Hyperthermia and Body Energy Store Effects on Estrous Behavior, Conception Rate, and Corpus Luteum Function in Dairy Cows

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ABSTRACT

The reproductive performance of 74 Israeli Holstein dairy cows was examined during summer. Cows were fed prepartum to reach high (3.8) and low (2.6) body condition scores by 1 mo prepartum. After calving, half of each group were cooled seven times a day for 30 min by sprinkling and ventilation. Cows were inseminated starting 60 d postpartum. Daily mean body temperatures of cooled and noncooled cows were 38.6 and 39.2°C, respectively, with differences between them reaching 1°C and more during the hot hours. Body condition affected only the time taken postpartum to the start of ovarian activity (26 d for high and 32 d for low body condition groups). Estrous behavior lasted longer in cooled (16 h) than in noncooled (11.5 h) cows of the low body condition group only. Conception rate was higher in cooled than in noncooled cows (59 vs. 17%). Pregnancy rate at 90 d postpartum was higher in cooled (44%) than in noncooled cows (14%). Progesterone concentrations were higher in inseminated nonpregnant and in noninseminated cyclic cooled cows than in noncooled cows and were similar in pregnant cows of both cooled and noncooled groups. The present cooling method appears to have a high potential for improvement of summer fertility.

INTRODUCTION

Summer depression of reproductive performance of dairy cattle is a worldwide problem and inflicts heavy economic losses on the dairy industry. Many studies have documented the negative effects of high environmental temperature and humidity on fertility. In Israel, for example, the average conception rate (CR) of dairy cows in 1981 was 52% in winter and 24% in summer (24). In the southern US and Mexico, CR of the order of 10% or lower has been recorded in summer (5, 15). As a result, seasonal breeding is common in extremely hot climates.

The method most common used to alleviate thermal stress is shading, which results in a higher CR than in nonshaded cows (22). However, even when shade is provided for all dairy herds, fertility in summer remains lower than in winter (24). Additional cooling systems, such as evaporative cooling of the air surrounding the cows under shade resulted in a higher CR than in cows provided with shade only [31% vs. 14%; (25)]. Air conditioning during the summer improved fertility relative to that of nonshaded cows but not of cows in shade, and the investment and operating costs were relatively high (26). Corral manger misting and shade coolers utilizing high pressure nozzles and fans were recently reported to improve fertility in the southwestern US (2).

Thermal stress in cows during the days preceding AI and during early pregnancy is detrimental to fertility. High environmental temperature and humidity on d −2 prior to AI (15), d 0 (5), and d1 after AI (12) were negatively correlated with CR. Heifers exposed to thermal stress for the first 72 h after AI did not conceive at all (6). On the basis of such reports, the effect on fertility of short-term cooling during early pregnancy was examined. Spraying of

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French Friesian cows with water during first 10 d post-AI was effective in promoting CR (11). Results were less effective with Holstein cows cooled in a climate controlled barn from d 1 to 6 following AI (25). Cooling of Holstein cows by a combination of sprinkling and ventilation from 1 d before AI to 8 d after it improved their estrous behavior and milk production but did not affect their summer fertility (14).

A system based on cooling periods during the hot hours of the day and comprising sequential cycles of sprinkling and forced ventilation recently proved to be an efficient cooling procedure (7). Use of this system increased milk production in long-term studies when animals were cooled during either the dry period or lactation (3, 29). Because CR was unaffected by short-term cooling (14), we decided to conduct a long-term study. The objective of this study was to evaluate the effects of cooling over 150 d postpartum on the reproductive performance of dairy cows during two summers.

MATERIALS AND METHODS

Animals

The study was carried out over two consecutive summers (1985 to 1986) in the experimental herd of the Agricultural Research Organization in Bet-Dagan, Israel. Included in the experiment were 82 Israeli Holstein dairy cows (38 cows in 1985 and 44 in 1986) in their second to sixth lactation, expected to calve between May 15 and July 30. Cows were paired by lactation number, previous milk production, and date of expected calving. They were randomly allocated to two groups fed during the last trimester of pregnancy to reach low or high body condition (BC) by 1 mo before parturition [2.6 to 3.8, respectively, on a scale of 0 to 5; (17)] . After parturition, half of each group were cooled (C) during the summer, while the other half served as noncooled (NC) controls.

Housing and Cooling System

Cows were housed in a two-sided open shed with adjoining unshaded yards, allowing a total of 15 m^2/cow, of which 6 m^2/cow consisted of shaded slatted flooring. Noncooled cows were kept on the southern side of the structure and C cows on the northern side where a cooling system (7) was installed. The cooling system consisted of four fans and an array of sprinklers, sequentially actuated to repeat cycles of wetting (30 s) and forced ventilation (4.5 min) for 7 half-hour periods at intervals of 2 h between 0730 and 1830. During the intervals between cooling periods, ventilation was continuous between 0600 and 2400 h.

Measurements

Daily minimum and maximum air temperatures and relative humidities were recorded from the nearby (1 km) meteorological station. Body temperatures (Tb) were recorded by thermistor probes (7) seven times per day on 8 representative d. Jugular blood samples were taken twice weekly, between 1030 and 1230 h, from calving until diagnosis of pregnancy, or 20 d after 3rd insemination. The samples were centrifuged and the plasma stored at $-20°C$. Cows were observed for behavioral estrus three times daily. Cows exhibiting standing behavior or cows manifesting pronounced mounting behavior on two or more consecutive observations were considered to be in estrus. The latter criterion was confirmed if progesterone (P_4) concentration on day of estrus (± 1 d) was below .5 ng/ml. Cows were first inseminated 60+ d after calving; on the average this was done 73 ± 3 d (SE) postpartum. All cows were inseminated with frozen semen obtained from a single ejaculate of one proven sire. Pregnancy was diagnosed by rectal palpation by 45 d following AI.

Progesterone concentrations in plasma were analyzed using a solid phase coated tube radioimmunoassay kit (Zer Science Industries, Jerusalem, Israel). Duplicate 50-μl aliquots of plasma were pipetted into polypropylene tubes in which antibody to P_4 had been covalently bound to the inner surface by the manufacturer. A volume of 1.0 ml 125-I-P_4 in phosphate-buffered saline (35000 cpm/tube) was added and tubes were shaken briefly. Following 15 h incubation at 20°C, the contents of the tubes were decanted, and radioactivity of bound P_4 was counted in a gamma counter. Standard curves were constructed in triplicate in ovariectomized cow plasma, by adding 15.6, 21.3, 62.5, 125, 200, 250, 500, and 1000 pg/tube of P_4 (4-pregnene-3, 20-dione; Sigma Chemicals

Co., St. Louis, MO). Standard data and interpolation of unknown samples were computed using a log-logit transformation. Average assay sensitivity was 20 pg/tube. At 50% displacement the antibody's crossreactivity for other steroids was testosterone, .08%; 17α-hydroxyprogesterone, .21%; deoxycorticosterone, 2.9%; 20α-dihydroprogesterone, 5%; cortisol, .003%; pregenolone, .12%. The mean ± SE of measured concentrations of two known quantities of P_4 (1.70 and 4.20 ng/ml) added to ovariectomized cow plasma were 1.72 ± .06 ng/ml and 4.15 ± .09 ng/ml, reflecting 1 and 1.1% differences from expected values, respectively. Plasma samples containing 6.8 ng/ml were assayed in sample volumes of 13, 25, and 38 μl; samples were made up to assay volume (50 μl) with ovariectomized cow plasma. Differences between expected and measured P_4 concentrations were .6, 10, and 1.1%, respectively. The intra-assay and interassay coefficients of variation were 11.2 and 5.6%, respectively.

Data Analysis

The interval from calving to resumption of ovarian cyclicity was calculated as the average time between date of last P_4 concentration below 1 ng/ml and that of first P_4 concentration above 1 ng/ml. Duration of estrous manifestation was calculated as the interval between consecutive observations when estrus was noted, plus half the intervals between first observation and that preceding it, and between last observation and that following it.

Body temperature, P_4 data, and estrous behavior characteristics were analyzed by least squares analysis of variance, using SAS General Linear Model procedures (SAS Institute, Cary, NC). For Tb data, the sources of variance in the model were postpartum treatment (C and NC); cows within treatment, date, and hour of Tb measurement; and their interactions. For P_4 data the sources of variance were postpartum treatment, cows within treatment, and days postestrus as a continuous independent variable; differences in day trends of P_4 curves between treatments were examined by a test of heterogeneity of polynomial regression. Statistical analysis of P_4 data relating to cycle preceding or following insemination was based on 1985 data only, because blood was less frequently sampled during the corresponding periods in 1986. In the preliminary analyses of Tb and P_4 data, BC was not significant either as a main effect or in its interactions and was therefore excluded from further models. For estrous behavior and onset of ovarian cyclicity the sources of variance in the model were BC, postpartum treatment, and their interaction. The year term was excluded since preliminary analysis indicated it was not significant.

Pregnancy rate and CR were analyzed by chi-square analysis; the data for the 2 yr were pooled as no difference was noted between them. Only summer inseminations (up to three, before October 10) were included in the CR analysis. Of the 82 cows at the start of the study, 8 were excluded from the analysis of CR: 1 sick cow was culled, 1 was not inseminated, 1 showed irregular ovarian activity and abnormally intensive estrous behavior, and 5 cows were inseminated in error when not in estrus according to the forementioned criteria for estrous behavior. In order to obtain a more detailed and accurate analysis, 5 more cows (4 C and 1 NC) of the remaining 74 cows were excluded because of their abnormal P_4 profiles; their P_4 concentrations remained at .5 ng/ml or below until at least d 10 following AI. It was assumed that these cows had not ovulated during the normal expected time.

RESULTS

Body Temperature

A detailed analysis of the effects of cooling on Tb will be presented in a separate paper. In the following are described only the major effects of the cooling regimen on the thermal state of the animals around the time of insemination. Environmental conditions during the insemination period are summarized in Table 1,

TABLE 1. Environmental temperatures (°C) and relative humidities (%) during the insemination period.

Environmental conditions	$\bar{X}$	SE	Range
Maximum temperature	30.6	.2	26–36
Minimum temperature	19.2	.2	12–25
Maximum humidity	71	.6	48–94
Minimum humidity	53	.5	35–70

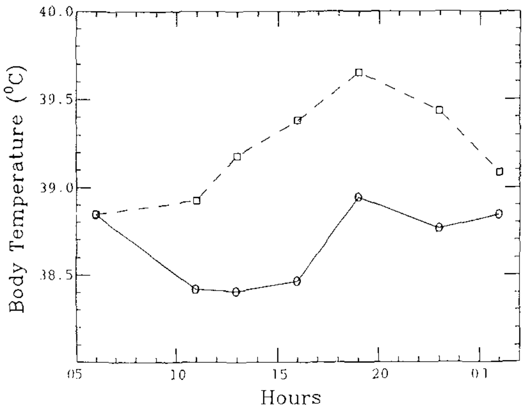

Figure 1. Body temperatures of cooled (○) and noncooled (□) cows during eight representative days. Standard errors for individual means ranged from .02 to .07°C.

and the average Tb of C and NC cows during this period are presented in Figure 1. Body temperature of C cows were lower ($P<.01$) than those of NC cows for most of the 24-h period. Temperature of NC cows was higher than 39.0°C during most hours of the day and night. It peaked at 1900 h, reaching about 39.7°C on average and declined thereafter. In contrast, Tb of C cows remained below 39°C throughout the 24-h cycle; it remained below 38.5°C during the hours of peak air temperatures and increased slightly (by .4°C) after cessation of cooling in the evening. A difference of about 1°C between C and NC cows was recorded in the hot afternoon hours. The relatively high Tb of both groups during the night and early morning hours might be attributable to the relatively warm (>25°C) and humid (>90%) night and early morning conditions, which probably impaired the cooling capacity of the animals.

Estrous Activity

Resumption of ovarian cyclic activity, as determined by the criterion of P_4, was affected by BC at parturition but not by postpartum cooling (Table 2). In high BC group it started about 6 d earlier than in low BC group (31.6 vs. 25.8 d, $P<.05$). Days to first observed estrus did not differ between low and high BC cows of either C or NC groups. The duration of estrus was longer in C (16 h) than in NC cows (11.5 h) in low BC group only (BC by cooling interaction, $P<.01$).

Conception Rate

The reproductive performance of C and NC cows is presented in Table 3. First insemination CR was 59% in the C group and only 17% in NC group ($P<.01$). This marked difference in CR was also evident for all inseminations (57 vs. 20% for C and NC cows, respectively; $P<.01$). Similar results were obtained with CR for all cows, including the 5 excluded from the analysis because of their abnormal P_4 profiles (55 and 22% for C and NC cows, respectively; $P<.01$). The pregnancy rates of C group were higher than those of NC group at 90, 120, and 150 d postpartum ($P<.01$). Body condition had no significant effect on CR; it was 40% for low and 34% for high BC groups. The number of animals was insufficient to allow reliable estimates of the BC by cooling interaction.

TABLE 2. Estrous behavior and resumption of cyclicity in cooled and noncooled cows of high and low body condition.

	High				Low			
	Cooled		Noncooled		Cooled		Noncooled	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Days to resumption of cyclicity[a]	25.1	2.7	26.5	2.8	29.9	2.7	33.3	2.6
Days to first estrus	43.2	4.8	43.7	5.1	45.3	2.7	47.9	4.6
Duration of first estrus,[b] h	9.9	1.2	13.0	1.2	15.0	1.2	10.1	1.1
Duration of estrus on AI,[b] h	12.6	1.2	13.6	1.0	16.0	1.0	11.5	1.0

[a]High and low body condition main effects differ ($P<.05$).

[b]Significant treatment by body condition interaction ($P<.01$).

TABLE 3. Conception rate[1] (%) and pregnancy rate[2] (%) of cooled and noncooled cows.

Group	Cooled		Noncooled	
	(%)	(n)	(%)	(n)
Conception rate				
First insemination	59[a]	20/34	17	6/35
All inseminations	57[a]	32/56	20	14/70
Pregnancy rate				
At 90 d	44[a]	15/34	14	5/35
At 120 d	59[a]	20/34	31	11/35
At 150 d	73[a]	25/34	31	11/35

[a]Cooled and noncooled groups differ, ($P<.01$).

[1]Number of cows diagnosed as pregnant by day 45 post-AI divided by number of inseminations.

[2]Percent of pregnant cows, by days after calving.

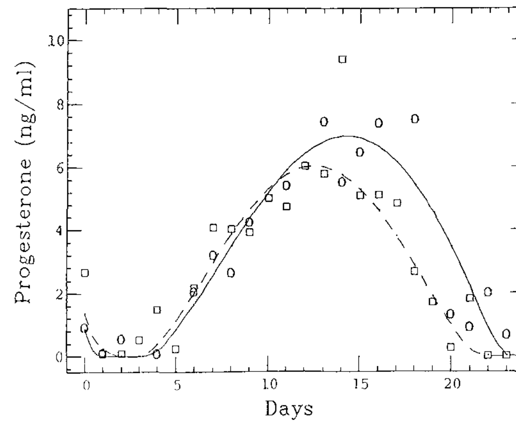

Figure 3. Polynomial regression curves and mean daily least square progesterone concentrations of cooled (○; n = 7) and noncooled (□; n = 11) nonpregnant cows during 23 d following first insemination.

Most nonpregnant cows of both C and NC groups were observed to be in estrus within 24 d after insemination: only 19% of them (5 C and 7 NC cows) manifested estrus later than 25 d post-AI. This was also the case when reoccurrence of cyclicity was estimated according to P_4 criteria; P_4 concentrations were below .5 ng/ml at 21 to 24 d post-AI in most nonpregnant cows of both groups and remained high (>3 ng/ml) until d 25 in only 13%.

Progesterone Concentration

Progesterone concentrations in C and NC cows are presented, respectively, as 2nd and 4th order polynomial regression curves of day trends. Analyses were performed for the cycle preceding insemination (Figure 2) and for cows that were not pregnant (Figure 3) or were pregnant (Figure 4) after first AI. For pregnant cows, there was no difference in least square

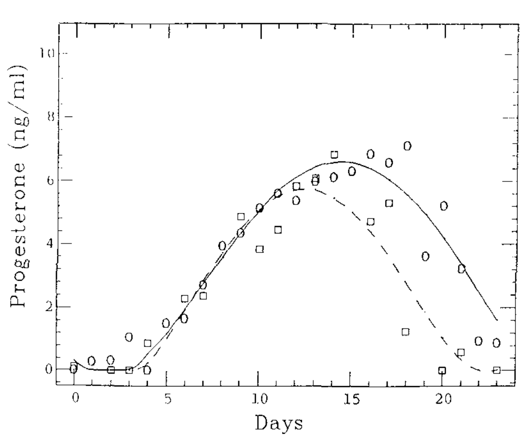

Figure 2. Polynomial regression curves and mean daily least square progesterone concentrations of cooled (○; n = 9) and noncooled (□; n = 8) cows during estrous cycle preceding first insemination.

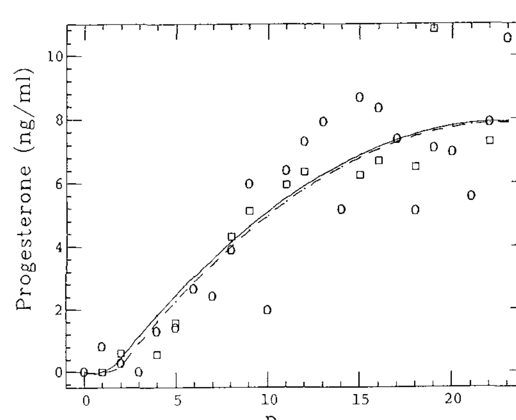

Figure 4. Polynomial regression curves and mean daily least square progesteron concentrations of cooled (○; n = 9) and noncooled (□; n = 4) pregnant cows during 23 d following first insemination.

means for P_4 and for P_4 day trends between C and NC groups. The means and curves for the cyclic cows and for the inseminated nonpregnant cows were very similar; in both groups, mean P_4 concentration during the second half of the luteal phase was higher in C animals than in NC ones ($P<.05$). The P_4 daily trends of the nonpregnant and the cyclic C cows were different from those of the corresponding NC cows ($P<.05$). The mean ± SE of estrous cycle length (interestrus interval) in cyclic C cows was slightly longer than that of NC cows (22.6 ± .6 and 21.9 ± .4 d). The P_4 curve for the pregnant cows was only slightly and not significantly higher than that for nonpregnant cows until about d 17 post-AI, when luteal regression started as expected in the nonpregnant cows.

DISCUSSION

Body temperature of NC cows was above 39°C for most of the day, in contrast to that of C cows, which remained below 39°C. On hotter days, Tb differences between C and NC groups were larger, more than 1°C, and the Tb of NC cows was higher than 40°C from midday to night. Because cooling was discontinued at night, the Tb in C cows was higher in the early than in the later morning hours. Cooling at night might be beneficial, especially on nights when temperature and humidity are in the higher range (Table 1). It was suggested that low morning Tb is important in maintaining homeothermia during the hotter hours later in the day (16).

Ovarian cyclic activity resumed in high BC animals about 6 d earlier than in low BC ones (Table 2). This is in agreement with the negative relation observed between prepartum nutrition and the interval between calving and resumption of ovarian activity (13). However, the number of days to first observed estrus was not affected by BC, as reported similarly elsewhere (10). This might indicate that prepartum nutrition has a different effect on the two phenomena involved in resumption of cyclicity. Duration of estrus was shorter in NC than in C cows, in agreement with other reports on the negative effect of heat stress on estrous behavior (9). This was, however, true for low BC cows only, for reasons unknown to us.

Cooling cows by means of the system described here completely prevented the summer depression of fertility. Conception rate on first AI was even higher (59%) than the average winter CR for the country (about 50%); this might however be partly attributable to the careful selection of cows included in our analysis. The pregnancy rate in cooled cows at 150 d postcalving was double that of control cows. Conception rate of C cows in the present experiment was higher than in cows cooled by evaporative cooler [31%; (25)], air-conditioning [40%; (26)] or shade [44%; (22)]. Improvement of CR in the present study is in contrast with the lack of effect after similar cooling between d 1 prior to AI and d 8 post-AI (14). This discrepancy suggests either that the effect of thermal stress on fertility is a long-term one, or alternatively, that the period before d −1, or after d +8, or both, is particularly sensitive to heat stress.

The decreased fertility observed in heat stressed cows might be partly due to ovarian dysfunction. This might include ovulatory failure, delayed ovulation, and impaired corpus luteum (CL) function. Pre- and postinsemination P_4 curves (relative to the day of estrus) were almost identical until d 10 in C and NC animals, whether they were pregnant or not. This suggests that the occurrence of ovulation was similar in these groups and that the low fertility of NC animals was thus probably not due to delayed ovulation. It should, however, be noted that this conclusion is based on relatively infrequent blood sampling, which permits detection of gross changes only.

This study was not designed to differentiate between fertilization failure and early embryonic losses in NC cows, both of which might have contributed to lower fertility. Recent studies (4, 20) in dairy and beef cows indicated that postfertilization heat stress impaired embryonic development by d 7 and d 16 post AI, respectively. A similar study in rabbits also revealed an increased incidence of impaired embryonic development and embryonic death (28). However, in the majority of nonpregnant cows in this study, the CL regressed or cows manifested estrus within 3 to 3.5 w after AI. Thus, most embryonic losses probably occurred by d 16, as the presence of the conceptus in utero after d 17 has been shown to prolong the estrous cycle in cows (19). Hence, it is probable that thermally depressed fertility is unlikely to be diagnosed by the presence of extended estrous cycles, if silent ovulations are accounted for.

Progesterone concentrations during the second half of the luteal phase in cyclic and in inseminated nonpregnant NC cows were lower than in C cows. This might be related to the earlier luteal regression in NC than in C cows, as also suggested by the slightly shorter duration of estrous cycle in NC cows. This indicates that the CL is probably susceptible to thermal stress, and that its earlier regression might reduce fertility in thermally stressed cows. In agreement with this suggestion is the finding that human chorionic gonadotropin administration on d 15 post-AI increased pregnancy rate of dairy cows in a hot summer climate (27). Lower plasma P_4 concentration during the second half of the luteal phase could be a result of heat stress during the earlier stage of CL development. This was recently shown for the pregnant rabbit; heat stress during the formative stage of the CL (d 3 to 5 postmating) was followed by a decrease in P_4 concentration only at the end of the period of heat exposure with further decline on the subsequent days (28). Progesterone curves for cows found pregnant on d 45 post-AI were similar in the C and the NC groups. This might suggest that presence of a live, normally developed conceptus could prevent the decline in P_4 concentration induced by thermal stress.

The effect of heat stress on progesterone concentration is equivocal. Increases (1, 23), no change (4), and decreases (8, 25) in P_4 concentration have been recorded. These differences might be related to the thermal state of the animals. Because plasma samples were collected around noon, when hyperthermia had only started to develop in NC cows, it is possible that the lower P_4 in NC than in C cows reflect mainly the P_4 output of the CL. In other studies, in which blood was sampled while the animals were heat-stressed and where increases in P_4 concentrations were found (26), the adrenal contribution to systemic P_4 concentrations might be more pronounced. A low P_4 concentration might be associated with reduced ovarian blood flow, as found in the rabbit during early pregnancy (18), or with morphological changes in CL tissue as found in the hyperthermic rabbit (28). Possible alterations in uterine environment during thermal stress as well as its relationship to embryo survival and corpus luteum maintenance need further investigation.

No effect of BC at calving on subsequent fertility was noted in this study. The absence of such an effect is important in view of a reported decline in fertility in cows with fatty livers (21). Examination of liver enzymes and other metabolites in cows in the present study (unpublished results) did not reveal the presence of fatty liver syndrome in cows brought to high body condition during the dry period.

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