson et al., 2003; Dikmen et al., 2014).

Other species show alterations in skin morphology that support increased heat loss. For example, Wind-nyer pigs have thinner epidermis, thinner dermis, thinner hypodermis, larger perimeter of sweat glands, and more superficial sweat glands, compared with Large White and Kolbroek pigs (Moyo et al., 2018). The epidermis is composed of 5 layers: the stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and SC and the thickness of the epidermis plays an important role in heat tolerance (Ross and Pawlina, 2016). Indian buffalo are better able to handle heat stress loads when they have a thinner epidermis, accounting for hair follicle density (Saravanakumar and Thiagarajan, 1992). In the present study, IUCL heifers had increased SC area at birth, the most superficial portion of the epidermis. However, we measured SC area, not thickness and we did not consider the hair follicle density at the surface layer. Yet, the SC area was not different between in utero treatments outside of birth measurements, and thus, it does not seem to be playing a major role in the postnatal thermoregulatory differences observed between IUCL and IUHT heifers.

When THI increases, blood flow to the skin surface increases and skin temperature increases (Hales et al., 1978) facilitating cutaneous evaporation through sweating (Johnson and Hales, 1983; Carvalho et al., 1995; Scharf et al., 2008). Zebu cattle have greater sweat gland density (Nay and Heyman, 1956) and these glands are localized in closer proximity to the skin surface relative to European breeds (Dowling, 1955). In the current study, IUCL heifers have larger and more numerous sweat glands at D63 and larger sweat glands at 12 mo of age. In addition, at d 63, IUCL heifers’ larger glands were localized closer to the skin surface. This pattern was also observed for the sweat glands of IUCL heifers at 12 mo. Interestingly, at this stage, the sweat glands were deeper in the skin, spanning a larger portion in comparison to their IUHT counterparts. For heat dissipation, evaporative cooling is an important mechanism in high temperatures (Yousef, 1985; Finch, 1986; Kadzere et al., 2002). Although the importance of sweat gland distribution is not well understood, it is evident that sweating is prominent and skin morphology is an important mechanism conferring heat tolerance. Nay and Hayman (1956) reported that in cattle, sweating efficiency is affected by size, density, number, and depth of sweat glands. This indicates that IUCL heifers, which are granted more, larger, and more
superficial sweat glands at d 63 and 12 mo, have an enhanced ability to sweat for heat dissipation.

Heifers exposed to intrauterine hyperthermia were born with more numerous but smaller sebaceous glands relative to IUCL heifers. Yet, at d 63 IUCL heifers had slightly more sebaceous glands in their skin relative to IUHT. The role of sebaceous glands in the thermoregulatory activity of the cattle skin is least understood. In the human, sebaceous glands are under hormonal control (Thody and Shuster, 1989) and the released sebum is involved in preventing dehydration (Porter, 1993). In addition, sebaceous glands of cattle are highly innervated and have a rich capillary blood supply (Goodall and Yang, 1954; Jenkinson et al., 1966). In hot conditions, sebaceous glands discourage sweat formation and loss of sweat from the skin (Porter, 2001). Because sebaceous glands are under hormonal control, it is tempting to speculate that IUHT heifers might experience some degree of endocrine programming for higher thermal conditions. It is possible that these factors combine to aid in the development of more SEB glands, observed at birth. This would possibly aid in combating dehydration in early life. However, as the heifer matures and hormonal profiles change this relationship reverts. Our data suggests that IUHT heifers may have altered SEB function potentially leading to lower sweating rate in the first few weeks of postnatal life.

Animal adaptations linked to tropical, warm weather include coat length and thickness alterations. Yeates (1955) suggests long-haired animals are more affected by heat stress. McManus et al. (2010) reported a positive correlation between SW and skin temperatures and respiration rate, which were also positively correlated with longer hair length. In the present study, at d 63 IUHT heifers had overall longer hairs, shorter short hairs and longer long hairs. Heifers exposed to in utero heat stress have a larger difference between their top and under coats, potentially trapping air in the coat and reducing the efficiency of heat dissipation from the skin surface. This hair coat difference between IUCL and IUHT heifers remained up to 8 mo of age. A short hair coat is a key thermoregulatory adaptation that allows cattle to lose heat more effectively. In contrast to the present study, Acharya et al. (1995) found that goats with shorter hair coats had higher increases in rectal and dermal temperatures, respiratory and pulse rates relative to long haired goats. That study implies long hairs in goats play more of an important protective role against solar radiation than it does in cattle. Dikmen et al. (2008) reported that Slick lactating Holstein cows had lower vaginal temperatures and respiration rates, compared with wild-type Holstein cows. Shorter bovine hair coats grant the animal an increased rate of heat loss via both convection and conduction (Berman, 2004) and are thought to reflect solar radiation, as opposed to hair coats of the goat. In the present study, the shorter hair coat of the IUCL heifers resembles that of the Slick phenotype and grants them improved thermotolerance in their postnatal life.

Intrauterine hyperthermia led to higher postnatal sweating rate, particularly when measured at the unshaved locations. However, during the hottest hours of the day IUCL heifers had the highest SR at the unshaved location compared with IUHT. Ahmed et al. (2017) observed a similar elevation in sweating rate in IUCL cows challenged with high THI at maturity. These observations exemplify the important role of the hair coat in sweating efficiency and the advantage of possessing more and larger sweat glands as an adaptation to dissipate heat. It is possible that IUHT heifers’ impaired ability to effectively dissipate heat is driven by their longer hair coat and altered skin gland characteristics. Notably, skin temperature was higher and RT was lower in the IUCL heifers across the preweaning period. These findings support the notion that when THI increases, blood flow to the skin surface increases, skin temperature rises, and sweating is induced allowing IUCL heifers to remain euthermic relative to IUHT heifers.

CONCLUSIONS

A relatively short period of fetal exposure to hyperthermic conditions elicited distinctive hair coat and skin morphology changes in the offspring, including fewer sweat glands and longer hair coats. These adaptations were not transient and remained evident at 1 yr of age. Consequently, prenatally heat-stressed heifers had elevated core body temperature during the preweaning period, despite being exposed to the same postnatal environment as their in utero thermoneutral counterparts. The molecular, hormonal, and physiological adaptations triggered by intrauterine hyperthermia that might lead to the observed outcomes, and the interplay between hair length, skin glands, and postnatal thermoregulation capacity warrants further investigation. Additionally, the biological importance of sweat and sebaceous gland distribution, density, and activity on the animal’s response to the it’s environment should be investigated.

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