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Research Article

Reproductive performance and serum concentrations of progesterone of heat-stressed Holstein heifers subjected to timed artificial insemination and progesterone supplementation

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Abstract: Sixty-nine Holstein heifers were used to evaluate the effect of progesterone supplementation after timed artificial insemination (TAI) on conception rates of heifers under heat stress. Heifers were randomly assigned to 1 of 3 treatments: 1) control treatment (C), visual detection of estrus. Heifers detected in estrous were inseminated according to the AM-PM procedure (n = 23); 2) TAI (n = 24), using a CIDR device (insert containing progesterone) placed intravaginally on day –10 and removed on day –2 with PGF2α given on day –3. Heifers were given 0.5 mg of ECP at CIDR insertion and a second injection (0.5 mg ECP) was given on day –2 and 48 h after all the heifers were inseminated (day 0); and 3) TAI with progesterone supplementation (TAI+S; n = 22), consisting of TAI plus progesterone supplementation with a used CIDR from day 4 to 14 after insemination. The maximum temperature humidity index during the study averaged 86, while the minimum was 77. Blood samples were collected from all heifers on days 5, 9, and 14 post-insemination for quantification of progesterone, thyroxine, and triiodothyronine levels. Respiration rate (RR) was measured at 1700 hours and rectal temperature (RT) at 1800 hours, 3 times per week. The conception rate did not differ ($P > 0.05$) among treatments (C = 40; TAI = 34.6; TAI+S = 44.4%). The levels of progesterone were not increased ($P > 0.05$) by progesterone supplementation. Values for RR, RT, and thyroid hormones were similar among treatments ($P > 0.05$). TAI combined with progesterone supplementation did not improve the conception rate of Holstein heifers under heat stress.

Key words: Conception rate, dairy heifers, heat stress, progesterone

Introduction

The negative effects of heat stress in dairy cows are associated with a reduction in the intensity of estrus (1), secretion of luteal progesterone (2), embryo development (3), and fertilization rate (4). The susceptibility to heat stress is greater in cows than in heifers. One reason for the greater susceptibility of cows to heat stress is that metabolic heat production by lactating cows leads to hyperthermia in the summer. However, heifers have a lower heat production

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derived from metabolism and consequently may not become hyperthermic at equivalent environmental temperatures. However, if equivalent hyperthermia is created between cows and heifers, reproductive responses to heat stress should be similar for both (5).

During heat stress heifers decrease their expression of estrus (6) and ovarian function changes (5), similar to cows. The use of timed artificial insemination (TAI) reduces the need for estrus detection, which is particularly important during heat stress conditions (7). Lower progesterone (P_4) concentration observed during the summer can affect conception rate because progesterone plays a major role in stimulating the production of several proteins and growth factors (8). On the other hand, early embryonic losses might be due to low levels of progesterone mainly during the first 5 days of pregnancy. Mann and Lamming (9) found that progesterone levels on days 4 and 5 after mating were correlated with uterine luminal concentrations of interferon-τ on day 16, which avoid luteolisis and consequently embryonic losses in pregnant animals. These authors increased conception rates when progesterone supplementation was administered prior to day 6 after AI in lactating dairy cows. Therefore, our objective was to evaluate the effects of a TAI protocol combined with progesterone supplementation on reproductive performance and serum concentrations of progesterone in Holstein heifers during summer.

Materials and methods

All procedures involving animals were carried out following approved local techniques of animal care (NOM-051-ZOO-1995: Humanitarian care of animals during mobilization of animals).

Experimental site and climatic conditions

The study was performed in a commercial heifer development farm located in Mexicali Valley, Baja California, Mexico (32°28′29″N, 115°24′07″W) with an altitude of 12 m above sea level. The climate is arid and hot and has average minimum and maximum temperatures in summer of 26 °C and 43 °C, respectively, while the average relative humidity ranges from 20% to 50% (10). The study was conducted from June to September, which is the hottest season in this area.

Environmental data

Daily ambient temperature and relative humidity were recorded every hour at the weather station of the University of Baja California located 15 km from the study area. The ambient temperature and humidity recorded were used to estimate the temperature–humidity index (THI) following the formula suggested by Hanh (11):

$$THI = 0.81\ T + RH\ (T - 14.4) + 46.4$$

where T = ambient temperature (°C) and RH = relative humidity expressed in decimals.

Animals and treatments

Sixty-nine virgin Holstein heifers aged 12–13 months and with a body weight average of 360 ± 26 kg were used in the study. Prior to the start of the experiment heifers exhibiting abnormalities in the reproductive tract, low body condition score, foot problems, or any other problem were excluded. Blood samples were collected (coccygeal venipuncture) 3 weeks before starting the study and 10 days after for serum P_4 analysis. Heifers with P_4 levels higher than 1 ng/mL in at least 1 sampling were considered cycling.

The heifers were stratified based on age and body weight and randomly assigned to 1 of 3 treatments: 1) control group (C), consisting of visual detection of estrus. Heifers detected in estrous were inseminated according to the AM-PM procedure (n = 23); 2) TAI group (n = 24) consisting of a TAI protocol (12) received an intravaginal progesterone releasing device, controlled internal drug release (CIDR; Eazi-Breed CIDR insert, Pfizer Animal Health, New York, NY) on day −10 plus 0.5 mg of ECP (Pfizer Animal Health). All heifers received 25 mg of $PGF_2α$ (5 mL of Lutalize, Pfizer Animal Health) on day −3 and the CIDR was removed on day −2 and they were given a second injection of ECP (0.5 mg). Heifers were inseminated at a fixed time (day 0), 48 h after CIDR removal; and 3) TAI with P_4 (TAI+S; n = 22), consisting of the TAI protocol plus P_4 supplementation with a CIDR used previously in the protocol and inserted vaginally from day 4 to 14 after insemination.

Housing and feeding

The heifers were housed in open, dry-lot corrals (20 m^2/heifer) with shades constructed of solid sides (3 m^2/heifer) and situated in the center of the pen with free access to drinking water. Heifers were fed twice daily with a total mixed ration and its chemical composition was dry matter (83.34%), crude protein (9.25%), ether extract (4.52%), ash (11.19%), and NDF (47.37%).

Hormone analysis, respiratory frequency, rectal temperature, and pregnancy diagnosis

Blood samples were collected (coccygeal venipuncture) from each heifer on days 5, 9, and 14 post-insemination to measure serum P_4, thyroxine (T_4), and triiodothyronine (T_3) levels. Immediately after collection the blood samples were placed on ice to be taken to the laboratory. Serum was separated by centrifugation at 1500 × *g* for 15 min and stored at –20 °C until assayed. Serum P_4 was determined by ELISA using a validated commercial kit for P_4 (BioCheck, Inc, Foster City, CA, USA) and thyroid hormones (Monobind, Inc, Lake Forest, CA, USA).

Respiration rate (RR) expressed as breaths per minute (bpm) was measured by observing the costal movements during 1 min at 1700 hours and rectal temperature (RT) was measured at 1800 hours using a digital thermometer (Electro-therm TC100A and 8 cm flexible probe). The RR and RT measurements were carried out 3 times per week (Monday, Wednesday, and Friday) during the study.

Days to first service (interval from the start of the experiment to the first insemination) and days to pregnancy (interval from the start of the experiment to pregnancy) were recorded for each treatment. Pregnancy diagnosis was performed by rectal palpation at 40 ± 2 days after insemination. Conception rate was defined as the percentage of heifers conceiving to AI that resulted in a confirmed pregnancy.

Statistical analyses

Hormonal concentrations and RR and RT data measured over time were analyzed with a repeated measurements design using the REPEATED and RANDOM statements of the MIXED procedure of SAS (13). Conception rate was analyzed by chi-squared test. The level of significance was 0.05; P values less than 0.10 were discussed as trends and values higher than 0.10 as only numeric differences.

Results

The thermal environment, in terms of ambient temperature, relative humidity, and temperature-humidity index, during the study is presented in Table 1.

Serum P_4 concentration was greater than 1 ng/mL in 85% of heifers (at least 1 sample), which was considered the proportion of heifers cycling in the experiment; however, the AI submission rate was 100% in the 3 treatments. The days to first service were reduced ($P < 0.05$) by 12 days for TAI and TAI+S treatments compared with C. The days to pregnancy were lower ($P < 0.05$) for TAI+S compared with C, while the conception rate was similar ($P > 0.05$) among treatments (Table 2).

Supplementation of P_4 (TAI+S) did not increase ($P > 0.05$) P_4 concentration compared with TAI and C (Figure). The treatments did not show differences in rectal temperature or respiration rate ($P > 0.05$), indicating no additional heat stress derived from the implementation of the TAI protocol. The levels of T_3 were lower ($P < 0.05$) for TAI compared with C, without differences ($P > 0.05$) in levels of T_4 among treatments (Table 3).

Table 1. Climatic measures recorded during the study.

Item	Day[a]	Night[b]	Maximum	Minimum
Ambient temperature, °C	36.6	33.0	41.1	28.4
Relative humidity, %	30.3	35.7	54.6	18.3
Temperature–humidity index	83	80	86	77

[a]Day: 0600–1700 hours
[b]Night: 1800–0500 hours

Table 2. Reproductive performance for control and timed artificial insemination groups.

Treatments	Days to first service	Days to pregnancy	Conception rate (%)
C	22.0[a]	47.0[a]	40.0[a]
TAI	10.0[b]	40.0[ab]	34.6[a]
TAI+S	10.0[b]	33.0[b]	44.4[a]

[ab]Different superscripts within column indicate significance (P < 0.05).

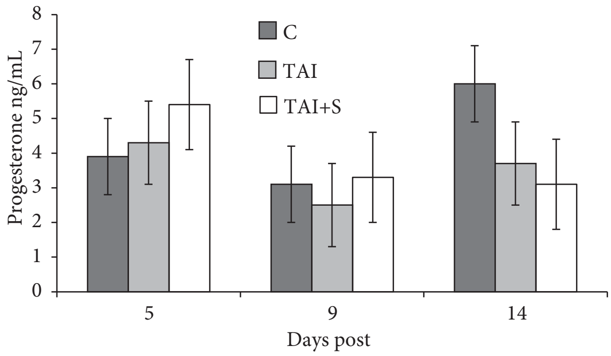

Figure. Effect of treatment on serum progesterone concentration after AI (P > 0.05).

Table 3. Physiological and hormonal responses for control and timed artificial insemination groups.

Treatment	Respiration rate (bpm)	Rectal temperature (°C)	T_3 (ng/mL)	T_4 (ng/mL)
C	97 ± 2.9[a]	39.4 ± 0.08[a]	2.9 ± 0.16[a]	77.9 ± 3.7[a]
TAI	99 ± 2.9[a]	39.4 ± 0.08[a]	2.3 ± 0.14[b]	84.0 ± 3.3[a]
TAI+S	99 ± 3.0[a]	39.2 ± 0.09[a]	2.6 ± 0.18[ab]	77.0 ± 4.2[a]

[ab]Different superscripts within column indicate significance (P < 0.05).

Discussion

A measurement for THI was recorded throughout a 24-h period and was considered moderate to severe, which can negatively affect reproduction in cattle (14). THI is used for assessing thermal stress by considering ambient temperature and air humidity. However, factors such as coat, air velocity, radiation, density of animals, and posture can influence the level of heat stress and is not considered in the THI (15); consequently, THI may under- or overestimate the effect of an adverse heat event (16).

Conception rate did not differ among treatments. Other studies have reported a reduction in the pregnancy rate of heifers under fixed-time artificial insemination compared with control groups, because of the lack of estrous synchronization (17,18). Lucy (19) suggested that cows inseminated following spontaneous estrous may have lower rates of embryonic death than those bred with timed AI. Studies have shown that TAI improves the pregnancy rate of cows under heat stress, but embryonic losses by day 40 to 50 after insemination are increased (20).

The pregnancy diagnosis results on day 40 in the TAI and TAI+S groups could have been affected by poor embryo survival. The TAI eliminates limitations induced by heat stress on estrus detection, but the program will not protect the embryo from temperature-induced embryonic mortality (21). Some reports have indicated that although under extreme heat stress conditions TAI is of little value it could be advantageous where estrous behavior is suppressed due to environmental factors without an increase in core body temperature (22).

Although conception rates may be reduced with timed AI, pregnancy rates could be improved by increasing the AI submission rate through these protocols. In the present study, the TAI and TAI+S protocols were beneficial because they allowed AI of heifers to reduce labor costs and days to first service by eliminating the need for detection of estrus, which is more difficult during summer months. Additionally, TAI+S reduced the days to pregnancy in comparison to the C group. De la Sota et al. (21) found an increase in net revenue ($118 per cow) with implementation of timed insemination for first service during the summer months.

Supplementation of P_4 may be more beneficial in cows that are less fertile due to the demands of lactation or by the effects of previous pregnancies or disease, but is less effective in normally fertile heifers (23). Folman et al. (24) showed that exogenous P_4 in the luteal phase prior to insemination enhanced fertility in lactating cows that had low endogenous P_4, but was not beneficial in cows with high endogenous P_4 prior to insemination. Van Cleef et al. (23) mentioned the possibility that the etiology of early embryonic mortality in heifers is less attributable to P_4 deficiency than other causes, which are not ameliorated by inducing higher systemic P_4 concentrations. The effects of incorporating a CIDR in TAI protocols have been inconsistent and these results could be attributed to differences in genetics, milk yield of cows, type of protocol used, proportion of anovular cows initiating the synchronization protocol, and the resulting low concentrations of progesterone in high-producing Holstein cows during treatment with CIDR (25,26).

In the present study, concentrations of P_4 did not increase in response to P_4 supplementation on either day of sampling; in contrast, in general P_4 concentration was numerically higher ($P > 0.10$) for the C group. Wolfenson et al. (27) concluded that chronic heat stress reduces P_4 concentrations, although P_4 concentrations may be elevated after acute heat stress; by consequence, the difference in conception rate resulting from experiments with P_4 supplementation may be due to the degree of heat stress that the cows were exposed to.

The intravaginal supplementation of P_4 in dairy cows between days 5 and 12 after insemination during summer and fall only increased the levels of this hormone on days 6 to 8 (28). These authors also observed a tendency for endogenous P_4 concentrations to be reduced when a progesterone-releasing intravaginal device was placed from days 5 to 12 and from days 10 to 17 after insemination. It is often assumed that a larger corpus luteum will be associated with high circulating plasma concentrations of P_4. Studies conducted by Assey et al. (29) and Spell et al. (30) found a significant relationship between corpus luteum size and plasma P_4 during corpus luteum development. However, other studies showed a decrease in plasma P_4 concentration in cows during summer, which was not associated with area of corpus luteum or with the presence of luteal cavities (2). The latter indicates that the decreased P_4 concentrations in plasma were directly related to heat stress. Plasma progesterone concentrations depend not only on the rate of production by the corpus luteum, but also on the rate of secretion to the circulation; the latter depends on ovarian luteal blood flow, which is reduced under heat stress conditions (31). The P_4 administered vaginally can be absorbed directly by nearby organs such as the uterus (28), reducing the problem of a possible low secretion of P_4 to circulation during heat stress conditions. These authors found a rise in fertility in dairy cows not heat stressed and supplemented with P_4 post-AI.

Some producers consider that the additional handling derived from TAI protocols application may increase the level of heat stress in the animals during the summer. Our study did not show any increase in RT or RR or changes in thyroid hormones levels as a consequence of the management during the implementation of TAI.

In conclusion, the results suggest that TAI maintained an acceptable conception rate in comparison to heifers inseminated to spontaneous estrus. Even though the supplementation of P_4 only improved the conception rate numerically, this practice could help to increase fertility under extreme heat stress conditions in lactating dairy cows compared to heifers because cows are more sensitive to high ambient temperatures. The additional management needed for the implementation of TAI did not increase the level of heat stress.

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