

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Alleviating Heat Stress Leads to Improved Cow Reproductive Performance

---

Siriwat Suadsong

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50881>

---

## 1. Introduction

Thailand, a country situated in the south eastern part of Asia, is situated in between 15° 00' North latitude and 100° 00' East longitude and located in a tropical area with high temperature and humidity. Crossbred Holstein dairy cattle are popular because, during times of high environmental thermal stress, their milk production and reproductive efficiency is not depressed as it is with purebred Holstein cattle. However, these crossbred cattle have been inseminated with purebred Holstein frozen semen to improve milk production. Although the genetic potential for milk yield has improved, the predominant dairy breed has now become Holstein and the impact of heat stress on production and reproduction has increased.

This chapter will be showed the finding of our studied that show the impact of heat stress on postpartum reproductive performance and milk production and evaluate the effects of utilizing an evaporative cooling system for improving cow comfort, energy balance, postpartum reproductive performance and milk production of early lactating dairy cows under hot and humid climatic conditions.

Climatic conditions in the tropics are such that the hot season is relatively long, there is intense radiant energy for an extended period of time, and there is generally high relative humidity. Thus heat stress is chronic in nature, there is often little relief from the heat during the night, and intense bursts of combined heat and humidity depress performance. Lactating dairy cows create a large quantity of metabolic heat and accumulate additional heat from radiant energy. Heat production and heat accumulation, coupled with a compromised cooling capability, because of environmental conditions, causes heat load in the cows to increase to a point when body temperature rises, feed intake declines and ultimately the cow's productivity falls.

During period of elevated temperature, animals show less physical activity and seek shelter to decrease radiant heat exposure. Elevated body temperatures will rapidly trigger adaptive mechanisms to restore body temperature to normal. These adaptations, including panting, sweating, reduced feed intake and lowered metabolism, may be necessary for survival, but they are not generally favorable to milk production [1]. Moreover, because of their relative size and their high metabolic rate, associated with milk production, dairy cows are particularly susceptible to the effects of heat stress.

Heat stress has a significant impact on dairy cattle in hot and humid climates. Environmental factors, which contribute to heat stress, include high ambient temperatures, radiant energy, and high humidity, all of which compromise the cow's ability to dissipate body heat. When the cow cannot dissipate sufficient heat to maintain thermal balance, body temperature rises and heat stress occurs. Ambient temperature is a major component of heat stress, however humidity must also be considered because evaporative heat loss is more effective when humidity is low. The temperature-humidity index (THI) combines these two factors into an indicator of cow comfort. Cows are beginning to be stressed when the THI exceeds 72 [2].

Dairy cows have several mechanisms to help dissipate body heat and maintain body temperature, such as; conduction, convection, radiation and evaporation. Conduction, convection, and radiation depend on a relatively large differential between the body and the environmental temperature, and evaporation works best at a low relative humidity. When the environmental temperature nears the cow's body temperature and is coupled with high relative humidity, all the cow's cooling mechanisms are impaired. As a result the cow's body temperature rises and the cows exhibit physiological responses to hot weather. Cows in hot climates generally produce additional heat, compared to those in cool climates, because of greater physical activity (such as panting) which is necessary to enhance cooling in hot conditions. In addition lactating dairy cows produce large amounts of heat from both ruminal fermentation and metabolic processes. As production increases, the total amount of heat produced increases. In order to maintain body temperature within the normal range, dairy cows must exchange this heat with the environment. The most noticeable response to heat stress is reduced feed intake, reduced milk yield, reduced activity, and increased respiration rate and water intake.

## **2. Effects of heat stress on production**

Temperature and humidity combine to decrease dry matter intake (DMI) in dairy cows as a physiological means of regulating internal body temperature. This is accomplished by decreasing rumen fermentation and the metabolic rate [3,4]. A reduction in DMI decreases the nutrients available for milk synthesis, milk production declines and many lactation parameters are affected [5-7]. High environmental temperatures also increase the respiration rate and the water intake, which consequently reduces DMI due to gut full [8]. Because of many dairy cows in hot weather are unable to consume enough feed to meet energy demands during early lactation, they typically mobilize body reserves to maintain their milk

production until the intake of feed can match or exceed nutritional requirements [9,10] thus, entering a state of negative energy balance (NEB).

In heat stressed dairy cows there is a reduction in DMI [11], which prolongs the period of negative energy balance. Negative energy balance leads to decreased plasma concentration of insulin, glucose and insulin-like growth factor-I (IGF-I), and increased plasma concentrations of growth hormone (GH) and non-esterified fatty acid (NEFA) [12,13]. All of these metabolic hormones can affect reproduction. Metabolic hormones acting on the hypothalamo-pituitary axis and the ovary probably mediate the inhibitory effects of negative energy balance on postpartum fertility.

### 3. Effects of heat stress on reproduction

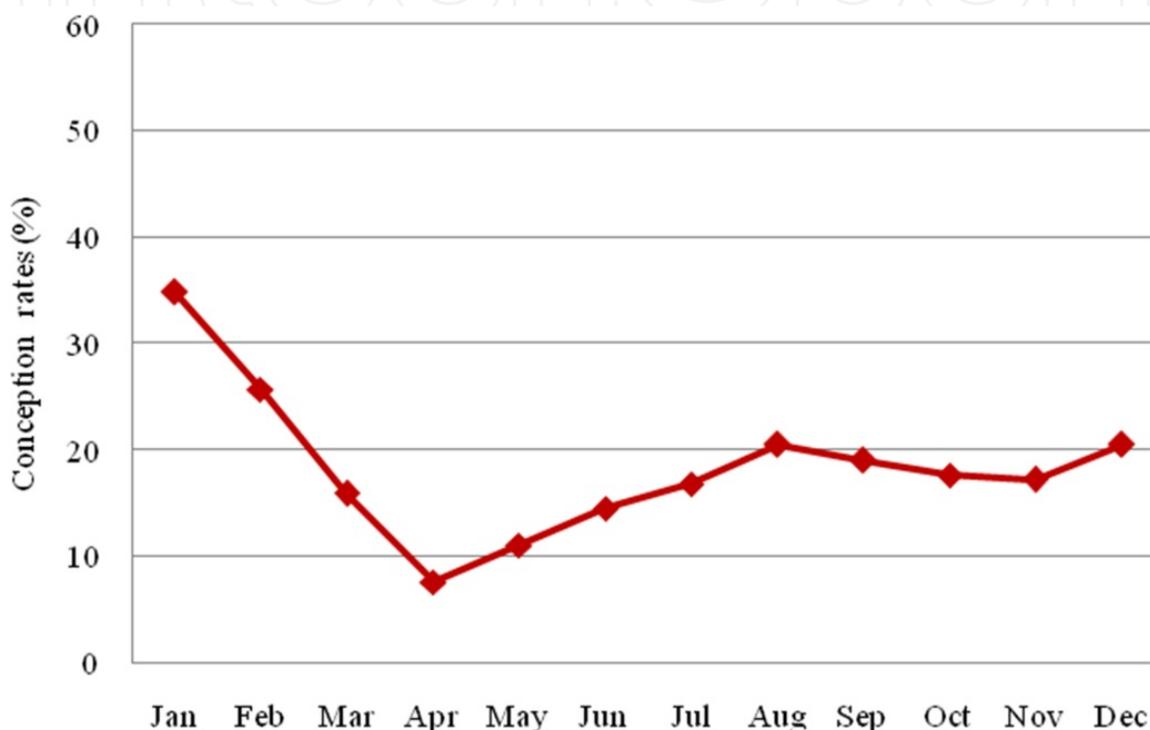
Heat stress affects reproductive performance both by direct action on reproduction and by indirect actions mediated through alterations in energy balance. There is an interaction between DMI, stage of lactation, milk production, energy balance and heat stress, that results in reduced luteinizing hormone (LH) secretion and a decreased diameter of the dominant follicle in the postpartum period [14], this results in reduced oestradiol secretion from the dominant follicle, leading to poor expression of oestrus. The postpartum anovulatory interval of dairy cow, is characterized by a variable period of negative energy balance that is reported to modulate the recrudescence of ovarian cyclicity [9,15,16]. In heat stressed cows, motor activity and other manifestations of oestrus are reduced [17] and the incidence of anoestrus and silent ovulation are increased [18].

There is a decrease in fertility in lactating dairy cows during summer in hot climate [19]. The magnitude of the depression depends on the geographical location and the milk yield [20-22]. In tropical climates, high ambient temperatures and humidity are important determinants of reproductive performance. Heat stress decreases the intensity and duration of oestrus, which in turn reduces both the number of inseminations and the pregnancy rates [23]. Heat stress alters the concentration of circulating hormones by increasing the circulating concentration of corticosteroids [24] and by reducing progesterone concentration [25]. The viability of pre-fixation embryos is reduced [26], and the uterine environment is altered by a decreased blood flow [27] and increased uterine temperature [28]. These changes are associated with increased early embryonic loss and a reduced proportion of successful inseminations. Cows exposed to heat stress have a high incidence of early embryonic mortality [26,29], and some of this effect is due to the direct effect of elevated temperature on the embryo [30].

Lactating dairy cows are susceptible to heat stress because of the elevated internal heat production which is associated with lactation. During periods of heat stress, milk production, feed intake, and physical activity are decreased [11]. At the same time, reproductive ability is compromised [31,32]. The exposure of lactating cows to heat stress has been shown to cause a decrease in follicular growth and to reduce serum estradiol [33], which also concluded that decreased follicular size or decreased dominant follicle function occurred in lactating cows that were exposed to heat stress [34,35]. Some of the reproductive losses, in heat stressed cattle, are associated with decreased expression of oestrus caused by anoestrus and silent ovulation

[36,37]. Heat stress delays follicle selection and lengthens the follicular wave having potentially adverse effects on oocytes quality [34] and follicular steroidogenesis [35].

Heat stress cause infertility and represented a major source of economic loss in dairy cows under tropical conditions. In recent study, the conception rate of dairy cows in Thailand decrease 20-30 % in hot season. The conception rate was lower in April and May when compared to other month, which lowest in summer and highest in winter (Figure 1). The effects of heat stress can be directly related to the increase in body temperature of dairy cow, which affects the reproductive function and embryonic development.



**Figure 1.** Conception rates of dairy cows in commercial farm located in the central part of Thailand.

The detrimental effects of heat stress on the reproductive performance of dairy cows have been well documented. These include a suppressed intensity of oestrus, a reduced preovulatory LH surge and decreased secretion of luteal progesterone [25], altered ovarian follicular development [33], decreased embryo development [38] and lower fertility [39]. In an attempt to minimize these effects, modifications to dairy cattle housing environments have been implemented to alleviate thermal stressors and improve cow comfort, through the use of shade, fans, sprinklers, and evaporative cooling [11,37,40]. These methods can enhance pregnancy rates significantly [24,41].

#### **4. Effect of environmental modification for dairy cows under tropical conditions**

Reducing heat stress in dairy cattle requires a multi-disciplinary approach. It involves breeding for improved heat tolerance, improved nutrition and improved reproductive

management for the animals, and improved the structural design and the environmental control of their housing.

Heat stress reduces milk production and reproductive efficiency. In an attempt to minimize these effects, modifications to dairy cattle housing environments have been implemented to alleviate thermal stressors and improve cow comfort [11,37,40,42]. The major objective of any cooling system is to keep the cow's body temperature as close to normal for as much of the day as possible. An acceptable range in rectal temperature is 38.5-39.3 °C. There are two general approaches to cooling dairy cows. One is to modify the environment to prevent heat stress or to utilize methods that increase heat dissipation from the skin surface of cattle. The easiest and most obvious way to help heat-stressed cows is to provide shade. Direct sunlight adds a tremendous heat load to the cow and can be blocked by shades, but shade alone is inadequate to reduce the effect of heat stress. A more economical method to reduce the effect of heat stress is by evaporative cooling. Evaporative cooling can be accomplished by two approaches; 1) direct evaporation from the skin surface of the cows (fan and sprinkler combinations) and 2) indirect evaporation which involve cooling the micro-environment of the cows, with cooling pads and fans, in an enclosed barn. When water evaporates it absorbs heat, thereby reducing the temperature and increasing heat dissipation from the skin of cattle. When water evaporates it also increases the relative humidity, due to the increased level of water vapor present. In hot and humid regions, evaporative cooling always requires the use of forced ventilation.

A number of studies have shown that housing systems in hot climates can be modified by the use of evaporative cooling to improve both milk production and reproductive efficiency of dairy cows [43-46]. There is a great potential to reduce temperature and THI. However, as relative humidity increases and or temperature decreases, the potential for evaporative cooling to modify the environment decreases. In hot and humid climates, high relative humidity reduces the potential of evaporative cooling. Therefore, there are questions regarding the effectiveness of evaporative systems in climates with high relative humidity. This chapter showed the impact of heat stress on postpartum cow performances and evaluated the effects of utilizing an evaporative cooling system for improved cow comfort and cow performance of early lactating dairy cows in a hot and humid climate.

## **5. Effect of evaporative cooling system on the environmental condition and physiological response of dairy cows**

The upper critical temperature for heat stress to begin was between 25 and 26°C [47]. Climatic conditions in the present study are such that the hot season is relatively long, and generally accompanied by high relative humidity. Thus heat stress is chronic in nature and there is little relief from the heat during the evening through to the morning, and also includes intense bursts of combined heat and humidity which further depresses performance.

Environmental modifications by evaporative cooling system equipped with tunnel ventilation in this study led to a decrease in the ambient temperature and an increase in the relative humidity, during the day. The air temperature in the tunnel barn was up to 6 °C

cooler ( $P<0.05$ ) during the daytime than that in the outside barn, while the relative humidity increased by up to 16%. As air was drawn through the wet cooling pads, water was evaporated into the air causing the temperature to be reduced while increasing the air moisture level. The amount of initial moisture in the outside air will directly impact on how much reduction in air temperature might be expected. Therefore, when the outside air is initially very high humid (relative humidity greater than 70%), the reduction in air temperature will be minimal (less than 2.8 to 5.6°C).

The average daily minimum and maximum temperatures were  $24.2\pm 0.1$  and  $28.4\pm 0.1$ °C, respectively in the evaporative, cooled tunnel, ventilated barn and  $24.7\pm 0.1$  and  $34.4\pm 0.1$ °C, respectively in the outside barn, indicating less variation and more consistency in this cooling system. This evaporative, cooled tunnel, ventilated barn reduced daily fluctuation in the ambient temperature, relative humidity and THI during hot and humid climatic conditions. Although, this system reduced ( $P<0.05$ ) afternoon barn temperature, but relative humidity increased ( $P<0.05$ ) when compared to the barn without a supplemental cooling system.

Although, high environmental relative humidity reduced the cooling capacity of the evaporative cooling, but on the days when animals were observed it reduced heat stress of the lactating dairy cows. This study shows that evaporative cooling with tunnel ventilation reduced the severity of afternoon heat stress in dairy cows. THI in the tunnel ventilated barn was decreased when compared to the outside barn. There was no thermoneutral zone ( $\text{THI}<72$ ) during the study but exposure to conditions of moderate heat stress ( $\text{THI}\geq 79$ ) that occurred outside was decreased by utilizing the evaporative cooling system. This difference in environmental conditions had a dramatic effect on the physiological response of the cows, as THI was highly correlated with both rectal temperature and respiration rate. The average rectal temperature and respiration rate in the cooled cows was lower ( $P<0.05$ ) than in the uncooled cows. Changing in cow body temperatures was most sensitive to same day climatic factors [48]. These results suggest that evaporative cooling and tunnel ventilation has the potential to decrease exposure to heat stress and alleviate the symptoms of heat stress.

The average daily THI in the morning and afternoon in the evaporative, cooled tunnel, ventilated barn were lower ( $P<0.05$ ) than that in the outside barn. However, the mean THI exceeded the critical point of 72 at daytime and nighttime, suggesting that the cows were exposed continuously to conditions conducive to mild heat stress for the cooled cows and moderate heat stress for the uncooled cows. In hot, arid conditions this system would work well and evaporative cooling has already been used very successfully to cool such dairy cows [46], but in high humidity locations its effectiveness would be limited by the evaporation potential. In this study the air temperature increases during the day and decreases in the evening until the next morning. As the air temperature rises during the day, the relative humidity will decrease. Accordingly during the hottest portion of the day, the outside relative humidity dropped to a level that allowed for maximum evaporation potential, making the system effective for reducing the severity of heat stress.

	Cooled (inside)	Uncooled (outside)	P-value
Number of animals (n)	17	17	
Environmental measurement :			
Daily minimum temperature (°C)	24.2±0.1	24.7±0.1	*
Daily maximum temperature (°C)	28.4±0.1	34.4±0.1	*
AM milking			
Daily mean temperature (°C)	25.3±0.1	25.6±0.1	*
Daily mean relative humidity (%)	88.5±0.3	91.6±0.2	*
Daily mean THI	76.0±0.2	76.7±0.2	*
Rectal temperature (°C)	38.4±0.0	39.2±0.1	*
Respiratory rate (breaths/min)	42.7±1.4	54.5±1.4	*
PM milking			
Daily mean temperature (°C)	26.5±0.1	31.4±0.1	*
Daily mean relative humidity (%)	86.5±0.3	74.6±0.8	*
Daily mean THI	78.0±0.2	83.1±0.2	*
Rectal temperature (°C)	38.7±0.0	39.7±0.1	*
Respiratory rate (breaths/min)	46.3±1.8	68.3±2.2	*

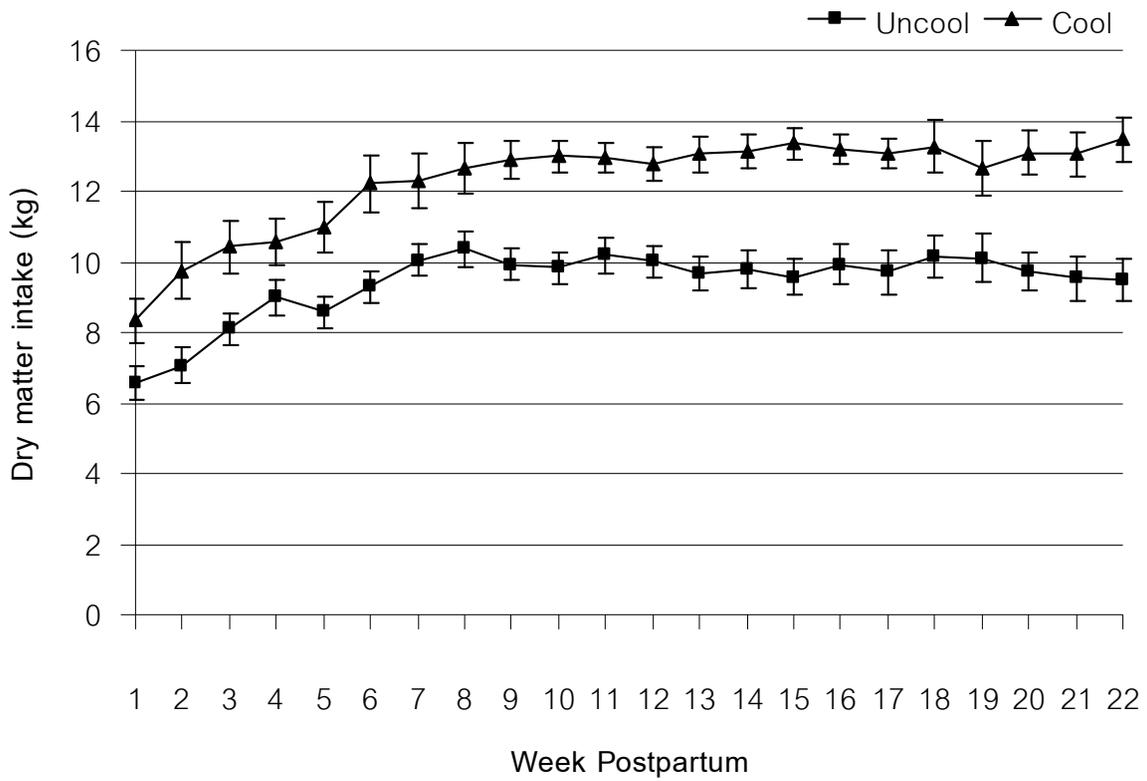
\* Means differ ( $P<0.05$ ) between groups

**Table 1.** The environmental measurement and physiological responses of dairy cows in the experimental housing. (Mean±S.E.M.)

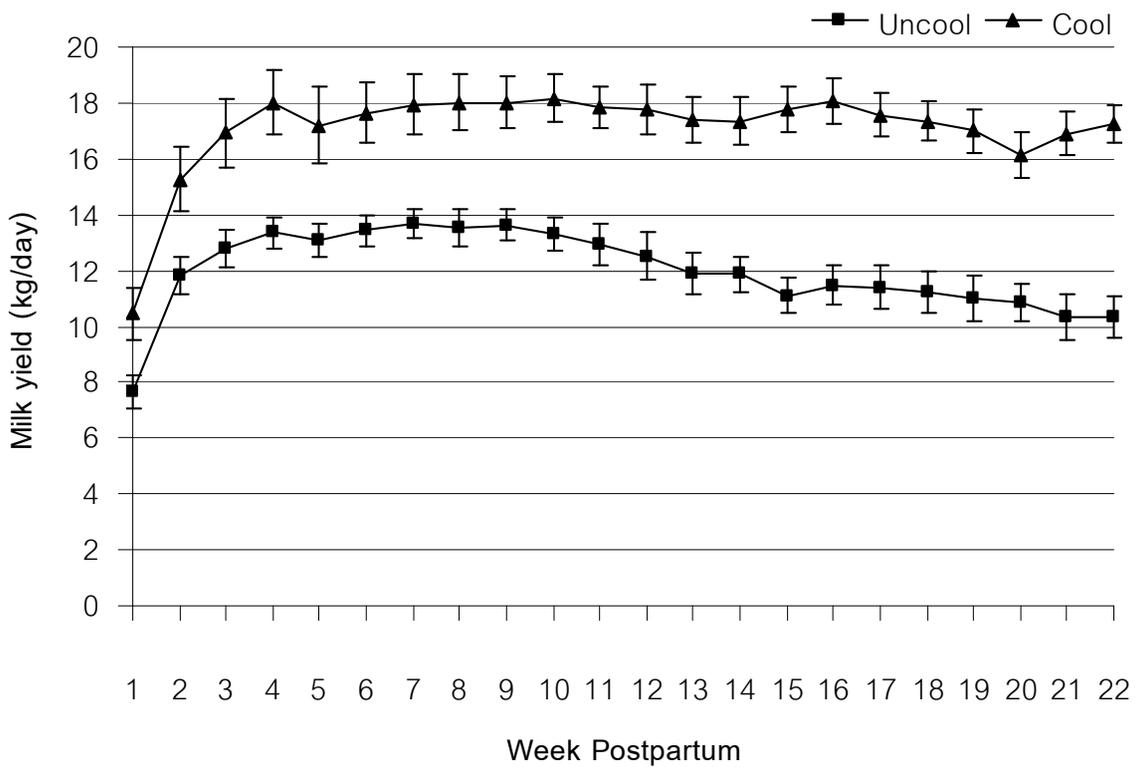
Air temperature had the greatest impact on physiological measurement, while radiation was second in importance, followed by vapor pressure and air movement [49]. Increasing the air temperature reduces the temperature differential between the cow's body temperature and the ambient temperature and decreases the transfer of heat to the environment. As ambient temperatures increase in the presence of low or high relative humidity, the cooling mechanisms employed by the cows shifted from a nonevaporative processes (convective, conductive, and radiation) to evaporative (sweating and panting) [48], this demonstrate that the percentage of cooling originating from the non evaporative processes declines as ambient temperature increases, while the evaporative process increase. As a result the uncooled cows had greater ( $P<0.05$ ) rectal temperatures and respiration rates than the cooled cows.

## 6. Effect of evaporative cooling system on dry matter intake and daily milk yield of dairy cows

Dry matter intake, expressed as kilograms per day (kg of DM/day) was greater ( $P<0.05$ ) in cooled ( $12.0 \pm 0.2$  kg/d) than uncooled cows ( $9.1 \pm 0.2$  kg/d). DMI increased ( $P<0.05$ ) from wk 1 to 22 of lactation in both groups of cows but treatment x week postpartum did not affect ( $P>0.05$ ) it (Figure 2) [50].



**Figure 2.** Weekly changes in average daily DMI for the cooled and uncooled cows during the first 22 week postpartum.



**Figure 3.** Weekly changes in average daily milk production for the cooled and uncooled cows during the first 22 week postpartum.

Daily milk yield was greater ( $P < 0.001$ ) in cooled ( $16.9 \pm 0.3$  kg/d) than uncooled ( $12.6 \pm 0.2$  kg/d) cows. Daily milk yield increased ( $P < 0.001$ ) from wk 1 to wk 22 of lactation in both groups of cows but treatment  $\times$  week postpartum did not affect ( $P > 0.50$ ) daily milk yield (Figure 3). The 4% FCM also differ ( $P < 0.001$ ) between cooled and uncooled cows. Cooled cows had more persistent milk production than uncooled cows. Milk composition did not differ ( $P > 0.50$ ) between the groups of cows over the 12 week study (Table 2) [50].

	Cooled (inside)	Uncooled (outside)	P-value
Dry matter intake (kg/d)	$12.0 \pm 0.2$	$9.1 \pm 0.2$	0.001
Milk yield (kg/d)	$16.9 \pm 0.3$	$12.6 \pm 0.2$	0.001
Fat (%)	$3.3 \pm 0.6$	$3.4 \pm 0.6$	0.810
Protein (mg/ml)	$3.2 \pm 0.3$	$3.1 \pm 0.3$	0.650
Lactose (mg/ml)	$5.0 \pm 0.2$	$5.0 \pm 0.2$	0.571
Solid not fat (mg/ml)	$8.9 \pm 0.4$	$8.8 \pm 0.4$	0.450

**Table 2.** Dry matter intake, milk production and milk composition of cooled and uncooled cows during the 12 week study.

In this study, rectal temperature was positively correlated with air temperature and THI but negatively correlated with DMI. Dry matter intake was positively correlated with milk production. Milk yield and DMI exhibited a significant decline when maximum THI reached 77 [51]. There is a significant negative correlation between THI and DMI [52,53], and the effect of THI is probably mediated through the effects of increasing body temperature on cow performance.

Mean air temperature had the greatest influence on milk yield for Holstein cows under hot conditions [48]. The mean daily ambient temperature was highly correlated with the p.m. rectal temperature and milk yield was highly correlated with the cows p.m. rectal temperature. The elevated p.m. rectal temperatures were associated with concomitant reductions in DMI and milk yield [54]. Uncooled cows, in this study, had rectal temperatures exceeded  $39^{\circ}\text{C}$  at both the a.m. and the p.m. milking, when cow temperatures should have been near their lowest and highest points, respectively. Such consistently elevated rectal temperatures, result in a significant decline in DMI and milk yield when compare with the cooled cows which had a lower rectal temperature.

The negative effects of heat stress on milk production could be explained by decreased nutrient intake and decreased nutrient uptake by the portal drained viscera of the cow [55]. Blood flow which moves to peripheral tissues for cooling purposes may alter nutrient metabolism and contribute to lower milk yields during hot weather. Providing cows with supplemental shade and cooling to mitigate heat stress has been evaluated in economic terms using the increase in milk production data [2]. Adding sprinklers and fan cooling increased the feed intake (7.1 to 9.2%) and milk production (7.1 to 15.8%), and decreased rectal temperature ( $-0.4$  to  $-0.5^{\circ}\text{C}$ ) and respiration rate (17.6 to 40.6 %) [56].

In this study the cooling system improved cow comfort and milk production. The cooling system decreased ambient temperatures and THI. The cows with this cooling system had decreased rectal temperatures and respiration rates, and increased feed intake (30.9 %) and milk production (42.5%). In addition, an analysis of the economics in the this study showed that the rate of return on investment in cooling equipment and additional feed plus electric costs of this cooling system, showed it was profitable in hot and humid conditions.

## 7. Economic analysis of the utilizing evaporative cooling system

There are questions that arise regarding the cost effectiveness of an evaporative cooling system over a period of years. In this study, an economic analysis of the evaporative cooling system showed that cows in the tunnel ventilation barn produced 5.1 kg/cow/day more than the cows housed outside. The milk price was 11.50 baht/kg. Therefore, the use of tunnel ventilation cooling increased revenue by 58.65 baht/cow/day and increased feed costs by 22.17 baht/cow/day. Thus, income over feed cost was 36.48 baht/cow/day.

The cost of the fan and water pump operation was 6.88 baht/cow/day. The total equipment and supply cost to build the tunnel barn facility was 125,000 baht (for 18 cows), which, when depreciated over the expected life of the equipment (including maintenance costs of 25,000 baht over 5 years), was 4.11 baht/cow/day. Thus, the cows housed in the tunnel barn earned 25.49 baht/day or 3823.50 baht more than the cows housed outside over the 22-week study (Table 3).

Item	Difference	Income-Cost (Baht/cow/day)
Milk yield (kg/cow/day)	5.1	58.65
DMI (kg/cow/day)	2.9	-22.17
Electric cost (fan & water pump operation)		-6.88
Equipment and maintenance cost (5 years)		-4.11
Total		25.49

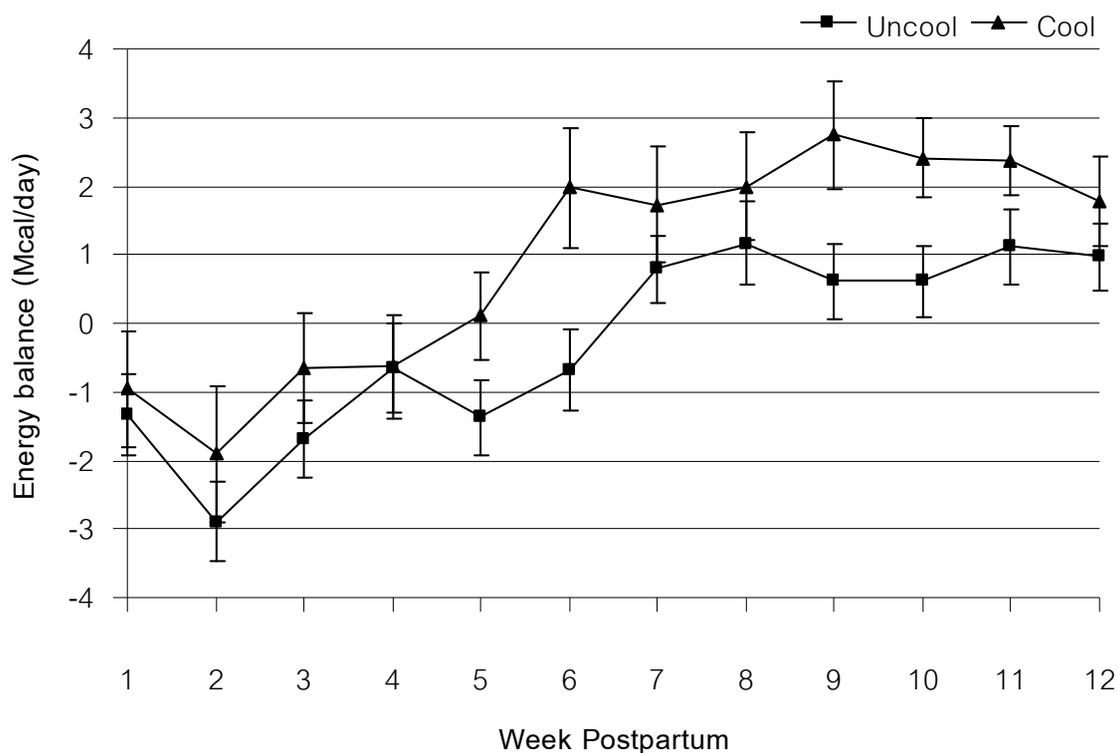
**Table 3.** The economic analysis of the evaporative cooling system over the first 22 week of lactation in dairy cows.

## 8. Effect of evaporative cooling system on energy balance and body weight of dairy cows

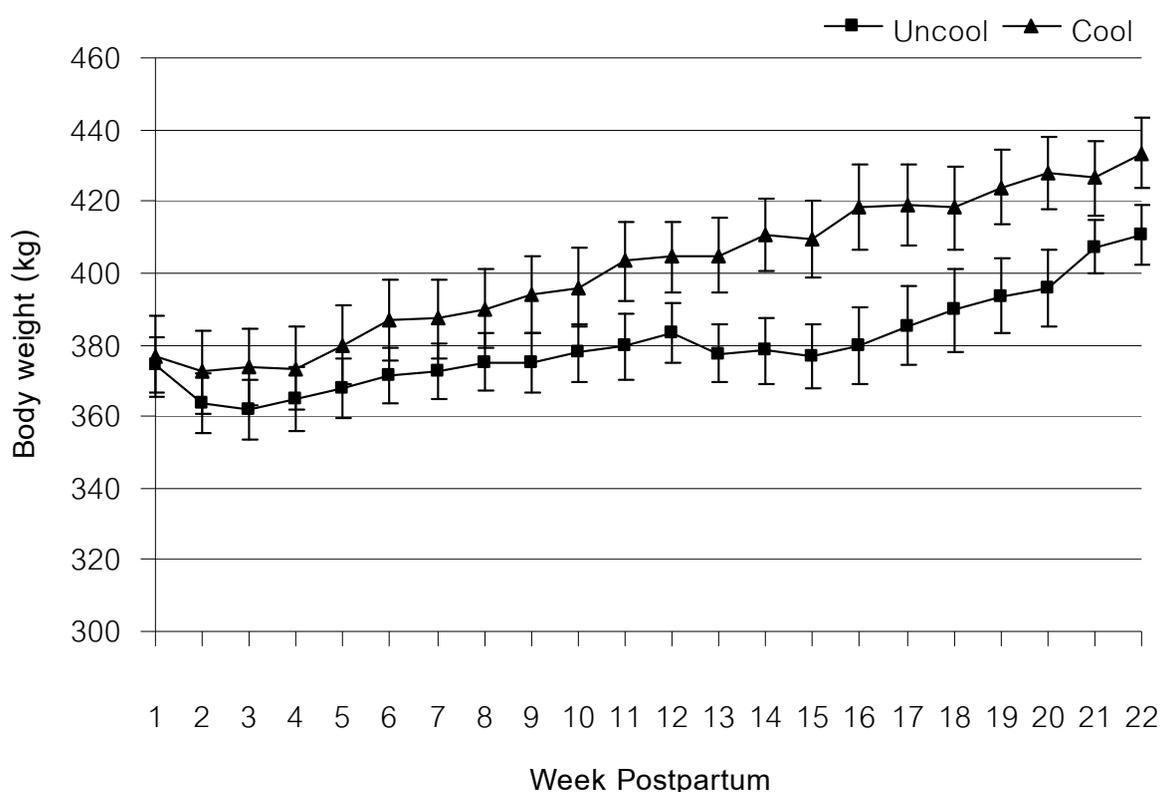
Responses of dairy cow to heat stress include panting and sweating. If these are not successful in alleviating the heat load, body temperature will rise. Increased body temperature will result in reduced feed intake, higher maintenance requirements (panting) and less efficient productive ability. The higher maintenance requirements dictates that cows need to increase feed intake to maintain milk production. However, this not possible as feed intake declines when ambient temperatures exceeded 26°C. For uncooled cows in this study, as a result of this increase in requirement and a decrease in intake, milk

production may decline as much as 25-30%, and typically they mobilize body reserves and lose body weight to maintain milk production until the intake of feed can match or exceed nutritional requirements [9,10], thus entering a state of negative energy balance.

The energy balance and body weight of dairy cows is presented in Figure 4 and Figure 5. During 12 wk of lactation, average body weight of postpartum cows was greater in cooled than uncooled cows. In both groups of cows, body weight decreased between wk 1 and 4, and increased between wk 5 and 22. Week postpartum ( $P<0.001$ ) and treatment ( $P<0.001$ ) affected EB but treatment  $\times$  week postpartum was not significant. Cows in both groups entered into NEB immediately after calving. Averaged EB was greater ( $P<0.001$ ) in cooled ( $0.916 \pm 0.194$  Mcal/day) than uncooled cows ( $-0.268 \pm 0.195$  Mcal/day). During the 12 week study, the week of EB nadir was at wk 2 in both groups and the degree of EB nadir did not differ significantly ( $P>0.50$ ) between the groups, although the average was lower in uncooled than cooled cows. After reaching the EB nadir, uncooled cows required more days to reach a positive energy balance than the cooled cows. The first week that EB was greater than zero was at wk 5 in cooled cows and at wk 7 in uncooled cows. Because of dry matter intake in uncooled cows was lower ( $P<0.05$ ) than in cooled cows. Resulting in uncooled cows having a prolonged period of negative energy balance and postpartum body weight in these cows were lesser ( $P<0.05$ ) than in cooled cows [50]. The negative energy balance is directly related to the postpartum interval to first ovulation, and follicle size was adversely affected by negative energy balance in early postpartum dairy cows [9]. The average EB during the first 4 week of lactation was negatively correlated to the postpartum interval to first ovulation [57].



**Figure 4.** Weekly changes in energy balance for the cooled and uncooled cows during the first 12 week postpartum.



**Figure 5.** Weekly changes in average body weight for the cooled and uncooled cows during the first 22 week postpartum.

## 9. Effect of evaporative cooling system on synchronization of follicular development and ovulation

The exposure of lactating cows to heat stress has been shown to cause a decrease in follicular growth and to reduce concentrations of serum estradiol [33]. In this study the cooled cows had a greater ( $P < 0.05$ ) average diameter of the largest ovulatory follicle than the uncooled cows. Heat stress inhibited follicular growth and dominance during the preovulatory period. Abnormal ovarian function in heat stressed cows was manifested as a decrease in the proestrus rise in estradiol, and the smaller size of the second wave dominant follicle [33]. Circulating estradiol concentration during the preovulatory period is necessary to produce an LH surge and ovulation. In addition, a reduced estradiol peak may also alter aspects of the LH surge that could account for some types of anovulation in lactating cows [58]. A reduction of the endogenous LH surge by heat stress was reported in heifers [59]. It has been suggested that these differences are related to preovulatory estradiol levels because the amplitude of tonic LH pulses and GnRH-induced preovulatory plasma LH surges are decreased in cows with low plasma concentrations of estradiol but not in cows with high plasma concentrations of estradiol [60]. Therefore, the synchronization rate in the uncooled cows tended to lower than in the cooled cows. In addition, the ovulation rate in response to a second injection GnRH of Ovsynch was reported to be between 87% [61] and 91% [62] in cycling cows. Therefore a second GnRH injection after the  $\text{PGF}_{2\alpha}$  treatment might be used to improve the ovulation rate in dairy cows under heat stress.

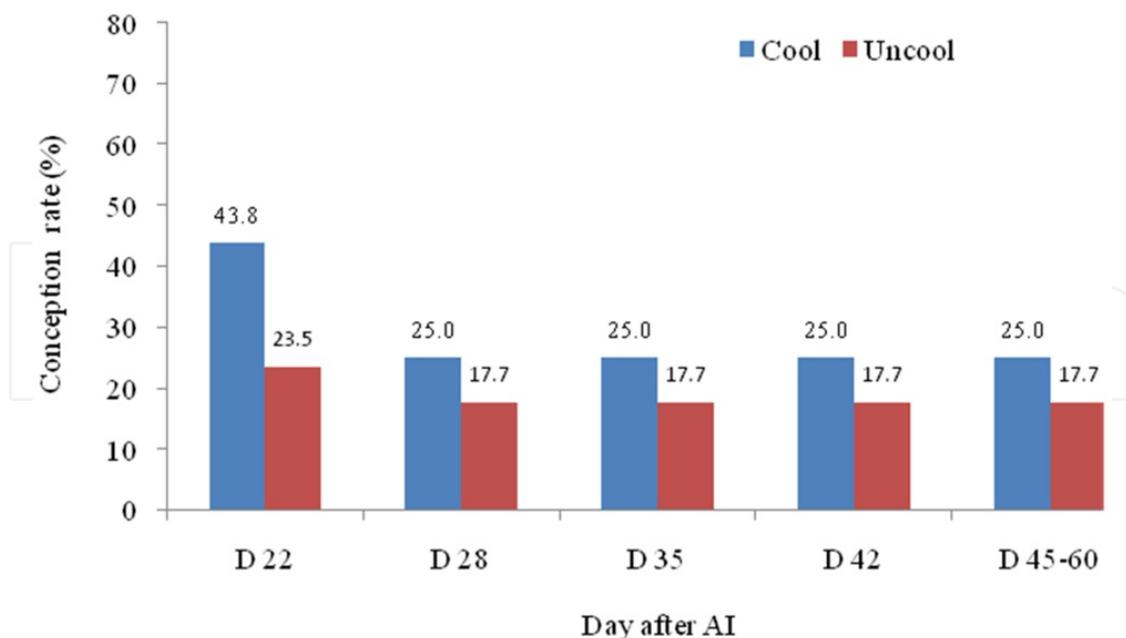
	Cooled	Uncooled	P-value
Number of animal (n)	17	17	
Synchronization rate (%)	82.4(14/17)	52.9(9/17)	0.08
Interval to new follicular wave emergence (day)	2.2±0.1	2.3±0.2	0.55
Size of the largest ovulatory follicle at PGF <sub>2α</sub> (mm)	11.5±0.6	10.2±0.5	0.12
Maximal size of the largest ovulatory follicle (mm)	14.6±0.5	14.2±0.4	0.57
Growth rate of the largest ovulatory follicle after PGF <sub>2α</sub> (mm/d)	0.9±0.1	1.1±0.1	0.17
Interval from PGF <sub>2α</sub> treatment to ovulation (h)	83.6±5.1	88.0±6.9	0.63

**Table 4.** The effect of evaporative cooling and tunnel ventilation system on follicular development, time of ovulation and the response rates of synchronized cows to GnRH and PGF<sub>2α</sub>. Results are expressed as mean±SEM.

## 10. Effect of evaporative cooling system on embryonic development and conception rate of dairy cows

Heat stress can compromise reproductive performance by decreasing the expression of oestrus behavior, altering follicular development, affecting oocyte competence, inhibiting embryonic development due to a reduced synchronization of ovulation response, lowering fertilization rates, and reducing embryo quality. In this study the modification of the barn environment and fixed TAI in lactating dairy cows in a hot and humid climate resulted in higher initial conception rates compared to cows housed in a barn without a supplemental cooling system (43.8 and 23.5%, respectively) (Figure 6). The results indicate that this method has the potential to attenuate some of the detrimental effects of heat stress on embryo survival during this period. Although, initial conception rates in cooled cows was higher than uncooled cows, it was still compromised by heat stress as the conception rate decreased further in both groups, indicating that embryo mortality may still have occurred after that. This was probably due to environmental heat stress [63], which causes maternal body temperature to rise leading to the impairment of embryo survival, or this cooling was not sufficiently cool to protect embryos from direct effect of high temperatures, or early embryos during this period might be sensitive to elevated temperature.

Climatic factors that may influence the degree of heat stress include: temperature, humidity, radiation and wind [32]. The upper critical temperature for heat stress begins between 25 and 26°C [47]. When environment temperatures exceed 30°C the day after insemination, pregnancy rates were adversely affected in lactating dairy cows than in heifers [64]. Maternal hyperthermia is detrimental to embryonic development and survival [65-67]. The oocyte and early cleavage stage embryo are the most sensitive to heat stress, while embryos that are 3 d or older are more tolerant [26,29]. Conception rates decline from 61 to 45% when rectal temperature 12 h, post breeding, increased by 1°C [68]. Furthermore, cattle with a rectal temperature of 40°C, as a result of exposure to 32.2 °C ambient temperatures for 72 h after inseminating, had conception rates of 0%, compared with a conception rate of 48% when rectal temperature was 38.5°C, for cows in an ambient temperature of 21.1 °C [68]. Given that exposure to conditions of high environmental temperature and humidity has been shown to elevate rectal and uterine temperatures [28].



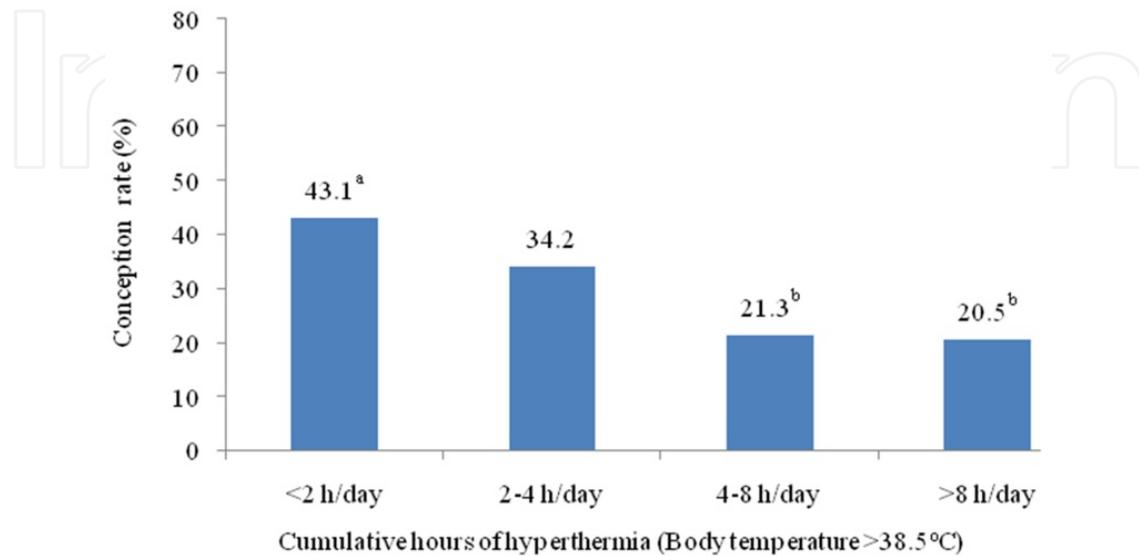
**Figure 6.** The conception rate after the synchronization of ovulation and fixed time AI of cooled and uncooled cows.

The modified environment had been used to reduce the effect of heat stress, however, this approach has not eliminated all problems. Timed AI might be particularly effective during heat stress periods because of the decreased incidence of missed oestrus, but heat stress has also a direct effect on the development of the embryo. Conception rates have ranged between 31 to 42 % in cows following the Ovsynch protocol [69-73]. However, conception rates in heat-stressed cows following the Ovsynch protocol were lower than in non stressed cows.

Timed AI programs based on follicular recruitment by synchronized ovulation have been developed [74]. The submission rates and pregnancy rates between d 27 and 30 were enhanced when a TAI program was used. However, the advantage was lost between d 40 and 50 due to increased embryonic mortality in the cows bred using TAI [75]. These results may indicate that cows were successfully induced to ovulate and subsequently conceive but had a reduced ability to maintain pregnancy. Previous studies also showed high (9.3 to 16.8 %) pregnancy losses between 28 and 56 d after AI [76,77].

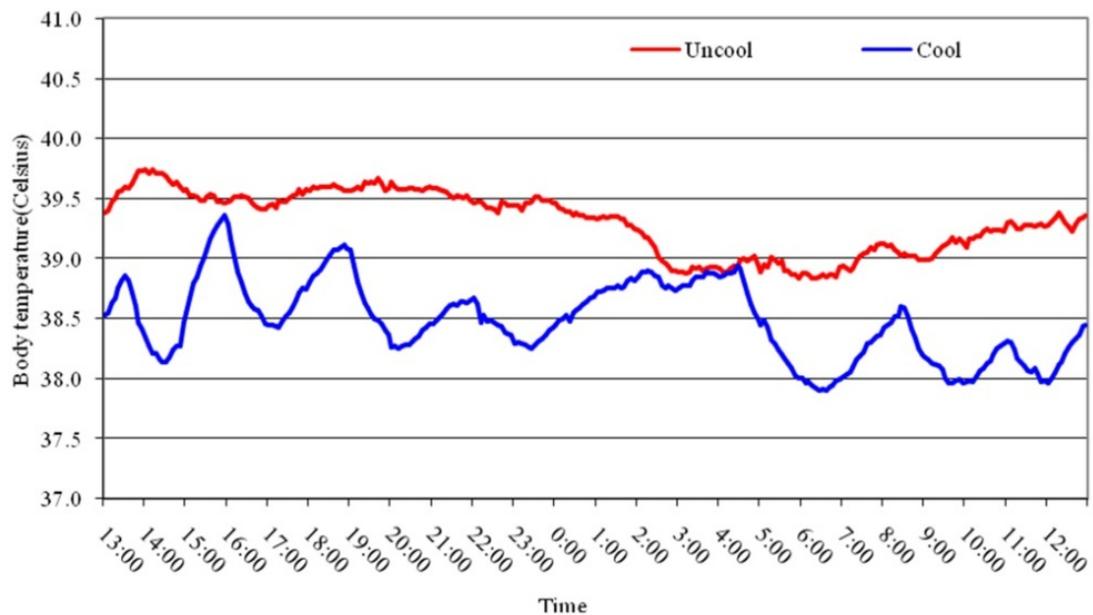
In recent study, reported that dairy cow had a cumulative hours of body temperature greater than 38.5°C more than 4-8 h per day, significantly ( $P < 0.05$ ) decline (Figure 7) in conception rate [78]. This study indicated that long period of hyperthermia (body temperature  $> 38.5^{\circ}\text{C}$ ) had an adverse effect on dairy cows and suggested that dairy cows in the tropical area need additional cooling system to completely eliminate heat stress result in close to normal fertility of dairy cows depend on the severity of the local environment conditions. In addition, *in vitro* heat stress during the critical stage of early embryo development significantly increases the incidence of early embryonic mortality [79]. These results indicate that increased maternal body temperatures adversely affect embryo quality and the conception rate. An increase in maternal body temperature may result in an increase

in the ambient temperature of oocytes, zygotes and embryos in the oviduct or the uterus. At temperature increase of 0.5°C above the basal body temperature has been associated with a decreased pregnancy rate [28]. These studies indicated that elevated environmental temperature lead to hyperthermia in lactating cows. Intensive cooling cows have the potential to eliminate the decline in conception rate of dairy cows under tropical conditions.



<sup>a, b</sup> Means with different superscripts differ ( $P < 0.05$ )

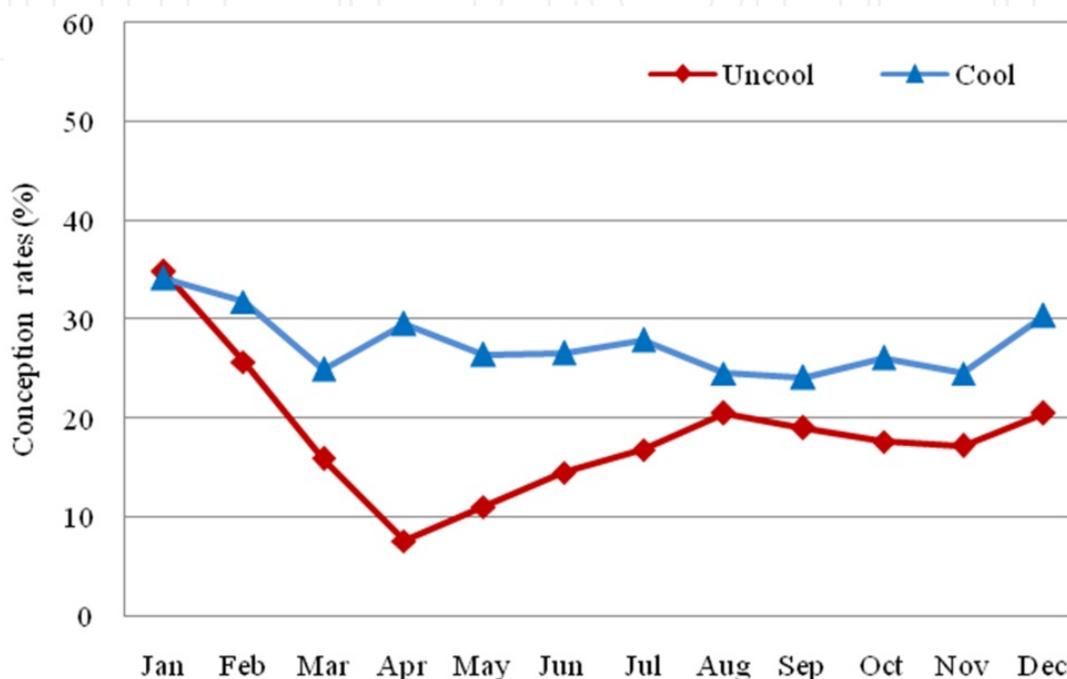
**Figure 7.** This chart show conception rates of dairy cows. It also illustrated that cow had cumulative hours of hyperthermia greater than 4-8 h per day that conception rate decline significantly ( $P < 0.05$ ).



**Figure 8.** This chart show changes in mean vaginal temperature of cooled and uncooled cows throughout the day.

Infertility of dairy cows under tropical conditions is primarily caused by elevated body temperature. Therefore, cooling cows should improve conception rates. A variety of cooling

systems are available for heat-stressed cows. The intensive cooling cows with the combination of sprinklers and fans were used for improved reproductive performance. Cows were cooled in the holding area for a total 6-8 cooling periods and 4-6 cumulative h/day. Each cooling period combined cycles of sprinkling (0.5 min) and forced ventilation (4.5 min). Intensive cooling had significantly affected on decreased body temperature and allowed cows to maintain normal body temperature throughout the day [78]. In the same study, uncooled cows had high body temperature, daytime significant portion of the day and returned to normal body temperature during the late night and early morning (Figure 8).



**Figure 9.** This chart show conception rates obtained in uncooled cows during 2004-2007 and cooled cows during 2008-2011 on commercial dairy farm in the central part of Thailand.

For large scale survey was carried out during a 8 yr period (2004-2011) in commercial dairy farm. The conception rate of intensively cooled cows with sprinkler and forced ventilation was significantly higher than that of uncooled cows. Conception rates obtained in intensively cooled cows in this study were similar to those obtained in that same winter in uncooled cows (Figure 9). This current finding confirmed this adverse effect of heat stress. In hot climates there is a large decrease in the fertility of dairy cows during summer months. In addition, intensively cooling cows has the potential to eliminate the decline in conception rate of dairy cows under tropical conditions. Therefore, intensive cooling is essential in dairy cows under tropical conditions to prevent hyperthermia and its harmful effects on those cows.

## 11. Summary

The exposure of dairy cows to elevate temperatures have a variety of effects, including decreased fertility, depressed appetite, and decreased milk production, all of which contribute to the goal of decreasing the production of metabolic heat in order to maintain thermo-neutrality. This chapter showed significant advantages for the evaporative cooled

barn can be used to reduce heat stress of dairy cows housed in hot and humid climates. The combined effect of higher milk production and increasing lactation persistency, with minimal costs could improve the financial status of dairy operations. The benefit demonstrated increased income over costs.

In addition, respiration rates and rectal temperatures, which affect both milk production and reproduction, were reduced by this environmental modification. These findings suggest that the evaporative cooling and tunnel ventilation system has the potential to decrease the exposure to heat stress, alleviate the symptoms of heat stress and improve milk production and metabolic efficiency during early lactation. This modification of the barn environment can reduce some of the detrimental effects of heat stress on follicular development and can improve the response rate to the synchronization of ovulation in dairy cows in hot and humid climates.

The implementation of evaporative cooling systems for dairy cows in hot and humid climates increased the percentage of cows that initially established a pregnancy and increased successful pregnancy while decreasing early embryonic mortality, if these cows were sufficiently cooled after breeding. It appears that modification of environment need to be developed further to improve reproductive performance of dairy cows in hot and humid climates. The finding that the cooling of cows did not alleviate all the effects of heat stress on pregnancy rates suggests that the degree of cooling was not sufficient to prevent the adverse effects of heat stress. It is also possible that the cooling of dairy cows needs to be done not only in the housing unit but also in the holding and milking areas to improve pregnancy rates. Dairy cows in hot and humid climatic condition need to be intensively cooled to completely eliminate heat stress to improve production and fertility close to normal. Therefore, additional research is needed to determine the effects of environmental modification or improved cooling system on postpartum reproductive performance and production when compared to conventional methods of cow cooling in the tropical area. However, cooling intensification should be combined with reproductive management, hormonal application and nutritional management to minimize the decline in cow performances under hot and humid climatic conditions.

## Author details

Siriwat Suadsong

*Department of Obstetrics Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand*

## Acknowledgement

This study was supported by the Thailand Research Fund and Faculty of Veterinary Science, Chulalongkorn University.

## 12. References

- [1] Stott GH. What is animal stress and how is it measured. *Journal of Animal Science* 1981;52:150-153.

- [2] Armstrong D. Heat stress interactions with shade and cooling. *Journal of Dairy Science* 1994;77:2044-2050.
- [3] Moody EG, Van Soest PJ, McDowell RE, Ford GL. Effect of high temperature and dietary fat on milk fatty acids. *Journal of Dairy Science* 1971;54:1457-1460.
- [4] Richardson CW, Johnson HD, Gehrke CW, Goerlitz DF. 1961. Effects of environmental temperature and humidity on the fatty acid composition of milk fat. *Journal of Dairy Science* 1961;44:1937-1940.
- [5] Bauman DE, Currie WB. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. *Journal of Dairy Science* 1980;63:1514-1529.
- [6] Collier RJ, Beede DK, Thatcher WW, Israel LA, Wilcox CJ. 1982. Influences of environment and its modification on dairy animal health and production. *Journal of Dairy Science* 1982;65:2213-2227.
- [7] Smith WA, Harris B, Van Horn JrHH, Wilcox CJ. Effect of forage type on production of dairy cows supplemented with whole cottonseed, tallow, and yeast. *Journal of Dairy Science* 1993;76:205.
- [8] Mallonee PG, Beede DK, Collier RJ, Wilcox CJ. Production and physiological responses of dairy cows to varying dietary potassium during heat stress. *Journal of Dairy Science* 1985;68:1479-1487.
- [9] Butler, W.R. and Smith, R.D. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *Journal of Dairy Science* 1989;72: 767-783.
- [10] Nebel RL, McGilliard ML. Interactions of high milk yield and reproductive performance in dairy cows. *Journal of Dairy Science* 1993;76:3257-3268.
- [11] Fuquay JW. Heat stress as it affects animal production. *Journal of Animal Science* 1981;52:164-174.
- [12] Lucy MC, Savio JD, Badinga L, de la Sota RL, Thatcher WW. Factors that affect ovarian follicular dynamics in cattle. *Journal of Animal Science* 1992;70:3615-3626.
- [13] Jolly PD, McDougall S, Fitzpatrick LA, Macmillan KL, Enwhistle K. Physiological effects of under nutrition on postpartum anoestrous in cows. *Journal of Reproduction and Fertility* 1995(Suppl.);49:477-492.
- [14] Jonsson NN, McGowan MR, McGuigan K, Davison TM, Hussain AM, Kafi M. Relationship among calving season, heat load, energy balance and postpartum ovulation of dairy cows in a subtropical environment. *Animal Reproduction Science* 1997;47:315-326.
- [15] Butler WR, Everett RW, Coppock CE. The relationships between energy balance, milk production and ovulation in postpartum Holstein cows. *Journal of Animal Science*. 1981;53:742-748.
- [16] Staples CR, Thatcher WW, Clark JH. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. *Journal of Dairy Science* 1990;73:938-947.
- [17] Nebel R.L, Jobst SM, Dransfield MBG, Pandolfi SM, Balley TL. Use of radio frequency data communication system, HeatWatch, to describe behavioural estrus in dairy cattle. *Journal of Dairy Science* 1997;179(Abst.)

- [18] Gwazdauskas FC, Thatcher WW, Kiddy CA, Pape MJ, Wilcox CJ. Hormonal pattern during heat stress following PGF<sub>2</sub>α-tham salt induced luteal regression in heifers. *Theriogenology* 1981;16:271-285.
- [19] Hansen PJ. Effects of environment on bovine reproduction. In: *Current therapy in large animal theriogenology*. Philadelphia: WB Saunders; 1997. p403-415.
- [20] Badinga L, Collier RJ, Wilcox CJ, Thatcher WW. Interrelationships of milk yield, body weight and reproductive performance. *Journal of Dairy Science* 1985;68:1828-1831.
- [21] Badinga L, Collier RJ, Thatcher WW, Wilcox CJ. Effects of climatic and management factors on conception rate of dairy cattle in subtropical environment. *Journal of Dairy Science* 1985;68:78-85.
- [22] Al-Katanani YM, Webb DW, Hansen PJ. Factors affecting seasonal variation in nonreturn rate to first service in lactating Holstein cows in a hot climate. *Journal of Dairy Science* 1999;82:2611-2616.
- [23] Thompson JA, Magee DD, Tomaszewski MA, Wilks DL, Fourdraine RH. 1996. Management of summer infertility in Texas Holstein dairy cattle. *Theriogenology* 1996;46:547-558.
- [24] Roman-Ponce H, Thatcher WW, Buffington DE, Wilcox CJ, Van Horn HH. Physiological and production responses of dairy cattle to a shade structure in a subtropical environment. *Journal of Dairy Science* 1977;66:424-430.
- [25] Howell JL, Fuquay JW, Smith AE. Corpus luteum growth and function in lactating Holstein cows during spring and summer. *Journal of Dairy Science* 1994;77:735-739.
- [26] Ealy D, Drost M, Hansen PJ. Developmental changes in embryonic resistance to adverse effects of maternal heat stress in cows. *Journal of Dairy Science* 1993;76:2899-2905.
- [27] Roman-Ponce H, Thatcher WW, Caton D, Barron DH, Wilcox CJ. Thermal stress effects on uterine blood flow in dairy cows. *Journal of Animal Science* 1978;46:175-180.
- [28] Gwazdauskas, F.C., Thatcher, W.W. and Wilcox, C.J. 1973. Physiological, environmental and hormonal factors at insemination which may affect conception. *Journal of Dairy Science* 1973;56:873-877.
- [29] Putney DJ, Drost M, Thatcher WW. Embryonic development in superovulated dairy cows exposed to elevated ambient temperatures between Days 1 to 7 post insemination. *Theriogenology* 1988;30:195-209.
- [30] Rivera RM, Hansen PJ. Development of cultured bovine embryos after exposure to high temperatures in the physiological range. *Reproduction* 2001;121:107-115.
- [31] Francos G, Macer E. Observations on some environmental factors connected with fertility in heat-stressed cows. *Theriogenology* 1983;19:625-634.
- [32] Gwazdauskas, F.C. Effects of climatic on reproduction in cattle. *Journal of Dairy Science* 1985;68:1568-1578.
- [33] Wilson SJ, Marion RS, Spain JN, Spiers DE, Keisler DH, Lucy MC. Effects of a controlled heat stress on ovarian function of dairy cattle. 1. Lactating cows. *Journal of Dairy Science* 1998;81:2124-2131.
- [34] Badinga L, Thatcher WW, Diaz T, Drost M, Wolfenson D. Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. *Theriogenology* 1993;39:797-810.

- [35] Wolfenson D, Thatcher WW, Badinga L, Savio JD, Meidan R, Lew BJ, Braw-Tal R, Berman A. Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. *Biology of Reproduction* 1995;52:1106-1113.
- [36] Her E, Wolfenson D, Flamenbaum I, Folman Y, Kaim M, Berman A. Thermal, productive, and reproductive responses of high yielding cows exposed to short-term cooling in summer. *Journal of Dairy Science* 1988;71:1085-1092.
- [37] Younas M, Fuquay JW, Smith AE, Moore AB. Estrous and endocrine responses of lactating Holsteins to forced ventilation during summer. *Journal of Dairy Science* 1993;76:430-436.
- [38] Hansen PJ, Arechiga CF. 1999. Strategies for managing reproduction in the heat-stressed dairy cow. *Journal of Animal Science* 1999;77:36-50.
- [39] Gwazdauskas FC, Wilcox CJ, Thatcher WW. Environmental and management factors affecting conception rate in a subtropical environment. *Journal of Dairy Science* 1975;58:88-92.
- [40] Roman-Ponce H, Thatcher WW, Wilcox CJ. Hormonal relationships and physiological responses of lactating dairy cows to a shade management system in a subtropical environment. *Theriogenology* 1981;16:139-154.
- [41] Thatcher WW, Gwazdauskas FC, Wilcox CJ, Toms J, Head HH, Buffington DE, Frederickson WB. 1974. Milking performance and reproductive efficiency of dairy cows in an environmentally controlled structure. *Journal of Dairy Science* 1974;57:304-307.
- [42] Flamenbaum I, Galon N. Management of heat stress to improve fertility in dairy cows in Israel. *Journal of Reproduction and Development* 2010;56:S36-S41.
- [43] Armstrong D, Wise M, Torabi M, Weirisma F, Hunter R, Kopel K. Effect of different cooling systems on milk production of late lactation Holstein cows during high ambient temperatures. *Journal of Dairy Science* 1988;71(Suppl. 1):212. (Abstr.)
- [44] Armstrong D, DeNise S, Delfino F, Hayes E, Grundy P, Montgomery S, Correa M. Comparing three different dairy cattle cooling systems during high environmental temperatures. *Journal of Dairy Science* 1993;76(Suppl. 1):24.(Abstr.)
- [45] Flamenbaum I, Wolfenson D, Mamen A, Berman A. Cooling cattle by a combination of sprinkling and forced ventilation and its implementation in the shelter system. *Journal of Dairy Science* 1986;69:3140-3147.
- [46] Ryan DP, Scland M, Kopel E, Armstrong D, Munyakazi L, Gorlke G, Ingergam R. Evaluating two different evaporative cooling management systems for dairy cows in hot dry climate. *Journal of Dairy Science* 1992;76(Suppl. 1):240. (Abstr.)
- [47] Berman A, Folman Y, Kaim M, Marnen M, Herz Z, Wolfenson D, Arieli A, Graber Y. Upper critical temperature and forced ventilation effects for high-yielding dairy cows in a subtropical climate. *Journal of Dairy Science* 1985;68:1488-1495.
- [48] West JW, Mullinix BG, Bernard JK. Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. *Journal of Dairy Science* 2003;86:232-242.
- [49] Legates JE, Farthing BR, Casady RB, Barrada MS. Body temperature and respiratory rate of lactating dairy cattle under field and chamber conditions. *Journal of Dairy Science* 1991;74:2491-2500.

- [50] Suadsong S, Suwimonteerabutr J, Virakul P, Chanpongsang S, Kunavongkrit A. Effect of improved cooling system on reproduction and lactation in dairy cows under tropical conditions. *Asian-Australasian Journal of Animal Sciences* 2008;21:555-560.
- [51] West JW. Effects of heat-stress on production in dairy cattle. *Journal of Dairy Science* 2003;86:2131-2144.
- [52] Holter JB, West JW, McGilliard ML, Pell AN. Predicting ad libitum dry matter intake and yields of Jersey cows. *Journal of Dairy Science* 1996;79:912-921.
- [53] Holter JB, West JW, McGilliard ML. Predicting ad libitum dry matter intake and yield of Holstein cows. *Journal of Dairy Science* 1997;80:2188-2199.
- [54] Maust LE, McDowell RE, Hooven NW. Effect of summer weather on performance of Holstein cows in three stages of lactation. *Journal of Dairy Science* 1972;55:1133-1139.
- [55] McGuire MA, Beede DK, DeLorenzo MA, Wilcox CJ, Huntington GB, Reynolds CK, Collier RJ. Effects of thermal stress and level of feed intake on portal plasma flow and net fluxes of metabolites in lactating Holstein cows. *Journal of Animal Science* 1989;67:1050-1060.
- [56] Bucklin RA, Turner LW, Beede DK, Bray DR, Hemken RW. Methods to relieve heat stress for dairy cows in hot, humid climates. *Applied Engineering Agriculture* 1991;7:241-247.
- [57] Spicer LJ, Vernon RK, Tucker WB, Wettemann RP, Hogue JF, Adams GD. Effects of inert fat on energy balance, plasma concentrations of hormones and reproduction in dairy cows. *Journal of Dairy Science* 1993;76:2664-2673.
- [58] Wiltbank MC, Gumen A, Sartori R. Physiological classification of anovulatory conditions in cattle. *Theriogenology* 2002;57:21-52.
- [59] Madan ML, Johnson HD. Environmental heat effects on bovine luteinizing hormone. *Journal of Dairy Science* 1973;56:1420-1423.
- [60] Gilad E, Meidan R, Berman A, Graber Y, Wolfenson D. Effect of heat stress on tonic and GnRH-induced gonadotrophin secretion in relation to concentration of estradiol in plasma of cyclic cows. *Journal of Reproduction and Fertility* 1993;99:315-321.
- [61] Vasconcelos JLM, Silcox RW, Rosa GLM, Pursley JR, Wiltbank MC. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrus cycle in lactating dairy cows. *Theriogenology* 1999;52:1067-1078.
- [62] Moreira F, Orlandi C, Risco CA, Mattos R, Lopes F, Thatcher WW. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *Journal of Dairy Science* 2001;84:1646-1659.
- [63] Putney DJ, Drost M, Thatcher WW. Influence of heat stress on pregnancy rates of lactating dairy cattle following embryo transfer or artificial insemination. *Theriogenology* 1989;31:765-778.
- [64] Thatcher WW, Collier RJ. Effect of climate on bovine reproduction. In: *Current Therapy in Theriogenology*, Morrow, D.A. (ed). Philadelphia: WB Saunders Co, 1986. p301-309.
- [65] Gordon I, Boland MP, McGovern H, Lynn G. Effect of season on superovulating responses and embryo quality in Holstein cattle in Saudi Arabia. *Theriogenology* 1987;27:231(Abstr.)

- [66] Monty DE, Racowsky C. In vitro evaluation of early embryo viability and development in summer heat-stressed, superovulated dairy cows. *Theriogenology* 1987;28:451-465.
- [67] Ulberg LD, Sheenan LA. Early development of mammalian embryos in elevated temperature. *Journal of Reproduction and Fertility* 1973;19:155-161.
- [68] Ulberg LD, Burfening PJ. 1967. Embryo death resulting from adverse environment on spermatozoa or ova. *Journal of animal Science* 1967;26:571-577.
- [69] Pursley JR, Kosorok MR, Wiltbank MC. Reproductive management of lactating dairy cows using synchronization of ovulation. *Journal of Dairy Science* 1997;80:301-306.
- [70] Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *Journal of Dairy Science* 1997;80:295-300.
- [71] Fricke PM, Wiltbank MC. Effect of milk production on the incidence of double ovulation in dairy cows. *Theriogenology* 1999;52:1133-1143.
- [72] Tenhagen BA, Drillich M, Heuwieser W. Analysis of cow factors influencing conception rates after two timed breeding protocols. *Theriogenology* 2001;56:831-838.
- [73] Pursley JR, Fricke PM, Garverick HA, Kesler DJ, Ottobre JS, Stevenson JS, Wiltbank MC. Improved fertility in noncycling lactating dairy cows treated with exogenous progesterone during Ovsynch. *Journal of Dairy Science* 2001;83(Suppl. 1):1563.(Abstr.)
- [74] Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF<sub>2α</sub> and GnRH. *Theriogenology* 1995;44:915-923.
- [75] Cartmill JA, El-Zarkouny SZ, Hensley BA, Lamb GC, Stevenson JS. 2001. Stage of cycle, incidence, and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. *Journal of Dairy Science* 2001;84:1051-1059.
- [76] Vasconcelos JLM, Silcox RW, Lacerda JA, Pursley JR, Wiltbank MC. Pregnancy rate, pregnancy loss, and response to heat stress after AI at 2 different times from ovulation in dairy cows. *Biology of Reproduction* 1997;56(Suppl 1):230.
- [77] Santos JEP, Thatcher L, Pool L, Overton MW. Effect of human chorionic gonadotropin on luteal function and reproductive performance of high-producing lactating Holstein dairy cows. *Journal of Animal Science* 2001;79:2881-2894.
- [78] Suadsong S, Chaikhun T, Suwimonteerabutr J. Effect of improved cooling system on diurnal body temperature patterns and conception rate in dairy cows under tropical conditions 2011: conference proceeding, July 26-29, 2011, Suranaree university of technology, Nakhon Ratchasima, Thailand. SAADC; 2011.
- [79] Sugiyama S, McGowan M, Kafi M, Phillips N, Yong M. Effects of increased ambient temperature on the development of in vitro derived bovine zygotes. *Theriogenology* 2003;60:1039-1047.