Short communication: Heat stress does not affect induced luteolysis in Holstein cows

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ABSTRACT

Heat stress (HS) has deleterious effects on bovine reproduction, including prolongation of the luteal phase in Holstein cows, perhaps due to compromised luteolysis. The objective was to characterize effects of HS on luteolytic responses of nonlactating Holstein cows given 25 or 12.5 mg of PGF_{2α} on d 7 of the estrous cycle. Cows were randomly distributed into 2 environments: thermoneutral (n = 12; 25°C) or HS (n = 12; 36°C). In each environment, cows were treated with 2 mL of saline, 25 or 12.5 mg of PGF_{2α} (n = 4 cows per group). The HS environment induced a significant increase in rectal temperature and respiratory rate compared with the thermoneutral environment. Heat stress did not have significant effects on luteolytic responses or circulating progesterone concentrations. Rapid and complete luteolysis occurred in all cows given 25 mg of PGF_{2α} and in 4 of 8 cows given 12.5 mg; the other 4 cows given 12.5 mg had partial luteolysis, with circulating progesterone concentrations initially suppressed, but subsequently rebounding. Therefore, we conclude that HS does not change corpus luteum sensitivity to PGF_{2α}.

Key words: cattle, corpus luteum, progesterone, prostaglandin

Short Communication

Heat stress (HS) has a negative effect on production and reproduction of dairy cows, reducing production efficiency during warmer months (Roush, 1994; Hulme, 1997), as it reduces ovarian follicular growth

rate and plasma estradiol concentrations (Wilson et al., 1998a,b), and inhibits fertilization, embryonic development, and maternal recognition of pregnancy (Sartori et al., 2002). The few available studies regarding the effects of HS on duration of the estrous cycle do not share a common conclusion. Some studies indicate that lactating Holstein cows and heifers subjected to HS had a luteal phase ~9 d longer than those in a thermoneutral (TN) environment, presumably due to effects of HS on luteolytic mechanisms (Wilson et al., 1998a,b). Another study reported lower circulating progesterone (P4) in the second half of the luteal phase in cows exposed to HS, attributing this effect to an earlier luteolysis (Wolfenson et al., 1988). On the other hand, one study found no effect of HS on estrous cycle length (Trout et al., 1998). Paradoxically, endometrial explants collected on the 17th day of the estrous cycle and subjected to in vitro HS produced more PGF_{2α} (Putney et al., 1988), which would presumably hasten luteolysis in vivo. However, in vitro HS increased P4 production by luteal cells without affecting their viability. Therefore, we hypothesized that changes in corpus luteum (CL) sensitivity to the luteolytic effects of PGF_{2α} account for the irregular luteal phases observed in heat-stressed cattle. The objective was to determine in vivo luteal responses of heat-stressed nonlactating Holstein cows challenged with 25 or 12.5 mg of PGF_{2α} on d 7 of the estrous cycle.

This experiment, conducted between October and December (2014) in the bioclimatic chamber of the Lageado Experimental Farm, was approved by the School of Veterinary Medicine and Animal Science, São Paulo State University (FMVZ-UNESP) Animal Care Committee No. 86/2013. Eighteen nonlactating, adult, clinically healthy Holstein cows with BCS 3, using a 1 (emaciated) to 5 (obese) scale according to Ferguson et

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al. (1994), were used. Cows were fed concentrate pellets and hay, with ad libitum access to drinking water and a mineral supplement.

After a 30-d interval for adaptation, ovulation was synchronized as previous described (Trevisol et al., 2015). Six cows with ovulation confirmed by ultrasonography (MyLab Five, Esaote, Italy) were selected and randomly assigned to 2 distinct environments: TN ($n = 3$, 24°C) or HS ($n = 3$, 36°C). On d 7 after GnRH challenge to initiate a new estrous cycle, cows from each environment were randomly allocated to 1 of the following treatments: saline (2 mL of 0.9% NaCl solution), 25 mg of PGF_{2 α} (Lutalyse, Zoetis, São Paulo, Brazil), or 12.5 mg of PGF_{2 α} . The study followed a completely randomized design with 4 replicates, with 12 cows in each environment, and 3 cows for each treatment per environment. Cows used in the first replicate of the HS environment were not used subsequently, whereas those assigned to the TN group were re-used in the next replicate for the HS environment. Every day during the experimental period, rectal temperature (RT) and respiratory rate (RR) were recorded. Temperature-humidity index (THI) for each environment was determined as described by McDowell et al. (1976).

Blood samples were collected into heparinized tubes by venipuncture of coccygeal vessels immediately before and every 6 h posttreatment until 24 h and then every 12 h until 48 h. Samples were immediately centrifuged ($1,500 \times g$ for 30 min at 4°C) and plasma stored at -20°C . Plasma P4 concentrations were determined using chemiluminescence (Immulin 1000, Immunoassay System, Siemens Healthcare, São Paulo, Brazil).

The variables THI, RT, RR, and circulating P4 were analyzed as repeated measures over time (Proc Mixed, SAS, version 9.1, SAS Institute Inc., Cary, NC). For RT and RR, the model included fixed effects of ambient temperature (AT), day, and their interaction. Because P4 data were not normally distributed, a natural log-transformation was performed. The transformed P4 data were then analyzed using a model with fixed effects of treatment, hour, and their interaction; ambient temperature was nonsignificant ($P > 0.05$) and was removed from the model. Covariance matrices were selected based on Akaike's information criterion, consistent Akaike's information criterion, and Bayesian information criterion, with comparisons between means done with pdiff. Results are presented as mean $\pm$ standard error of the mean, with $P < 0.05$ considered significant.

We observed AT and day effects ($P < 0.001$) for RT and RR, with an AT $\times$ day interaction for RT ($P < 0.005$). On all evaluation days, values were greater ($P < 0.05$) for HS compared with TN for THI (87.8 ± 0.3 vs. 75.0 ± 0.4), RT (39.2 ± 0.2 vs. $38.3 \pm 0.1^{\circ}\text{C}$), and RR (65.6 ± 5.2 vs. 35.1 ± 5 breaths per min; Figures 1A and B). For these end points, we also observed effects of treatment, hour, and their interaction ($P < 0.0001$).

Ambient temperature had no effect on circulating P4 on d 7, 5 d after allocating cows to TN or HS environments (2.4 ± 0.3 and 2.8 ± 0.4 ng/mL, respectively). After starting the treatments, greater circulating P4 ($P < 0.05$) was observed for saline when compared with the other treatments from 12 to 36 h (Figure 2A and 2B). Furthermore, half of the cows ($n = 4$) given 12.5

Figure 1. Mean $\pm$ SEM for (A) temperature-humidity index (THI), rectal temperature (RT), and (B) respiratory rate (RR; bpm = breaths per minute) of nonlactating Holstein cows exposed to thermoneutral (TN, $n = 12$) or heat stress (HS, $n = 12$) conditions. Probabilities for main effects [ambient temperature (AT) and day] and the interaction between AT and day are shown. Significant differences ($P < 0.05$) between TN and HS conditions are indicated by an asterisk (*).

mg of $\text{PGF}_{2\alpha}$ had complete luteolysis, whereas the remaining cows treated with 12.4 mg of $\text{PGF}_{2\alpha}$ ($n = 4$) underwent partial luteolysis. The cows that had partial

luteolysis had greater circulating P4 at 36 (Figure 2A) and 48 h after treatment compared with cows treated with 25 mg of $\text{PGF}_{2\alpha}$ or cows treated with 12.5 mg of

Figure 2. Circulating progesterone (P4) concentrations (%) in relation to 0 h (A) and mean $\pm$ SEM of P4 concentration (ng/mL; B) of nonlactating Holstein cows after $\text{PGF}_{2\alpha}$ treatments on d 7. Saline ($n = 6$), 25 mg of $\text{PGF}_{2\alpha}$ ($n = 7$), 12.5 mg of $\text{PGF}_{2\alpha}$ regressed ($n = 4$), and 12.5 mg of $\text{PGF}_{2\alpha}$ nonregressed ($n = 4$). Probabilities for main effects [treatment (TRT) and hour], the interaction between TRT and hour, and the significant differences ($P < 0.05$) between treatments (different lowercase letters) and hours (#) are indicated.

PGF_{2α} that had complete CL regression (Figure 2A and 2B). Cows with partial luteolysis had a decline in circulating P4 until 18 h posttreatment, followed by a subsequent rebound (Figure 2A). Last, circulating P4 was similar between cows given 25 versus cows given 12.5 mg of PGF_{2α} that had complete regression (Figure 2A and B), except before treatment (0 h) when cows subsequently given 25 mg of PGF_{2α} had greater P4 concentrations.

Furthermore, we detected that in the TN environment THI exceeded 72, regarded as the homeothermic limit for lactating Holstein cows (Staples and Thatcher, 2002). Regardless, cows in the present study did not display behavior indicative of HS and their RT and RR were ~38.5°C and 37.3 breaths per minute, respectively, within physiological limits for Holstein cows (≤39.5°C and <40 breaths per minute; Dirksen et al., 1993). We attributed the lack of detectable HS symptoms to cows being nonlactating and nonpregnant. Greater thermotolerance and thermoregulatory capability of nonlactating cattle were also observed by Sartori et al. (2002) who reported Holstein heifers at ~25°C ambient temperature had a RT <38.8°C, whereas lactating cows had RT >39.1°C.

We observed that in the HS environment, during the first 48 h of exposure (average THI of 88), cows were able to maintain their RT within physiological limits. However, thermoregulatory capability was subsequently lost and RT increased, peaking on the 6th experimental day (~39.7°C), indicating that these cows were in the immediate phase of the HS response, characterized by poor adaptation to the environment and failure to maintain body temperature within physiological limits (Horowitz, 2002; Bernabucci et al., 2010).

Despite effectively inducing HS, we observed that the luteolytic responses to PGF_{2α} treatment (25 and 12.5 mg) were not affected by the HS environment. Therefore, we concluded that changes in luteal susceptibility to PGF_{2α} were not the primary pathophysiological cause of the irregular luteal phase length in Holstein cows and heifers subjected to HS, which is in agreement with other studies (Wolfenson et al., 1988; Trout et al., 1998; Wilson et al., 1998a,b).

In addition, the similar circulating P4 concentrations observed on d 7 in cows assigned to TN or HS environments suggest that HS does not affect the production and metabolism of P4 during the early luteal phase. Another study (Trout et al., 1998) reported that circulating P4 in HS cows, from d 11 to 14 of the estrous cycle, was greater than that observed in control animals kept under TN conditions. However, these results are not necessarily comparable to our data due to the different diestrus period investigated. Last, the reduced circulating P4 (<1 ng/mL) observed at 48 h posttreat-

ment in cows treated with 12.5 mg of PGF_{2α}, followed by a subsequent rebound, was consistent with partial luteolysis, a well-described phenomenon in cattle (Meira et al., 2006; Wiltbank et al., 2012; Trevisol et al., 2015).

We conclude that exposure of nonlactating Holstein cows to an environment with a THI of ~75 did not induce HS. In contrast, a THI averaging 88 caused marked HS, characterized by increased RT and RR. Notwithstanding, HS had no significant effect on CL sensitivity to PGF_{2α}. Independent of the environment, 25 mg of PGF_{2α} induced complete luteolysis in all cows, whereas 12.5 mg induced complete luteolysis in only half of the cows with the remainder having incomplete luteolysis and a subsequent rebound in circulating P4. These data did not support the assertion that HS changes CL sensitivity to the luteolytic effects of PGF_{2α}.

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