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Relationship of Environmental Factors, Cooling Treatment, Blood Metabolites, and Intensity of Heat Signs at Insemination with Conception Rate of Water Buffalo *(Bubalus bubalis)*

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ABSTRACT. *A study was carried out to examine the relationship of thermal environment, cooling treatment, circulating metabolites and intensity of heat signs at the time of insemination with conception rate (CR) of water buffalo. A total of 226 oestrous induced postpartum buffalo cows were used. Randomly selected 102 cows were used for the cooling experiment (sprinkling of 10 l water cow^{-1} for 10 minutes at -1 h, 0 h, and +1 hr post insemination). Environmental temperature (ET), relative humidity (RH), rectal temperature (RT), vaginal temperature (VT), pulse rate (PR), respiration rate (RR), and heat sign score of cows at the time of insemination were measured. Blood samples were obtained at insemination to quantify circulating D-3-hydroxybutyrate (BHB). Conception was assessed by per rectal palpation at 90 d and 120 d fallowing insemination. Mean CR (54%) was adversely affected by E>29.9°C, temperature humidity index (THI)>79, and RT>38°C. Cooling treatment lowered ($p<0.05$) RT, which was positively related to VT ($r= 0.55$; $p<0.05$). Cooling did not affect BHB, intensity of heat signs or milk yield, but improved ($p<0.05$) CR. Negative relationship between CR and BHB suggested the importance of energy nutrition on CR. Heat sign score was positively related to CR. The results suggest that CR in buffalo is adversely affected by hot thermal environmental conditions, and poor energy nutrition. Cooling at insemination to reduce RT<38°C, and inseminating the cows having low BHB and high intensity of heat signs at insemination can improve CR in buffalo.*

INTRODUCTION

Improving the performance of dairy sector is one of the primary goals of the livestock development policy in Sri Lanka. Buffalo plays a significant role in the dairy sector of Sri Lanka by contributing approximately 25% of the national milk production (Perera, 2001). One of the main factors affecting lactation performance is postpartum reproduction of the buffalo cow. In Sri Lanka, the average calving interval of buffalo is reported to be 540 d, which may vary from 480 d to 1005 d (Perera, 2001). Postpartum anoestrous, high incidence of silent heat and poor conception rate are some of the factors contributing to this status. Conception rate of buffalo is reportedly affected by body temperature, days postpartum, timing of insemination, and skill of the inseminator (Pagthinathan *et al.*, 2002).

Body temperature of buffalo is usually lower than that of cattle, but has shown great sensitivity to changes in ambient thermal status compared to cattle under Sri Lanka field conditions (Perera *et al.*, 1993; Perera *et al.*, 1994). Other than wallowing, intermittent sprinkling of water for 10 minutes at hourly intervals effectively alleviated

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heat stress (Perera and Perera, 1995), and improved milk yield and body condition even under free grazing conditions in the Dry Zone of Sri Lanka (Perera and Perera, 1996). It is of interest to examine whether such cooling technique can improve the conception rate of buffaloes.

In addition to body temperature, conception rate in cattle is affected by the nutritional status of the animal. Negative energy balance (NEB) in adult cows delayed the onset of ovarian activity after calving (Jainudeen and Hafez, 1987), and reduced the synthesis of progesterone and PGF $_{2\alpha}$, the two hormones which are directly linked to the conception in cows (Hafez, 1986). Information is lacking on the relationship between nutritional status and conception rate in buffalo.

Circulating beta hydroxybutyrate (BHB) is a blood metabolite (Russel and Wright, 1983) resulted from disrupted or abnormal energy metabolism (Spain, 2000), and closely related to the energy status of the animal (Russel and Wright, 1983). Increased BHB concentration in plasma was observed during NEB, especially in late pregnant and high yielding cows (Kunz *et al.*, 1985). High level of circulating BHB was negatively related to conception rate in cows (Miettinen, 1990). However, information is lacking on the relationship between BHB and conception rate in buffalo. This study was designed to examine the relationship of thermal environmental factors, cooling, circulating metabolites, and intensity of heat signs at the time of insemination with conception rate in buffalo cows.

MATERIALS AND METHODS

Location and animals

The study was conducted from January 2001 to March 2003. Field experiment was carried out at the Melsiripura and Nikaweratiya National Livestock Development Board (NLDB) farms located in the Kurunegala district, in North Western Province of Sri Lanka. A total of 226 postpartum buffalo cows belonging to *Nili-Ravi and Surti* breeds and their crosses were selected for the study after subjecting all the breedable cows maintained in the two farms to per rectal palpation to ascertain the reproductive stage.

Synchronization of oestrus and insemination

Non-pregnant postpartum cows having active ovaries and in normal physiological condition, were subjected to oestrus synchronization. For this purpose, progesterone releasing intra-vaginal device (PRID: 1.38 g Progesterone + 10 mg estrogen) was inserted to each cow (on day 0), removed on day 8, 500 mg PGF $_{2\alpha}$ injection was given on day 7. The animals were closely monitored during the next several days for the appearance of heat signs, and artificially inseminated using deep frozen semen of the same bull, on d 11 or 12. Based on the expression of heat signs, the date of insemination was determined. Two artificial insemination (AI) technicians inseminated all the buffalo cows included in the study.

Cooling treatment (Sprinkling of water)

A total of 102, randomly selected cows were used to determine the effect of cooling treatment. Sixty-four cows randomly selected at one hour before the time of insemination, were subjected to the cooling treatment (sprinkling of water), while 38 cows inseminated on the same days served as the control. Each treatment cow was sprinkled with 10 l of water for 10 minutes, at 1 h before, at the time of and at 1 h after insemination. The average water temperature was 28°C.

Data and measurements

For all the cows, information was collected on age and milk production on the day of insemination. Individual rectal temperature (RT), vaginal temperature (VT), pulse rate (PR) and respiration rate (RR) at the time of insemination were obtained from each cow. Rectal and vaginal temperatures were obtained by using a digital thermometer placed in the rectum and vagina, respectively. Pulse rate was assessed by palpation of the *coccigeal* artery. Respiratory rate was estimated by visual observation of chest and abdominal movements.

Heat signs of each cow at the time of insemination were assessed by visual observation. The scoring system used in this regard is given in Table 1. Ambient temperature and relative humidity at the time insemination were recorded using a dry and wet bulb thermometer.

Information on the level of education, duration of formal training in AI and experience in AI of the two technicians were obtained

Blood samples

Blood samples were collected into vaccutainer tubes by jugular venipuncture from the inseminated animals just after insemination. Blood was transported to the laboratory in ice within 6 h of collection.

Table 1. Scoring system used to assess heat symptoms.

Heat signs		Score
Color of Vulva	Red	4
	Light red	3
	Pinkish red	2
	Light pink	1
	Normal color	0
Mucus discharge	Thick	3
	Moderate	2
	Thin	1
	Less or None	0
Urination	Frequent	1
	Normal	0
Total		8

Blood was centrifuged at 2500 rpm for 20 minutes (Fick *et al.*, 1979) and serum was separated. Aliquots of serum were stored at –20°C until used for BHB quantification.

D-3- hydroxybutyrate (BHB) analysis

A commercial kit (Ranbut D-3 hydroxybutyrate) was used to quantify BHB content in serum samples. This kit uses colorimetric method to determine the BHB content based on the change of absorption associated with conversion of NAD^+ to NADH with oxidization of D-3 hydroxybutyrate to acetoacetate.

Pregnancy determination

All the inseminated cows were closely monitored for re-exhibition of heat signs between 18-21 d after insemination. The cows, which did not re-exhibit heat signs, were considered pregnant. They were further subjected to per- rectal examination at 90 d and 120 d after insemination to confirm pregnancy.

Statistical analysis

Data were tabulated and analysed by using Correlation and Regression, Analysis of Variance and Categorical Data Analytical procedures of the Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

Environmental parameters (ET, RH and THI)

The mean ± SD of Environmental temperature (ET) at the time of insemination of 226 cows in the two farms was 28.4 ± 2.3 (range 22.5 °C - 33.3 °C). The mean RH and THI at the time of insemination were 75.6 ± 2.5% (range 50%-96%) and 79.6 ± 2.7 (range 71.8 -87.8). respectively. Majority of the inseminations (80%) have been performed during the morning hours. Temperature humidity index at insemination was positively related to ET ($r=0.88$, $p<0.01$), although ET and RH had negative correlation ($r=-0.71$, $p<0.01$), suggesting a greater role of ET on THI.

Physiological parameters

Overall mean of RT, VT, PR and RR of buffalo cows at the time of insemination were 38.2±0.54°C, 38.6±0.44°C, 58.4±3.73 $minute^{-1}$, and 21.0±2.93 $minute^{-1}$, respectively. These values were within the physiological norms reported for buffalo (Perera, 2001). Significant positive relationship existed between RT and VT ($r=0.55$, $p<0.01$), and RR and PR ($r=0.25$, $P<0.05$) at insemination. No significant relationship could be detected between the environmental parameters and physiological parameters at insemination. This could be due to the use of large number of animals over a period of about 14 months with no repeated measurements on any animal.

The mean value of physiological parameters recorded from water sprinkled (cooled) and control cows at the time of insemination are given in Table 2. The mean values of VT, PR and RR of cooled animals were slightly lower than those of the control cows, but not significantly different. However, the RT of cooled cows was significantly lower than that of control cows suggesting the beneficial effects of sprinkling of water on lowering of body temperature.

Circulating D-3- hydroxybutyrate (BHB)

In this study, circulating BHB concentration at the time of insemination varied from 0.005 mmol L^{-1} to 0.5 mmol L^{-1}, and assumed an overall mean value of 0.17± 0.09 mmol L^{-1}, suggesting high degree of variation among animals. Circulating BHB was not significantly different between cooled and control cows. This was understandable, because hourly application of a cooling treatment for three hours cannot change a metabolite such as BHB, which reflects the status of long-term energy nutrition of the animal.

Table 2. Mean ± SD Values of physiological parameters at the time of insemination in cooled and non-cooled cows.

Parameter	Treatment group	
	Cooled	Non-cooled
Vaginal temperature (VT) °C	38.51 ± 0.22	38.59 ± 0.25
Rectal temperature (RT) °C	38.07± 0.46*	38.25 ± 0.34
Pulse rate (PR) per minute	58.40 ± 3.45	58.53 ± 2.58
Respiration rate (RR) per minute	21.02 ± 2.47	21.37 ± 2.43

* $p<0.05$

Heat sign score

Heat sign score of the cows at the time of insemination, varied from 1 to 5 in a scale that could range from 0-8 (Table 1), and assumed an overall mean value of 2.8 ± 0.9. Heat sign score was assigned based on the intensity of expression of visually observable behavioral and physiological oestrus signs at the time of insemination. Expression of heat signs depends on circulating estrogen and progesterone ratio (Thatcher *et al.*, (1985). Usually, the intensity of heat signs of buffalo is low, and the animal is said to be having 'silent heat' (Perera, 2001). The low mean value of heat sign score suggests that expression of oestrus is weak in buffalo even with administration of PRID and $PGF_{2\alpha}$ for synchronization, and close monitoring to detect heat. However, expression of at least some heat signs rather than silent heat facilitates detection of heat. Hence, synchronization of estrus using PRID and $PGF_{2\alpha}$ and timed insemination following close monitoring of heat signs can be considered as a satisfactory assisted reproductive technique to be employed for buffalo.

The relationship between the heat sign score and cooling treatment was not significant. This can be expected because cooling treatment was commenced only an hour before the intended time of insemination. Further investigation using a longer period of cooling to examine the relationship between cooling treatment and heat sign score is suggested.

Conception rate (CR)

Overall conception rate of 226 cows was 55.38%. This value is higher than the values (30 %) of conception rate of buffalo reported by Perera *et al.*, (1977) in Sri Lanka. Higher conception rate achieved in this study could be due to many factors such as oestrus synchronization, better timing of insemination and greater skill of technicians. The relationship of CR with the environmental parameters, cooling treatment, heat sign score and blood metabolites is discussed under the sections a, b, c and d given below.

a) Environmental parameters and conception rate

Relationship of overall CR with ET, RH, and THI were more pronounced under hot thermal conditions. As ET and THI increased above 29.9 and 78, the CR declined, suggesting adverse effects of increased heat load on CR of buffalo.

b) Cooling treatment and conception rate

Mean conception rate achieved by the 102 cows included in the part of the cooling treatment (cooled and non cooled cows) was 66.6%. This was superior to overall mean conception rate (55.4 %) of 226 cows used in the major part of the study. The cows that were subjected to sprinkling of water one hour before, at the time of insemination and one hour after insemination had better conception rate (78.2% *vs.* 47.4%) compared to non cooled control cows. Sprinkling of water on the body surface improves the efficiency of heat dissipation and thereby reduces the body heat load resulting in a lower body temperature. As indicated previously, in this experiment, the cows that were subjected to sprinkling of water had significantly lower rectal temperature compared to control non-cooled cows at the time of insemination (Table 2), suggesting the effectiveness of even a short-term cooling treatment in lowering of body temperature. Cooling improves the reproductive performance, if body temperature can be lowered (Her *et al.*, 1988; Wolfenson *et al.*, 1984). Conception rate was greater in buffalo cows having < 38° C rectal temperature at the time of insemination (Pagthinathan *et al.*, 2002). The findings of this study agree with those reports, and support the suggestion of Pagthinathan *et al.* (2002), that 38° C as the critical rectal temperature determining the success of conception rate in buffalo. The findings further suggests that sprinkling of water around the time of insemination to reduce body temperature of buffalo as a means for improving conception rate when inseminations are performed during hot climatic conditions that prevail during day time in buffalo rearing countries such as Sri Lanka.

c) Heat sign score and conception rate

Heat sign score of the cows used in this study was positively associated with conception rate ($p<0.05$, Fig. 1). This finding agrees with reports of De Silva et al., (1980). Expression of heat signs depends on circulating oestrogen: progesterone ratio. Thatcher *et al.*, (1985) reported that concentration of oestrogen and progesterone affect the blood flow rate to the reproductive tract and its microenvironment, which control the gamete transport, sperm capacitation, fertilization and development of the formed embryo. Poor expression of heat has been recognized as a major contributory factor to lower conception rate in buffalo and cattle (Van Eerdenburg *et al.*, 2002). The positive relationship between heat sign score and conception rate achieved by buffalo cows in this experiment is explained by above. The results suggest the use of timed insemination following PRID and $PGF_{2\alpha}$ administration accompanied with close monitoring of heat as a satisfactory technique for improving conception rate in buffalo.

d) D-3- hydroxybutyrate (BHB) and conception rate

Circulating BHB at insemination was not significantly ($p>0.05$) associated with conception rate in buffalo cows. However, the cows that became pregnant had lower BHB concentration (0.202 ± 0.13 mmol L^{-1}) at insemination compared to the cows that did not become pregnant (0.254 ± 0.42 mmol L^{-1}).

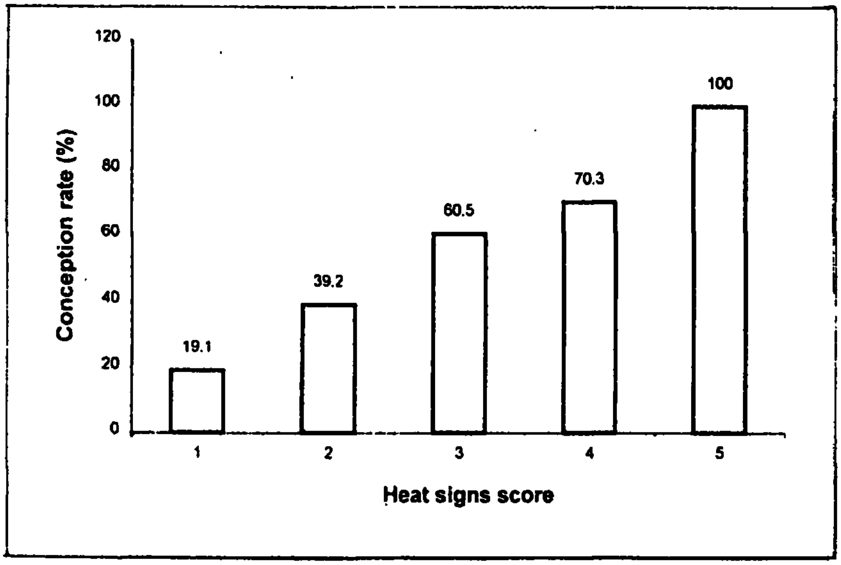

Fig. 1. Relationship between heat signs score and conception rate.

Miettinen (1990) reported that higher concentration of BHB to be positively related to negative energy balance, and negatively related to conception rate. Although the BHB concentrations of the buffalo cows that became pregnant and those that did not become pregnant in this study were not significantly different, a negative relationship between BHB and conception rate was evident indicating the adverse effects of poor energy nutrition on conception rate, and supporting the reports of Miettinen (1990). If more cows were included in the study, a significant relationship between BHB and conception rate could have been established.

CONCLUSIONS

The finding of study indicates that the conception rate in buffalo cow is adversely affected under hot thermal environmental conditions, and poor energy nutrition. Cooling of the cows around the time of insemination to reduce the rectal temperature to a level below 38°C, and inseminating the cows that exhibit more intense heat signs at insemination can help in achieving superior CR in buffalo. Synchronization of oestrus facilitates detection of heat and timed insemination.

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