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# Interrelationships of Heat Stress and Reproduction in Lactating Dairy Cows

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## INTRODUCTION

Efficient reproductive performance of lactating dairy cattle in tropical/subtropical and arid environments throughout the world is impacted by a multiplicity of factors such as: the physical environment, social-economic status of producers, available nutrients, adaptability and genetic composition of cattle, intensive or extensive management systems, and available reproductive technology. Seasonal summer reductions in reproductive performance of lactating cows has been well-documented and is associated with decreased thermoregulatory competence of lactating dairy cows, partially due to intensive genetic selection for high milk production (Al-Katanani et al., 1999). With higher production, the associated increase in dietary dry matter intake (**DMI**) enhances heat increment, which when coupled with increases in metabolic heat production to produce milk, aggravates thermal balance during the stressful summer period.

Elevated environmental temperature, solar radiation, and relative humidity leads to hyperthermia or heat stress. Heat stress is defined as any combination of environmental conditions that will cause the effective temperature of the environment to be higher than the temperature range of the animal's thermal neutral zone. When rectal temperature is greater than 39.2 °C and breaths exceed 60/min, cows are on the verge of experiencing significant heat stress; this is the point at which the cow will heat up exponentially if exposed to further increases in temperature and humidity. Temperature Humidity Index (**THI**) has been used to integrate environmental temperature and relative humidity i.e., $THI = Td - (0.55 - 0.55RH/100) (Td - 58)$, where **Td** is the dry bulb temperature in °F and **RH** is relative humidity expressed as a percentage.

Lactating cows are thought to experience no stress when THI is less than 72 and severe stress when THI exceeds 88. These guidelines may shift somewhat depending on amount of milk produced, degree of air movement, and direct solar radiation. For example, Zimbelman et al. (2009) reported that dairy cows producing more than 35 kg/d of milk need additional cooling when average THI is 68 for more than 17 hr/d. Seasonal periods of reduced fertility are associated with concurrent increases in temperature and humidity, availability of nutrients, and elevations in body temperature detrimental to ovarian function, oocyte competence, embryo development, placental-fetal growth, and postpartum reproductive function.

In this review, a series of approaches are presented to partially alleviate harsh environmental stresses on productivity and fertility of lactating dairy cows.

- The first area involves utilization of classical genetics that also includes introduction of heat tolerant genes into less heat tolerant breeds.
- A second complementary approach is the use of various environmental facilities and management to provide heat abatement in order to enhance performance.

- A third thrust is identifying thermal sensitive reproductive responses coupled with reproductive technologies and their application to manage seasonal periods of infertility associated with heat stress.

## GENETIC STRATEGIES TO INCREASE MILK PRODUCTION AND REPRODUCTIVE EFFICIENCY

Conventional crossbreeding between breeds of *Bos taurus* cattle and *Bos taurus* with local *Bos indicus* cattle (**$F_1$**) has been a strategy to improve resistance to thermal stress; but always lowers milk yields in the $F_1$ generation compared to the *Bos taurus* purebred dairy cow. Fluctuations in environmental conditions (climate, feeding, management, etc.) from year-to-year at the same tropical location can be important in determining the preferred genotype of dairy cows.

Within the Holstein breed in the USA, the Predicted Transmitting Ability (**PTA**) for milk yield and heat tolerance was determined from 172,411 sires and 10.5 mil cows (Bohmanova et al., 2005). Heat tolerance PTA of sires ranged from -0.48 to 0.38 kg milk per THI unit above 72/d; milk yield PTA for sires were between -8.9 and 7.9 kg/d. Based on estimated heat tolerance PTA, the 100 most and 100 least heat tolerant sires were examined. Bulls that transmit a high tolerance to heat stress have daughters with higher pregnancy rates and a longer productive life; but lower milk yields (Bohmanova et al., 2005). Continued selection for milk yield without consideration of heat tolerance likely will result in greater susceptibility to heat stress. Conversely, selection of bulls for heat tolerance will likely result in a decrease in milk yield. This is to be expected because as average production per cow increases the metabolic heat output increases, making cows more susceptible to heat stress.

Since genetic variance for heat tolerance exists in dairy cattle, there is the likelihood that specific genes controlling heat tolerance can be introduced into the gene pool of the population. One such gene is the slick hair gene (*slick hair*) originally described in Senepol cattle, subsequently identified in Carora cattle, and introduced into Holsteins by crossbreeding (Olson et al., 2003). The gene has been mapped to chromosome 20 (Mariasegaram et al., 2007). Animals with the dominant allele have a very short and sleek coat. Holstein (75 %) by Carora (25 %) crossbred dairy cows in Venezuela with slick hair coats had lower body temperatures in heat stress conditions than those with the wild-type hair coat ($38.58\ ^{o}C < 39.09\ ^{o}C$) and produced more milk ($6389 > 5579$ kg, 305-d milk yield; Olson et al., 2003). The superior thermoregulatory ability associated with the slick phenotype is apparently the result of increased convective and conductive heat loss and decreased absorption of solar radiation.

During an experimentally imposed heat stress, slick haired lactating Holstein cows (i.e., out of Holstein cows sired by 75 % Holstein and 25 % Senepol bulls heterozygous for the slick hair gene) had lower vaginal temperatures and respiration rates than wild-type lactating cows (Dikmen et al., 2008). In either the indoor environment of a freestall barn with sprinklers and fans or the outside environment during the heat stress period, sweating rates were greater for the slick haired cows (indoor: 57 vs. 43 $g{\cdot}h^{-1}/m^2$; outdoor 82 vs. 61 $g{\cdot}h^{-1}/m^2$). Lactating cows with the slick hair gene had a greater sweating rate, which is deemed very important during hot stressful periods; since at an air temperature above 30 °C, 85 % of heat loss from the skin is through evaporation. Consequently, the slick hair gene is a candidate gene for incorporation into dairy cows that would improve regulation of body temperature and production potential in heat stress environments.

Differences between thermal adapted breeds and non-thermal adapted breeds extend to early developmental stages of the embryo (Hansen, 2007). *Bos indicus* embryos are less adversely affected by elevated temperature in culture than Holstein or Angus embryos. Furthermore, *Bos taurus***Bos indicus* embryos, in response to an *in vitro* heat shock, have a higher rate of blastocyst development acquired through *Bos indicus* genes that contribute to presence of thermotolerance factors from the oocyte or imprinting of certain embryonic paternal genes. For example, embryos produced by insemination of Brahman oocytes with Angus semen were more thermotolerant than embryos produced by insemination of Holstein oocytes with Angus semen. However, there was no difference in thermotolerance between Day 4 embryos produced by insemination of Holstein oocytes with Brahman semen versus embryos produced by insemination of Holstein oocytes with Angus semen. With the known gene sequences of the bovine genome, future identification of heat tolerance genes of *Bos indicus* breeds offers the potential of introducing these genes into less heat tolerant breeds.

## MODIFICATION OF THE ENVIRONMENT

Implementation of heat abatement facilities can enhance both pregnancy rates and milk production. Heat abatement is dependent upon optimizing heat exchange via convection, conduction, radiation, and evaporation. The system to be used depends upon the local environment (e.g., arid to tropical) and includes the use of: shades (reduction in solar radiation); sprinklers and fans under shade structures (enhances evaporative cooling from the skin surface); fans and sprinklers in the holding areas and/or exit lanes from the milking parlor; fans and sprinklers in free-stall facilities (e.g., cooling cows along the feed lines with sprinklers and fans); and evaporative cooling systems (i.e., cool the air that ultimately surrounds the cow).

Although a shade structure partially alleviates heat exposure from solar radiation, there is no alteration in air temperature or relative humidity; consequently, additional cooling strategies are required for lactating cows in a tropical/subtropical environment. Collier et al. (2006) reviewed extensively the dynamics of environmental management and subsequent impacts on the lactating dairy cow. A benchmark reference point for lactating cow status is a surface skin temperature of 35 $^{o}$C. Below this temperature all 4 routes of heat exchange are possible, and the micro environment to sustain a skin temperature at or below 35 $^{o}$C avoids reductions in milk yield.

These types of environmental management systems need to be optimized for the region of application and integrated with the production potential of the area. For example, in many tropical areas, the period of stress most often extends for an extended period of the year and is coupled with diseases, parasites, and low nutritional inputs. Obviously, a system under this environment needs to incorporate a management plan that not only protects animals from periods of thermal stress; but provides more stringent health care, well being, and nutritional inputs to reach the production potential of the animal unit in the system. Such systems involve increased investment of monies to allow maximal performance of high-producing animals.

A system of environmental management comprised of intermittent cooling with sprinkling and forced ventilation throughout the heat stress period in Israel, improved conception rates (Berman and Wolfenson, 1992). However, fertility levels were not restored to levels typical of what is found during the winter months. An additional study intensified the period of cooling, such that cows were cooled 7 times a day for 30 min by sprinkling and ventilation in the summers (i.e., 1985-1986; Wolfenson at al., 1988a). The cooling system consisted of fans and sprinklers, sequentially actuated to repeat cooling cycles of wetting (30 s) and forced ventilation (4.5 min) for 7, 30 min-periods with intervals of 2 h between cooling periods. Cooling began at 0730h. During the intervals between the cooling periods, ventilation was continuous between 0600 and 2400 h. Pregnancy rate at 90 d postpartum was higher in cooled (44 %) than in non-cooled cows (14 %). The authors felt that complete elimination of heat stress with the cooling system of intensive and frequent use of sprinkling and ventilation was able to achieve summer conception rates comparable to that recorded in winter.

A similar approach was conducted earlier in Florida (1970 and 1971) with the use of an environmentally controlled structure that would house 48 cows (Thatcher et al., 1974). Effects of a controlled environment on production and reproduction were evaluated with 4 groups of cows: T1, remained outside 24 h; T2, inside from 1930 h to 0530 h; T3, inside from 0530 h to 1930 h; T4, inside 24 h. Average daily degree-hours above 21 °C (T 1 to T4) for 1970 (148 d) and 1971 (79 d) were 100, 84, 41, 11, and 71, 62, 19, 0. Milk yield, fat percentage, fat yield, 4 % fat-corrected milk, and conception rates were improved by controlled environment. Least squares means for daily adjusted 4 % fat corrected milk yields (kg, 208 cows overall) were T1, 14.23; T2, 14.48; T3, 14.96; T4, 15.57 kg. Conception rates to all services (n=305) were T1, 28.1 %; T2, 28.4 %; T3, 40.0 %; T4, 38.8 %. It was clear that cooling cows during the daylight hours or 24 hr/d in summer sustained conception rates of 40 %.

Both the intensive cooling study in Israel and the air-conditioned experiment in Florida suggested that perhaps winter pregnancy rates may be achieved with very intensive cooling. However, this is highly unlikely in today's lactating dairy cows with greater milk production. The 4 % fat corrected milk production for lactating dairy cows of Israel in 1984 and the USA in 1971 (i.e., ~21.4 and 14 Kg/d, respectively; Kadzere et al., 2002) are considerably lower than the high producing cows of 2008 in Israel and the USA (36.2 and 36.8 Kg/d, respectively). The estimated increase in energy expenditure of a USA cow to produce an increase in milk production/d between 1971 and 2008 (i.e., from 14 kg/d to 36.8 kg /d) is 21.55 Mcal/d (NEL + NEM + HI). This amount of energy will increase the temperature of 6331 gallons of water by 1 °C. Consequently a tremendous increase in heat exchange needs to be accommodated within a controlled environment of elevated summer temperatures to maintain a normal body temperature without decreasing DMI, milk production, and pregnancy rate. This is a management challenge, and it is not surprising that heat abatement systems may not totally sustain normal pregnancy rates in the summer period.

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Particularly vulnerable is pregnancy rate, because of the sensitivity of the oocyte and sperm at the time of insemination and the early developing embryo to an elevation in body temperature for a short period of time. When uterine temperature the day after insemination is elevated 0.5 °C (0.9 °F) above the mean of 38.3 °C (100.9 °F), conception rate is reduced 6.9 %. Thus a 38.8 °C (101.8 °F) uterine temperature was a temperature associated with a decrease in conception rate. It is important to realize that mean uterine temperature was 0.2 °C (0.3 °F) higher than rectal temperature. This uterine temperature relationship with conception rate is basically inherent in lactating dairy cows of the *Bos taurus* species. However, the higher milk production and feed intake of cows today likely lowers the environmental conditions (e.g., THI) at which the cow begins to elevate her rectal and/or uterine temperature. Conversely, more intensive heat abatement systems are necessary to attenuate a diurnal rise in rectal or uterine temperature.

Low profile cross ventilated **(LPCV)** freestall buildings are 1 option for dairy cattle housing (Smith et al., 2008). These facilities allow producers to control a cow's environment during all seasons of the year. As a result, an environment similar to the thermoneutral zone of a dairy cow is maintained in the summer, resulting in more stable core body temperatures. For example, a heat stress audit was conducted on a North Dakota dairy to evaluate the impact of a changing environment on the core body temperature of cows (Smith et al., 2008). Vaginal temperatures were collected from eight cows located in the LPCV facility with evaporation pads and fans and 8 cows located in a naturally ventilated freestall facility with soakers and fans. Vaginal temperature was recorded every 5 min for 72 hr using data loggers (HOBO® U12) attached to a blank intravaginal CIDR device. The environmental conditions and vaginal temperatures during the evaluation period are presented in Figures 1 and 2. Vaginal temperatures of cows housed in the LPCV facility were more consistent and did

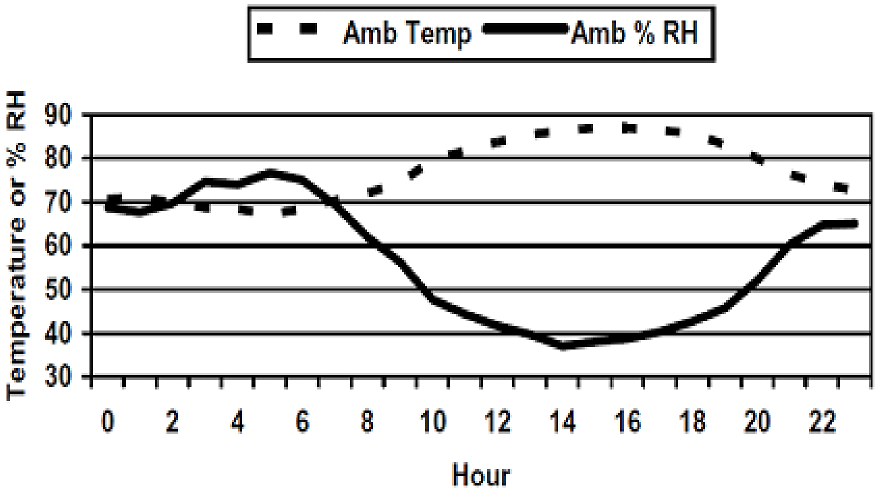

**Figure 1**. Ambient (Amb) temperature and percent (%) relative humidity for Milnor, North Dakota, USA (Smith et al., 2008).

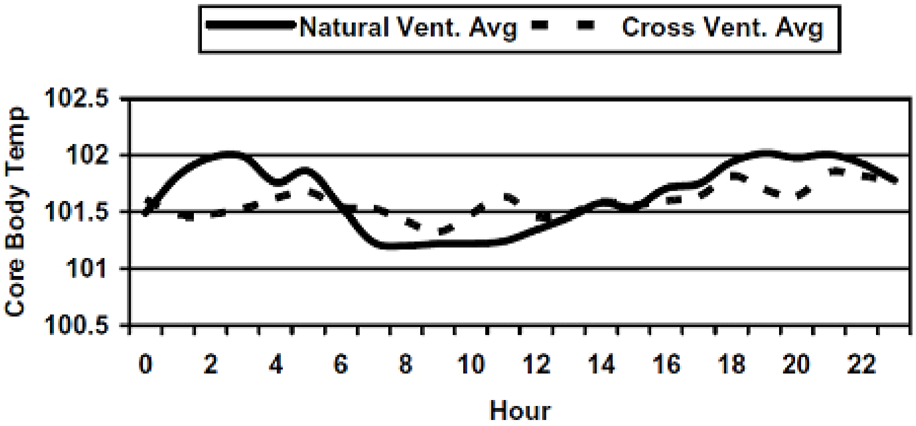

**Figure 2.** Vaginal body temperature of cows housed in naturally ventilated freestalls with fans and soakers and low profile cross ventilated freestalls with evaporative pads (Smith et al., 2008).

not reach the 102 °F temperature recorded in the cows of the naturally ventilated facility with fans and soakers. Based upon the benchmark measurements presented for uterine temperatures, cows in the naturally ventilated facility would experience a decrease in conception rate.

Feed-line soakers in naturally ventilated buildings are effective in cooling cows, but they require the cows to walk to the feed-line to be soaked. In contrast, cows in an LPCV facility experience temperatures that are considerably lower than the ambient temperature and have reduced fluctuations in vaginal body temperature, which will benefit milk production and reproduction. However, even cows in the LPCV facility have an attenuated fluctuation in vaginal temperature that is comparable to 101.8 °F. Thus one would anticipate that there will be some reduction in pregnancy rate, but not to the extent expected in the naturally ventilated free-stall facility with soakers and fans. Clearly, environmental modification with intensive whole animal cooling provides a means to enhance both milk production and reproductive efficiencies during the summer months.

## ASSESSMENT OF INTENSIVE COOLING IN HIGH YIELDING DAIRY HERDS OF ISRAEL

Over the last decade, Flamenbaum and co-workers conducted extensive surveys of dairy farms in cooperation with the Israel Cattle Breeders Association (**ICBA**). The aim of these studies was to evaluate the effect of cooling systems, installed on commercial farms, as to the response of productive and reproductive traits of high yielding Holstein lactating cows. Specifically, ratios between summer and winter daily production of economical corrected milk (**ECM**) and conception rates were examined.

A complete summary of the various surveys was described recently (Flamenbaum, 2009). For example, one survey quantified the overall effect of better management of dairy farms, which intensively cool cows in the summer, on their annual productive and reproductive results (Flamenbaum and Ezra, 2009). Summer to winter ECM ratios for each herd served as the response indicator by which the 24 top farms for ECM ratio were

**Table 1.** Summer and winter averages of economical corrected milk (ECM) and conception rate (CR) for first 3 inseminations and their ratios in high and low ratio herds.

| | Group | |
|---|---|---|
| Parameter | Low S : W ratio | High S : W ratio |
| No. Herds | 24 | 24 |
| Winter milk production, kg/d | 39.5 | 39.7 |
| Summer milk production kg/d | 34.4 | 38.9 |
| S:W ECM ratio | **0.87** | **0.98** |
| Winter conception rate, % | 0.36 | 0.40 |
| Summer conception rate, % | 0.19 | 0.27 |
| S:W ratio | 0.53 | 0.68 |

**Table 2.** Average 305-d production for milk, economically corrected milk (ECM), milk fat, and milk protein for herds with high and low S:W ratio.

| | Group | | | |
|---|---|---|---|---|
| | Low S : W ratio | High S : W ratio | Difference (kg) | Added production (%) |
| Milk, kg | 11,346 | 12,017 | 671 | 6.0 % |
| ECM, kg | 11,081 | 11,807 | 726 | 6.5 % |
| Milk, kg | 402.6 | 430.1 | 27.5 | 6.8 % |
| Milk protein, kg | 360.9 | 385.3 | 24.4 | 6.8 % |

compared to the 24 farms with the lowest ECM ratios. It was assumed that the difference between the productive ratios of these 2 groups represented the combined effect of cooling and better summer management on annual milk yield and reproduction traits.

The average herd size of farms in the study was 400 cows, so the comparison includes nearly 10,000 cows in each group. Initial averages for productive and reproductive traits for the *high* and *low* ratio groups are presented in Table 1. The fact that the average winter milk production was similar in both groups supported the supposition that most of the differences in the S:W ratio among farms in the 2 groups can be related to better management in the summer for the high ratio group. Indeed summer conception rates in either grouping were not equivalent to the winter period. However, the mean decrease in conception rate of the High S:W ratio group was less, such that this grouping achieved 68 % of the winter conception rate. In contrast, farms in the low S:W ratio group for ECM had a lower summer conception rate achieving 53 % of their winter conception rate.

Least squares means for Milk, ECM, milk fat, and milk protein for 305 d lactations for high and low S:W ratio farms are presented in Table 2. The data indicated that intensive cooling of high yielding dairy cows under Israeli summer conditions had the potential to add approximately 700 kg ECM to every cow's lactation, an increase of 6.5 % in annual production. Cows in high ratio herds that calved in spring and early summer reached higher lactation peaks, while those calving in winter had more persistent lactations compared to cows on low ratio farms, probably due to being intensively cooled in summer.

Collectively, this type of record analysis of S:W ratios identifies farms that can improve their intensive cooling systems with perhaps closer attention to proper maintenance of the systems to get maximal cooling and management of the cows during the heat stress period. Basically the results indicate that cows in both groupings did not differ in either milk production or conception rate in the winter months. Results further demonstrate that in high producing cows it is difficult to get sufficient heat stress abatement in summer to sustain reproductive function at the level obtained during the winter. Comparison among farms identifies those farms that need closer attention to their existing high intensity cooling systems and management and provides a means to evaluate one's system to those who use comparable systems; or for producers with moderate cooling systems to glean what can be achieved by upgrading their heat stress management system.

## REPRODUCTIVE PHYSIOLOGICAL RESPONSES ASSOCIATED WITH SEASONAL AND ACUTE HEAT STRESS

To further improve reproductive efficiency under environmental conditions leading to heat stress, it is essential to understand what physiological reproductive responses are altered during this stressful period of the year. Studies completed in Spain provide valuable insight into seasonal differences in reproductive responses collate with changes in reproductive tract responses, such as ovarian activity and disorders (Lopez-Gaitus et al., 2001; Lopez-Gaitus, 2003). Pregnancy (P)/AI was lower in the warm season (27.4 < 44.4 %). Furthermore, the incidence of inactive ovaries (i.e., detection of no CL or ovarian cyst at 2 consecutive rectal palpations 7 d apart) and ovarian cysts were higher in the warm seasons (i.e., 12.9 > 1.2 % and 12.3 > 2.4 %, respectively).

The P/AI during the warm season had a greater decrease per 1000 kg increase in milk production than that observed for all cows (i.e., 6 vs 3 % per 1000 kg increase in milk production). When this relationship was examined in cows only during the cool season, there appeared to be no change in P/AI with increases in milk production (Figure 3). Inactive ovaries were subsequently associated with persistent follicles with no CL or ovarian cysts (Lopez-Gatius et al., 2001). These findings confirm on a farm basis that aberrant follicle development occurs during the warm season.

### *Follicle Dynamics*

Lactating dairy cows express a less intense behavioral estrus than non-lactating cows or heifers. Indeed, the metabolic and hormonal changes of lactating dairy cows reduce the proestrus concentrations of estradiol (de la Sota et al., 1993), and this is further reduced by heat stress (Gwazduskas et al., 1981). Collectively, these changes reduce the probability of detecting cows in estrus. The transitional patterns of reduced fertility in early summer and the restoration of fertility in the fall are strikingly different. Conception rates drop precipitously in the beginning of the stress season and return gradually (e.g., from October to December) with the end of the summer stress. Heat exposure of lactating cows during the entire estrous cycle induced a 50 % increase in the number of large (>10mm) follicles during the first follicular wave (Wolfenson et al., 1995), and a similar trend was observed in

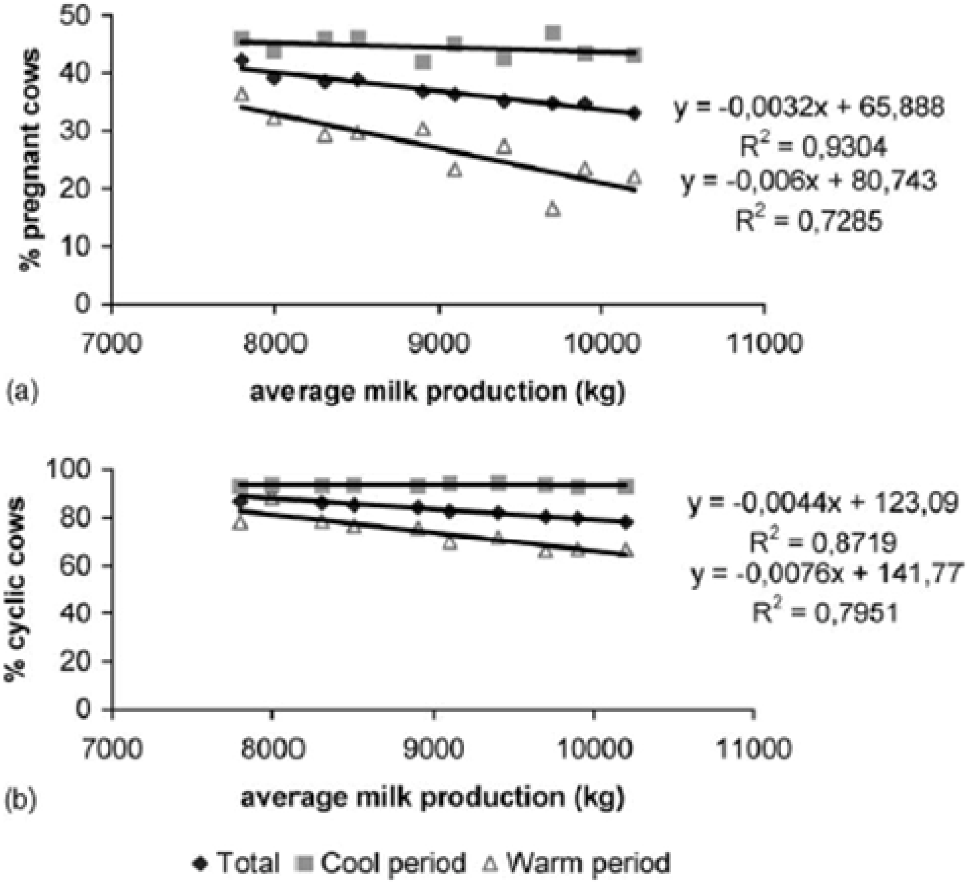

**Figure 3.** Percent pregnant per AI (a) and percent cyclic cows (b) regressed with average milk production (Kg) of cows during the cool and warm seasonal periods, as well as the total for the year.

heat stressed heifers during d 17-21 of the cycle (Wilson et al., 1998).

An additional observation supporting diminished follicle dominance is the lack of a decline in the number of medium-size follicles during the period of dominance of the first-wave (Badinga et al., 1993) or preovulatory follicle (Wolfenson et al., 1995; Wolfenson et al., 2000). A depression of follicular dominance induced by heat stress also appears to be associated with a 2-3 d earlier emergence of the second-wave dominant-preovulatory follicle (Wolfenson et al., 1995). With earlier emergence of the second-wave dominant follicle and consequently a longer persistence to ovulation, it is anticipated that P/AI to that ovulation will be less, since a slightly longer period of dominance is associated with lower embryo quality (Cerri et al., 2009).

Seasonal studies report lower steroid concentrations in the follicular fluid obtained from large follicles during the hot season are associated with reduced viability of granulosa cells and impaired aromatase activity (Badinga et al., 1993; Wolfenson et al., 1995). Alterations of steroidogenic capacity induced by summer heat stress carry over to the final stage of follicle development, as evidenced by reduced androstenedione production by thecal cells and low estradiol concentrations in follicular fluid collected from dominant follicles in the autumn (Wolfenson et al., 1997). Sensitivity of steroidogenesis to heat stress is reflected by decreased estradiol and androstenedione production from granulosa and thecal cells obtained from follicles 3 to 4 wk after acute heat stress (Roth et al., 2000). Similarly, estradiol content in the follicular fluid aspirated from cows was relatively low in late summer and increased throughout the autumn (Roth et al., 2004). Thus the extent of the heat stress effects on follicular function is transient, as also reflected by the spontaneous improvement of fertility throughout the fall and early winter (Al-Katanani et al., 1999).

Based upon these adverse effects of heat stress on follicular estradiol production and follicle dynamics, it is not surprising that heat stress reduces the length and intensity of estrus. For example, in summer motor activity and other manifestations of estrus are reduced (Hansen and Arechiga, 1999) and incidence of anestrous and silent ovulations are increased (Gwazdauskas et al., 1981). Nebel et al. (1997) reported that Holsteins in estrus during the summer had 4.5 vs. 8.6 mounts/estrus for those in winter. On a commercial dairy in Florida, undetected estrous events were estimated at 76 to 82 % during June through September compared to 44 to 65 % during October through May (Thatcher and Collier, 1986).

A tendency for a reduction in plasma inhibin concentrations was detected in heat stressed lactating dairy cows (Wolfenson et al., 1995). Furthermore concentrations of plasma inhibin were reduced in the summer for cyclic buffaloes (Paltra et al., 1997). Collectively these findings suggest that depression of follicular dominance during heat stress involves a decrease in inhibin secretion by granulosa cells and subsequent alterations in FSH that leads to an increase in development of large follicles. This may account for the increase in twinning rate (May-July; Ryan and Boland, 1991) following insemination of cows during the summer, in that increases in the number of large follicles occur in the summer. However, due to elevated body temperature, early embryo losses occur and pregnancies are not sustained. When cows cool off in the early fall and are undergoing a transitory recovery of follicle dominance, the incidence of double ovulations and twin pregnancies occurs with calving occurring in the following summer.

Induction of acute heat stress on the day of estrus for just 15 h reduced subsequent embryo survival following insemination (Putney et al., 1989). Heat stress at this time is during the pre-ovulatory period in which the oocyte has commenced maturation following the LH surge and is still in the microenvironment of the follicle exposed to granulosa cells and follicular fluid.

Presently, it is not known at what stage in the follicular hierarchy of the ovary that heat stress damages the ovarian follicle and/or oocyte. This is an important area for future investigation, since heat damage of the ovary may be analogous to damage of the testis in which a time lag of approximately 45 d is required before completion of the spermatogenic cycle leading to the production and ejaculation of new sperm that were not damaged by heat stress. A comparable time lag in the female ovary may account for or contribute to delays in restoration of fertility in the fall. From a holistic perspective, heat stress can compromise follicular growth and steroidogenic capacity due either to direct actions of elevated temperature and/or alterations of follicular function, such that the oocyte can be compromised leading to a reduction in P/AI.

### *Heat Stress Alters Early Embryo Development: Role of Timed Embryo Transfer to By-Pass the Window of Embryo Sensitivity*

Heat stress at d 1 or d 1 to 3 after breeding reduced embryonic survival (Ealy et al., 1993). Heat stress of superovulated cows at d 3, 5, or 7 after estrus did not affect embryonic development or survival at d 8 (Ealy et al., 1993). Consequently, effects of heat stress on embryonic survival decrease as embryos proceed through development. Hansen (2007) reviewed various strategies to improve embryonic survival in dairy cattle during heat stress, and utilization of timed embryo transfer was effective in studies in both Florida and Brazil. Embryos transferred into recipients at d 7 - 8 after either an estrus or injection of GnRH to induce a programmed ovulation will by-pass the thermosensitive periods of the oocyte or early embryo. Pregnancy rates are enhanced with embryo transfer during periods of heat stress because transfers are made with embryos considered transferable that were not exposed to heat stress or were developmentally competent to be transferable. Natural embryos that were cryopreserved from superovulated donors or fresh embryos produced *in vitro* improved pregnancy rates, but cryopreserved embryos produced *in vitro* did not enhance pregnancy rates.

Current studies by Block, Bilby, and Hansen in Texas (unpublished observations, 2009) document on farm benefit of transferring fresh *in vitro* produced embryos. The experiment was conducted in the summer of 2009 on 2 commercial dairies in Texas. Embryos were produced *in vitro* using X-sorted semen. The vitrification process of freezing embryos was done on embryos harvested on d 7 after insemination in which embryos were vitrified (**VIT**) using the open-pulled straw method. Fresh embryos were harvested, also on d 7 after insemination, and then transported to the farms for transfer. Experimental recipients were lactating dairy cows and blood samples were collected to determine plasma progesterone concentrations on the day of AI (AI group) and comparable day for ET cows (d 0; VIT Embryo and Fresh [**FR**] Embryo Groups). All cows were palpated on d 7 using ultrasonography to confirm the presence or absence of a CL.

Embryos were transferred only to those cows with a CL. Cows were considered synchronized (i.e., both AI and VIT, FR embryo transfer groups) if they had less than 1.0 ng/mL progesterone on day 0 and a CL confirmed by ultrasound on day 7. The AI cows were inseminated with non-sorted commercial semen. Pregnancy was diagnosed by palpation at d 34-46 and again at d 90 - 107 of gestation. Pregnancy rates for the synchronized cows at 34 to 46 d were: AI:22.9 % (36/157); VIT: 30.9 % (58/188); and FR: 45.5 % (61/134). Reconfirmed pregnancy rate at days 90-107 were: AI 21.2 % (33/156); VIT: 27.0 % (50/185); FR: 39.4 % (52/132). These findings under commercial conditions are very encouraging.

Embryo transfer of female sexed embryos resulted in basically a 40 % pregnancy rate by 90 to 107 d of pregnancy. Compared to AI embryo transfer of fresh embryos resulted in 40 % pregnancies or 34 % female fetuses (i.e., 40 % x 85 % female enriched semen) as opposed to 21.2 % pregnancies or 10.5 % female fetuses (31.2 % x 50 % sperm carrying the X-chromosome). This demonstrates the power of using available reproductive technology. Clearly embryo transfer by-passed the early period of embryo sensitivity on the 2 commercial dairies.

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An area needing further development is the technique of freezing *in vitro* produced embryos. Nevertheless, the VIT embryo group tended to have a higher pregnancy rate than the AI group, indicating some progress compared to an earlier study in which frozen *in vitro* produced embryos gave exactly the same low pregnancy rate as the AI group and both were lower than the cows receiving non-sexed fresh embryos (Ambrose et al., 1999). A by-pass of early embryo sensitivity to heat stress has occurred with the 7 d embryo transfer because transfers are made with embryos considered transferable that were not exposed to heat stress or were developmentally competent to be transferred. Several studies have documented the beneficial effects of adding IGF-1 to bovine embryos produced *in vitro* on both enhancing the rate of blastocyst development and reducing the magnitude of elevated temperature effects on inhibition of blastocyst development and apoptosis (Moreira et al., 2002; Hansen, 2007). Furthermore, *in vivo* embryo transfer of *in vitro* produced embryos that were cultured with IGF-1 increased pregnancy and calving rates during heat stress, but not during the non-heat stress seasons.

Heat stress can also reduce embryo growth up to d 17 (Biggers et al., 1987), which is a critical time point for embryo production of interferon-tau. Adequate amounts of interferon-tau are critical for reducing pulsatile secretion of prostaglandin $F_{2\alpha}$ in order to block CL regression and maintain pregnancy. Putney et al. (1988) incubated embryos and endometrial explants obtained on d 17 of pregnancy at thermoneutral (39 °C, 24 h) or HS (39 °C, 6 h; 43 °C, 18 h) temperatures. The HS conditions decreased protein synthesis and secretion of interferon-tau by 71 % in embryos; however, endometrial secretion of prostaglandin $F_{2\alpha}$ and embryo secretion of prostaglandin $E_2$ increased in response to HS by 72 %. Collectively these studies demonstrate that both the embryo and the uterine environment can be disrupted due to HS inhibiting the embryo's ability to secrete interferon-tau (signal to block CL regression) and maintain pregnancy, manipulating production of potentially important proteins from the uterine endometrium, and stimulating prostaglandin $F_{2\alpha}$ that would antagonize maintenance of pregnancy.

Bilby et al. (2006) reported that supplementing lactating dairy cows with recombinant growth hormone at the time of AI and 11 d later increased growth factors, conceptus lengths, interferon-tau production, and pregnancy rates in lactating dairy cows compared to cows without bST supplementation. Possibly bST supplementation during heat stress may increase availability of important growth factors to improve embryo growth and survival. This strategy combined with feeding by-pass fats enriched in EPA and DHA may benefit reproductive performance during the summer heat stress period in well managed herds with good heat abatement facilities (Thatcher et al., 2010). This last point is very important because application of reproductive technology and nutritional management will not rescue dead embryos.

High environmental temperatures increase late embryonic-fetal losses. The likelihood of pregnancy loss (i.e., from first diagnosis of pregnancy at 35 to 45 d to a second diagnosis at d 90) increased by a factor of 1.05 for each unit increase in mean maximum THI calculated from D 21 to 30 of gestation. Pregnancy losses with maximum THI of 55, 55-59, 60-64, 65-69, and > 69 were 0, 1, 2, 8 and 12 %, respectively (Garcia-Ispierto et al., 2006).

Several reproductive management strategies have been implemented to improve P/AI during seasonal periods of heat stress. Implementation of a timed AI program to compensate for attenuated expression of estrus and reduced heat detection rates is an alternative. During the summer months, a standard timed AI program (i.e., Ovsynch) increased pregnancy rate at 120 d postpartum (27 vs. 16.5 %, respectively), decreased days open, and reduced both the interval from calving to first breeding and services per conception compared to lactating dairy cows inseminated at estrus (de la Sota et al., 1998). Injection of lactating dairy cows with GnRH at detected estrus during summer in Mississippi (Ullah et al., 1996) and summer/autumn in Israel (Kaim et al., 2003) increased conception rates from 18 to 29 % and 41 to 56 %, respectively. These findings are encouraging; however, if implemented it is vital that lactating dairy cows be managed under an efficient heat abatement system of intensive cooling. Otherwise, the reproductive manipulation has a low chance of success.

### *Placental and Fetal Development*

When dairy cattle are heat stressed during the last 2 to 3 mo of pregnancy, there are clear reductions in placental function (reduced concentrations of estrone sulfate), calf birth weight, and subsequent milk production during the ensuing lactation (Collier et al., 1982; Moore et al., 1992; Wolfenson et al., 1988b). Indeed, cooling of dry cows during the latter stages of pregnancy is an efficient means to improve animal productivity; this is a physiologically sensitive period that often is ignored by producers. It is possible that the secretion of bovine placental lactogen has been reduced due to reduced placental function.

Additional research is needed to determine if administration of recombinant bovine placental lactogen during late pregnancy in heat stressed cows would enhance both fetal growth and mammary development of the maternal unit. This would potentially compensate for a potential deficiency in placental hormonal secretion induced by heat stress. Maintenance of fetal growth and maternal mammary gland function after parturition likely would enhance neonatal survival and production of milk. Recent research by Dahl and coworkers at the University of Florida suggests that heat-stress abatement in the dry period improves subsequent lactation, possibly via suppression of plasma prolactin surge around calving (Amara et al., 2009) and alters peripheral lymphocyte function (Amaral et al., 2010).

## CONCLUSION

Multi-scenarios of management can improve reproductive performance of lactating dairy cows during seasonal periods of thermal stress. Implementation of various extensive to intensive heat abatement systems to improve productivity depends on the severity of the local environment. A system that sustains a skin temperature at or below 35 $^{o}$C avoids reductions in milk yield. Intensive cooling to completely eliminate heat stress results in close to normal fertility, indicating that short periods of hyperthermia compromise fertility. However fertility is not restored to winter levels.

Israeli researchers have documented the utility of calculating Summer:Winter ratios for milk production and P/AI to assess differences in performance of the herds' heat abatement management system.

Conventional crossbreeding between breeds of *Bos taurus* cattle and *Bos taurus* with local *Bos indicus* cattle ($F_1$) has been a strategy to improve resistance to thermal stress, but always lowers milk yields in the $F_1$ generation compared to the *Bos taurus* purebred dairy cow. Genetic variance for heat tolerance exists among bulls of the Holstein breed such that more heat tolerant bulls have daughters with higher pregnancy rates. Indeed heat tolerant genes are being identified, such as the slick hair gene, which improves heat tolerance and productivity. *Bos indicus* embryos are more heat tolerant, that offers the potential of introducing possible heat tolerant genes into *Bos taurus* breeds.

Various reproductive responses have been identified that are sensitive to heat stress (e.g., estrous behavior, follicle development, oocyte and embryo development, placental function and fetal growth). Knowledge of these thermal sensitive reproductive responses has led to the development of reproductive technologies that can be applied to manage seasonal periods of infertility associated with heat stress. These include timed embryo transfer of *in vitro* produced normal embryos that are sexed or such embryos treated with factors (e.g., IGF-1) that improve fertility. Cooling of cows during the dry period enhances postpartum calf birth weight and milk production. The above technological approaches provide an array of alternatives for producers in different socio-economic and environmental locations to improve productivity and fertility of dairy cattle.

## REFERENCES

Al-Katanani, Y. M., D. W. Webb, and P. J. Hansen. 1999. Factors affecting seasonal variation in 90-day nonreturn rate to first service in lactating Holstein cows in a hot climate. J. Dairy Sci. 82:2611-2616.

Ambrose, J.D., M. Drost, R.L. Monson, J.J. Rutledge, M.L. Leibfried-Rutledge, M-J. Thatcher, T. Kassa, M. Binelli, P.J. Hansen, P.J. Chenoweth, and W.W. Thatcher. 1999. Efficacy of timed embryo transfer with fresh and frozen in vitro produced embryos to increase pregnancy rates in heat stressed dairy cattle. J. Dairy Sci. 82:2369-2376.

Amaral, B.C. do., E.E. Connor, S. Tao, J. Hayen, J. Bubolz, and G.E. Dahl. 2009. Heat-stress abatement during the dry period: Does cooling improve transition into lactation? J. Dairy Sci. 92:5988-5999.

Amaral, B.C. do., E.E. Connor, S. Tao, J. Hayen, J. Bubolz, and G.E. Dahl. 2010. Heat stress abatement during the dry period influences prolactin signaling in lymphocytes. Dom. Anim. Endoc. 38:38-45.

Badinga, L., W.W. Thatcher, T. Diaz, M. Drost, and D. Wolfenson. 1993. Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. Theriogenology 39:797-810.

Berman, A., and D. Wolfenson. 1992. Environmental modifications to improve production and fertility. *In:* Large Dairy Herd Management. H.H. Van Horn and C.J. Wilcox., Eds., American Dairy Science Association, Champaign, IL. pp 126-134.

Biggers, B. G., R. D. Geisert, R. P. Wettemann, and D. S. Buchanan. 1987. Effect of heat stress on early embryonic development in the beef cow. J. Anim. Sci. 64:1512-1518.

Bilby, T. R., A. Sozzi, M. M. Lopez, F. Silvestre, A. D. Ealy, C. R. Staples, and W. W. Thatcher. 2006. Pregnancy, bovine somatotropin, and dietary n-3 fatty acids in lactating dairy cows: I. Ovarian, conceptus and growth hormone – Insulin-like growth factor system responses. J. Dairy Sci. 89:3375-3385.

Bohmanova, J., I. Misztal, S. Tsuruta, H.D. Norman, and T.J. Lawlor. 2005. National genetic evaluation of milk yield for heat tolerance of United States Holsteins. Interbull Bul. 33:160-162.

Cerri, R.L.A., H.M. Rutigliano, R.C. Chebel, R.G.S. Bruno, and J.E.P. Santos. 2009. Period of dominance of the ovulatory follicle during synchronization programs influences embryo quality. Reproduction 137:813–823.

Collier, R.J., G.E. Dahl, and M.J. VanBaale. 2006. Major advances associated with environmental effects on dairy cattle. J. Dairy Sci. 89:1244-1253.

Collier, R.J., S.G. Doelger, H.H. Head, W.W. Thatcher, and C.J. Wilcox. 1982. Effects of heat stress during pregnancy on maternal hormone concentrations, calf birth weight and postpartum milk yield of Holstein cows. J. Anim. Sci. 54:309-319.

de la Sota, R.L., M.C. Lucy, C.R. Staples, and W.W. Thatcher. 1993. Effects of recombinant bovine somatotrophin (Sometribove) on ovarian function in lactating and nonlactating dairy cows. J. Dairy Sci. 76:1002-1013.

de la Sota, R.L., C.A. Risco, F. Moreira, J.M. Burke, and W.W. Thatcher. 1998. Efficacy of a timed insemination program in lactating dairy cows during summer heat stress. Theriogenology 49:761-770.

Dikmen, S., E. Alava, E. Pontes, J.M. Fear, B.Y. Dikmen, T.A. Olson, and P.J. Hansen. 2008. Differences in thermoregulatoroy ability between slick-haired and wild-type lactating Holstein cows in response to acute heat stress. J. Dairy Sci. 91:3395-3402.

Ealy, A.D., M. Drost, and P.J. Hansen. 1993. Developmental changes in embryonic resistance to adverse effects of maternal heat stress in cows. J. Dairy Sci. 76:2899-2905.

Flamenbaum, I. 2009. Reproductive management of high yielding dairy herds in a hot climate: the case of Israel. Proc. Dairy Cattle Reproduction Council Conference, E.R. Jordan, ed. pp 7-14.

Flamenbaum, I., and E. Ezra. 2009. How much milk adds; intensive cooling of high-yielding dairy cows during hot season? *In:* The Dairy Industry In Israel, Israel Cattle Breeders Association Herd Book report. pp. 14.

Garcıa-Ispierto, I., F. Lopez-Gatius, P. Santolaria, J.L. Yaniz, C. Nogareda, M. Lopez-Bejar, and F. De Rensis. 2006. Relationship between heat stress during the peri-implantation period and early fetal loss in dairy cattle. Theriogenology 65:799-807.

Gwazdauskas, F.C., W.W. Thatcher, C.A. Kiddy, M.J. Paape, and C.J. Wilcox. 1981. Hormonal patterns during heat stress following $PGF_{2\alpha}$-Tham salt induced luteal regression in heifers. Theriogenology 16:271-285.

Hansen, P.J. 2007. Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress. Theriogenology 68S:S242-S249.

Hansen, P. J., and C. F. Arechiga. 1999. Strategies for managing reproduction in the heat-stressed dairy cow. J. Anim. Sci. 77(Suppl. 2):36–50.

Kadzere C.T., M.R. Murphy, N. Silanikove, and E. Maltz. 2002. Heat stress in lactating dairy cows: a review. Livestock Prod. Sci. 77:59-91.

Kaim, H., A. Bloch, D. Wolfenson, R. Braw-Tal, M. Rosenberg, H. Voet, and Y. Folman. 2003. Effects of GnRH administered to cows at the onset of estrus on timing of ovulation, endocrine responses, and conception. J. Dairy Sci. 86:2012-2021.

Lopez-Gatius, F. 2003. Is fertility declining in dairy cattle? A retrospective study in northeastern Spain. Theriogenology 60:89–99.

Lopez-Gatius, F., P. Santolaria, J. Ya´niz, J. Rutllant, and M. Lo´pez-Be´jar. 2001. Persistent ovarian follicles in dairy cows: a therapeutic approach. Theriogenology 56:649–59.

Mariasegaram, M., C.C. Chase Jr., J.X. Chaparro, T.A. Olson, R.A. Brenneman, and R.P. Niedz. 2007. The slick hair coat locus maps to chromosome 20 in Senepol-derived cattle. Anim. Gen. 38:54–59.

Moore, R.B., J.W. Fuquay, and W.J. Drapala. 1992. Effects of late gestation heat stress on postpartum milk production and reproduction in dairy cattle. J. Dairy Sci. 75:1877-1882.

Moreira, F., C., Orlandi, C.A. Risco, R. Mattos, F. Lopes ,and W.W. Thatcher. 2001. Effects of pre-synchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J. Dairy Sci. 84: 1646-1659.

Moreira, F., F.F. Paula-Lopes, P.J. Hansen, L. Badinga, and W.W. Thatcher. 2002. Effects of growth hormone and insulin-like growth factor-I on development of in vitro derived bovine embryos. Theriogenology 57:895-907.

Nebel, R. L., S. M. Jobst, M.B.G. Dransfield, S. M. Pandolfi, and T. L. Bailey. 1997. Use of radio frequency data communication system, HeatWatch®, to describe behavioral estrus in dairy cattle. J. Dairy Sci. 80 (Suppl. 1):179 (Abstr.).

Olson T.A., C. Lucena, C.C. Chase Jr., and A.C. Hammond. 2003. Evidence of a major gene influencing hair length and heat tolerance in *Bos taurus* cattle. J. Anim. Sci. 81:80–90.

Paltra, P., S. Mondal, B.S. Prakash, and M.L. Madan. 1997. Peripheral inhibin levels in relation to climatic variations and stage of estrus cycle in buffalo (Bubalus bubalis). Theriogenology 47:989-995.

Putney, D.J., S. Mullins, W.W. Thatcher, M. Drost, and T.S. Gross. 1989. Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between the onset of estrus and insemination. Anim. Reprod. Sci. 19:37-51.

Putney, D. J., J. R. Malayer, T. S. Gross, W. W. Thatcher, P. J. Hansen, and M. Drost. 1988. Heat stress-induced alterations in the synthesis and secretion of proteins and prostaglandins by cultured bovine conceptuses and uterine endometrium. Biol. Reprod. 39:717-728.

Roth, Z., A. Bor, R. Braw-Tal, and D. Wolfenson. 2004. Carry-over effect of summer thermal stress on characteristics of the preovulatory follicle of lactating cows. J. Therm. Biol. 29:681– 685.

Roth, Z., R. Meidan, R. Braw-Tal, and D. Wolfenson. 2000. Immediate and delayed effects of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows. J. Reprod. Fertil. 120:83–90.

Ryan, D.P., and M.P. Boland. 1991. Frequency of twin births among Holstein-Friesian cows in a warm dry climate. Theriogenology 36:1-10.

Smith, J.F., J.P. Harner, and J.P. Bradford. 2008. Opportunities with low profile cross ventilated freestall facilities. *In:* Proc. Dairy Housing of the Future. pp 1-21.

Thatcher, W. W., and R. J. Collier. 1986. Effects of climate on bovine reproduction. *In:* D. A. Morrow (Ed.) Current Therapy in Theriogenology 2. W. B. Saunders, Philadelphia. pp 301−309.

Thatcher, W.W., F.C. Gwazdauskas, C.J. Wilcox, J. Toms, H.H. Head, D.E. Buffington, and W.B. Fredriksson. 1974. Milking performance and reproductive efficiency of dairy cows in an environmentally controlled structure. J. Dairy Sci. 57:304-307.

Thatcher, W.W., F.T. Silvestre, J.E.P. Santos, and C.R. Staples. 2010. Nutritional modulation of immune-function and compatibility with pregnancy. Proc. 25th Ann. SW Nutr. Mgmt. Conf. pp. 25-42.

Ullah, G., J.W. Fuquay, T. Keawhoong, B.L. Clark, D.E. Pogue, and E.J. Murphy. 1996. Effect of gonadotrophin-releasing hormone at estrus on subsequent luteal function and fertility in lactating Holstein during heat stress. J. Dairy Sci. 79:1950–1953.

Wilson, S.J., C.J. Kirby, A.T. Kenigsfeld, D.H. Keisler, and M.C. Lucy. 1998. Effects of controlled heat stress on ovarian function of dairy cattle. 2. Heifers. J. Dairy Sci. 81:2132-2138.

Wolfenson, D., I. Flamenbaum, and A. Berman. 1988a. Hyperthermia and body energy store effects on estrous behaviour, conception rate, and corpus luteum function in dairy cows. J. Dairy Sci. 71:3497-3504.

Wolfenson, D., I. Flamenbaum, and A. Berman. 1988b. Dry period heat stress relief effects on prepartum progesterone, calf birth weight and milk production. J. Dairy Sci. 71:809-818.

Wolfenson, D., Z. Roth and R. Meidan. 2000. Impaired reproduction in heat-stressed cattle: basic and applied aspects. Anim. Reprod. Sci. 60-61: 535-547.

Wolfenson, D., W.W. Thatcher, L. Badinga, J.D. Savio, R. Meidan, B.J. Lew, R. Braw-Tal, and A. Berman. 1995. Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. Biol. Reprod. 52:1106-1113.

Wolfenson, D., B.J. Lew, W.W. Thatcher, Y. Graber, and R. Meidan. 1997. Seasonal and acute heat stress effects on steroid production by dominant follicles in cows. Anim. Reprod. Sci. 47:9–19.

Zimbleman, R.B., R.P. Rhoads, M.L. Rhoads, G. C. Duff, L.H. Baumgard, and R.J. Collier. 2009. A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. Proc. 24th Ann. SW Nutr. Mgmt. Conf., pp.158-168.