UNIVERSITY OF PERADENIYA
17 - 1 - 2002
LIBRARY

EFFECT OF ENVIRONMENTAL, PHYSIOLOGICAL, AND MANAGEMENTAL PARAMETERS AT INSEMINATION ON CONCEPTION RATE OF EXOTIC (EUROPEAN)*INDIGENOUS COWS

E.R.K. PERERA, H.N.R. UDAYANGA, N. JAYAWEERA* and A.N.F. PERERA
Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya.
** Veterinary Surgeon's Office, Pannala.*

ABSTRACT

One hundred and one Exotic (European) * Indigenous crossbred cows presented for artificial insemination (AI) at Pannala Veterinary Range from April to July, 2000 were used to examine the effect of environmental temperature (ET), various physiological parameters of the cows, and management factors at the time of insemination, on conception rate (CR) of Exotic (European) * Indigenous crossbred cows in Sri Lanka.

Data on breed, age, parity, date of last calving, average daily milk yield and interval from detection of heat to insemination (IDI) of each cow were recorded based on the information provided by the owner. Environmental temperature (ET), vaginal temperature (VT), rectal temperature (RT), pulse rate (PR), respiration rate (RR), length and width of vulva, and body condition score (BCS) of individual cows at the time of insemination were measured. Heat signs (HS) of each cow at the time of insemination were assessed by adopting a scoring system, which assigned a score for each heat symptom examined, based on its intensity of expression. Two experienced technicians performed all 101 inseminations. Time of insemination and inseminator number were recorded for each insemination. Per rectal pregnancy diagnosis was performed at 75 days after insemination. Data were subjected to correlation and regression analysis, and analysis of variance procedures.

Mean ET at the time of insemination was 31.2 ±1.5 °C, resembling the typical daytime temperature conditions prevailing during these months in the intermediate zone of Sri Lanka. Mean age, parity, days postpartum (DPP) and BCS of cows were 5.39 ±2.74 yr, 2.52 ±1.55, 213±115 d and 2.74 ± 0.65, respectively. These values suggest that the cows included in the study were a representative sample of the normal population of breedable cows. Mean PR, RR, VT and RT of 101 cows at the time of insemination were 68.4 ±2.4 beats/min, 24.6 ±1.5 breaths/min, 39.45 ±0.38 °C and 38.5 ±0.4 °C, respectively. The values of physiological parameters were within the normal range for cattle. Relationships between ET at the time of insemination, and individual PR, RR, RT, and VT were positive, indicating increase in body temperature at higher ET despite induced thermoregulatory responses. Mean VT was 0.9 ± 0.1 ° C higher than mean RT in cows, and VT and RT were positively correlated ($r = 0.58$, $p<0.05$). Conception rate (65.4±1.2 %) was not significantly different between the two technicians. CR was highest in cows with BCS of 2 to 3, parity of 2 to 3, between 61 and 150 DPP, and when IDI was <13 hr. Inseminations performed after 19 hr lapse from detection of heat were not successful. CR declined ($p < 0.05$) by 8%, 3.9%, 5.2% and 4.9% with every 1°C, 0.1°C, 0.1°C and 1 hr increase in ET, RT, VT, at the time of insemination, and delay in time from detection of heat to insemination, respectively. CR was lowest when ET, RT, VT, and IDI at insemination were above 31.7 °C, 38.6 °C, 39.6 °C, and 19 hr. Highest CR resulted from inseminations performed when ET, RT, VT, and IDI at insemination were below 30.9 °C, 38.2 °C, 39.3 °C, and 13 hr, respectively. These trends are in the same direction, but the values are different to those reported for Holstein cows.

The results suggest that CR in Exotic (European) * Indigenous cross bred cows in the sample was affected by ET, RT, VT, BCS, parity, DPP and IDI. Highest CR was resulted from inseminating cows having BCS of 2-3, parity of 3, between 61 to 150 DPP, within 13 hours of detection of heat, and when ET, RT, VT were below 30.9 °C, 38.2 °C, and 39.3 °C, respectively.

INTRODUCTION

Conception rate is one of the key determinants affecting efficiency of reproduction in cows. Reduced fertility resulted from failure to conception, or from early embryonic mortality, is a common phenomenon among dairy cows reared in tropical countries (Stott and Williams, 1962). Successful fertilization depends on synchronous transport of gametes in the female reproductive tract to reach the site of fertilization. This process can be affected by many environmental, physiological and managerial factors. For example, conception rate in Holstein cows declined from 66% to 35% as the temperature humidity index (THI) increased from 68 to 78 (Ingraham *et al.*, 1976). The maximum temperature on the day after estrus and uterine temperature on the day of insemination significantly affected conception rate (Gwazdauskas *et al.*, 1973). Heat signs (De Silva *et al.*, 1981), and body condition (Domecq *et al.*, 1997) of the cow also affected conception rate in cattle. Although such information has been generated on the factors affecting, and the critical temperatures to be maintained to achieve superior conception rate in European breeds of cattle, information is lacking on the interrelationships among natural thermal environmental conditions, cow parameters, managemental factors and conception rate of tropical cattle and their crosses. This experiment was conducted to examine the effect of environmental temperature (ET) at the time of insemination, physiological parameters (body temperature, pulse rate, respiration rate, heat signs, body condition) of the cow at insemination, and management factors (time lapse from detection of heat to insemination) on conception rate (CR) of Exotic (European) * Indigenous crossbred cows in Sri Lanka.

MATERIALS AND METHODS

Location and animals: The study was conducted in the Pannala Veterinary Range, located in the intermediate zone of the Kurunegala district of Sri Lanka. A total of 101 Exotic (European) * Indigenous crossbred cows presented for artificial insemination (AI) to the Pannala veterinary surgeon's office, between April and July, 2000 were studied. All the European*indigenous crossbred cows presented for AI during this period were used for the study to avoid any discrimination or biased sampling.

Parameters measured: Data on breed, age, parity, date of last calving, average daily milk yield, and interval from the time of detection of heat to insemination (IDI) of each cow, were recorded based on information provided by the owner. Body condition score (BCS) of each cow at the time of insemination was assessed by adopting the scoring system of Jones et al (1982). Heat signs (HS) expressed by each cow at the time of insemination were evaluated by adopting the scoring system given in Table 1, which assigned a score of 0 to 4 for each heat symptom examined, based on its intensity of expression. The length and width of the vulva of individual cows, were measured just before insemination with the use of a vernier caliper. Environmental temperature (ET) of the area, vaginal temperature (VT), and rectal temperature (RT) of the cow at the time of insemination, were measured using a dry bulb thermometer and a digital clinical thermometer respectively. Pulse rate (PR), and respiration rate (RR) of individual cows at insemination were determined by adopting the standard methods of counting the number of pulses/minute in the tail vein, and the number of flank movements/minute, respectively. Two experienced technicians designated as technician I and

performed all 101 inseminations. The time of insemination and inseminator number were recorded for each insemination. Per rectal pregnancy diagnosis was performed at 75 days after insemination for the 101 cows, by a veterinary surgeon. Conception rate (CR) at different values of each independent variable was computed using the following equation:

Conception rate = (number of cows conceived / number of cows inseminated) * 100

Table 1. Scoring system adopted to assess heat signs in cows

Heat sign	Score
Colour of vulva:	
Red	4
Light red	3
Pink	2
Light pink	1
Normal colour	0
Mucous discharge:	
Thick	3
Moderate	2
Thin	1
None	0
Frequent urination:	
Present	1
Absent	0
Restlessness:	
Present	1
Absent	0
Bellowing:	
Present	1
Absent	0
Mounting:	
Present	1
Absent	0
Loss of appetite:	
Not eating	1
Eating normal	0
Total maximum score	12

Statistical analysis: Data on ET, RT, VT, PR, RR, HS and IDI were subjected to correlation analysis to assess the inter-relationships among environmental, physiological and managemental parameters at the time of insemination. For the significant correlations that were revealed, regression analysis was performed to compute the best fitting regression equation and line. To compare the CR at different ranges of independent variables, analysis of variance (ANOVA) procedures were performed on completely randomized design.

RESULTS AND DISCUSSION

Environmental parameters: The environmental temperature prevailed at the time of 101 inseminations varied between 28.0 °C and 33.5 °C, with a mean value of 31.2±1.5 °C. These values resemble the typical daytime temperature conditions in April to July months in the intermediate zone of Sri Lanka.

Cow parameters: The 101 crossbred cows presented for artificial insemination during the study period, were of 2 to 12 yr in age, while the majority (71%) were below 8 yr of age as reflected by the mean value of 5.39±2.74 yr. Parity ranged between 1 and 8 among the 74 cows for which the parity was reported, while the majority of them (90%) were below parity of 5 (mean parity 2.52±1.55). Data on DPP were available only on 60 cows, which were within 42 to 480 DPP. Sixty five percent of the cows were between 90 and 240 DPP, resulting in a mean value of 213±115 DPP. This DPP indicates the existence of an exceptionally long open period among cows, considering the facts that, the recovery of hypothalamic pituitary ovarian axis is completed within 15 DPP (Moss *et al.*, 1985), and that involution of the uterus, which is a pre-requisite for next conception is completed before 28 DPP in cattle (Butler and Smith, 1989). Negative energy balance, under-nutrition (Butler and Smith, 1989), and suckling (Smith *et al.*, 1981) are reported to prolong resumption of ovarian function following parturition in cows. These factors could have contributed to the lengthy DPP observed in the cows used in the study. The body condition of most of the cows (88 %) assumed a score of 2 to 3, (mean 2.74 ± 0.65), although the BCS varied between 1 and 4. These values suggest that the cows included in the study can be considered as a representative sample of the normal population of breedable cows, while the majority were reasonably young and had satisfactory body condition.

Physiological parameters: The mean values of PR, RR, VT and RT of cows (n=101) at the time of insemination were 68.4±2.4 beats/min, 24.6±1.5 breaths/min, 39.45±0.38 °C, and 38.5±0.4 °C respectively. The values of physiological parameters were within the normal range reported for cattle. Mean VT was 0.9 ± 0.1° C higher than mean RT in cows. This is in agreement with the findings of Gwazdauskas *et al.*, (1973). Mean VT reflects the thermal conditions prevailing in the female reproductive tract to which the gametes are exposed prior to fertilization. Heat sign score of the cows varied between 3 and 12, while the majority (72%) had a score between 6 and 9.

Relationships among environmental and physiological parameters: The resulted correlation coefficients between prevailed ET and RT, VT, PR and RR of cows (n=101) at the time of insemination are given in Table 2. Environmental temperature at the time of insemination showed positive correlations to RR, PR, RT, and VT. These physiological parameters serve as the indices of internal thermal status of the cows. The positive correlations between ET and physiological parameters are in agreement with the findings elsewhere (Munida and Yamamoto; 1996). The correlation coefficients (Table 2) suggest that, despite various thermoregulatory mechanisms employed such as changes in respiration rate (RR), increased peripheral blood flow (PR) to maintain thermal balance, the body temperature (as reflected by RT and VT) elevated with increasing ET. Linear regression between RT and ET indicated that with every 1 ° C increase in ET, RT increased by 0.06 ° C.

$$RT = 0.0599\ ET + 36.63 \quad (1)$$

Among the physiological parameters, the highest correlation was found between VT and RT ($r = 0.58$, $p < 0.01$). Linear regression between VT and RT (Fig. 1) indicated that, for each 1° C increase in RT, VT which reflects the temperature within the reproductive tract, increased by 0.5 ° C.

$$VT = 0.5027\ RT + 20.09 \quad (2)$$

Apart from the above relationships between ET and physiological parameters, HS of the cows declined with increasing ET (r = -0.18) and with increasing IDI (r = -0.36) as follows.

$$HS = -\ 0.2303\ ET + 14.746 \quad (3)$$

$$HS = -\ 0.1324\ IDI + 9.6172 \quad (4)$$

Such reduction in intensity of heat signs under hot climatic conditions, and with time due to alterations in estrogen: progesterone hormonal balance has been reported (De Silva *et al.*, 1981; Thatcher *et al.*, 1985).

Table 2: Correlation coefficients among environmental temperature and physiological parameters at the time of insemination

Parameter	Environmental temperature	Rectal temperature	Vaginal temperature	Pulse rate
Respiration rate	0.25*	0.29*	0.08	0.27*
Pulse rate	0.14	0.15	0.17	
Vaginal temperature	0.13	0.58**		
Rectal temperature	0.21*			

* $p < 0.05$; ** $p < 0.01$

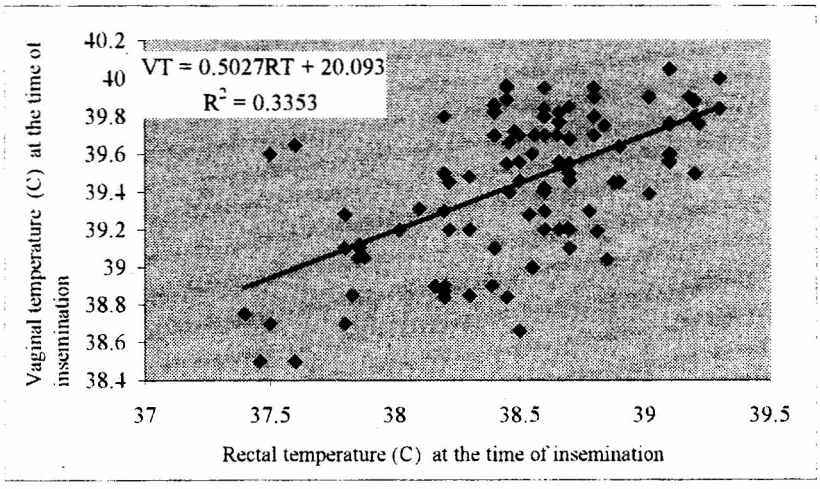

Fig. 1. Relationship between vaginal temperature and rectal temperature at insemination

Conception rate changes with environmental, physiological and management factors: Of the 101 cows inseminated, 66 cows conceived, resulting in a conception rate of 65.3%. Conception rate exhibited significant correlations with several environmental, physiological, and management parameters (Table 3). In addition, CR varied with body condition score, parity, and days postpartum (DPP). The following (a), (b), and (c) sub-sections of this paper, discusses the significant changes exhibited by conception rate in relation to environmental parameters, cow and physiological parameters, and management parameters respectively.

a). Changes in conception rate with environmental temperature at insemination: Conception rate declined from 100% to 38% as environmental temperature increased from 28° C to 33.5° C (Fig. 2). Linear regression revealed 8% decline in CR with every 1° C increase in ET. Such inverse relationships between ET and CR in cows have been reported (Ingraham *et al.*, 1974). Conception rates achieved from inseminations performed when ET was below 30.9° C, were significantly ($p < 0.05$) greater than those achieved from inseminations performed when ET was above 31.9° C. Although no information is available regarding the above on tropical cattle for comparison purpose, the results of this study agree with those of Dunlap and Vincent (1971), who reported zero conception rate in beef cattle when exposed to 32° C temperature for three days post-breeding, and those of Thatcher *et al* (1985), who reported greater decline in CR in lactating Holsteins when ET exceeded 30° C.

Table 3. Correlation coefficients of conception rate, with environmental, physiological and managemental parameters at insemination

Parameter	Conception rate
Environmental temperature	-0.92**
Physiological parameters	
Rectal temperature	-0.87**
Vaginal temperature	-0.86**
Management parameters	
Time from detection of heat to insemination	-0.98**

** $p < 0.01$

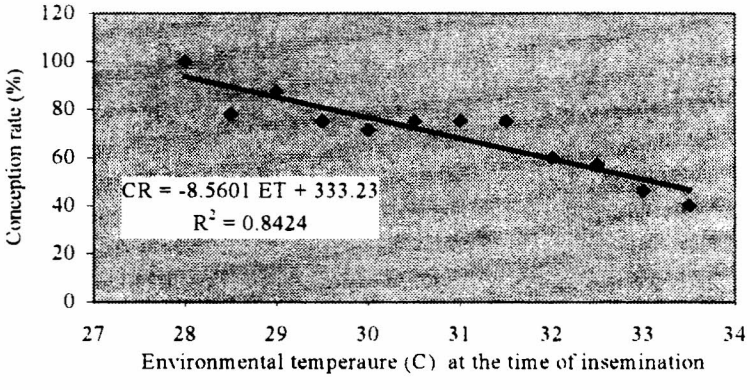

Fig. 2. Relationship between conception rate and environmental temperature at insemination

b) Changes in conception rate with cow (physiological) parameters at insemination: Several cow parameters and several physiological parameters affected the CR of cows in this study.

Body condition score (BCS): Conception rate varied with BCS of the cows (Table 4). Conception rate was higher among cows which had a BCS of 2 to 3 at insemination. Domecq *et al.*, (1997) reported that, although the BCS at insemination was not related to conception, cows experiencing loss of body condition had negative influence on conception. This may partly explain the lower conception rate among cows with BCS of 1.

Table 4. Changes in conception rate and body condition score at insemination

Body condition score	Number of cows inseminated	Number of cows conceived	Conception rate %
1	2	1	50.0
2	32	23	71.8
3	57	37	64.1
4	10	5	50.0

Parity: Changes in conception rate with parity is given in Table 5. Conception rate varied and tended to increase with parity but this linear relationship was not significant. Higher conception rates were achieved by cows with parity of 2 to 3, and by those with a parity above 6 at insemination. The number of cows having a parity above 5 were few, possibly because the farmers get rid of older unproductive cows, keeping only the very best older ones. This partly explains the superior CR in cows with parity above 6.

Table 5. Changes in conception Rate with Parity

Parity	Number of cows Inseminated	Number of cows conceived	Conception rate %
1	19	11	57.8
2	21	15	71.4*
3	17	13	76.4*
4	9	6	66.6
5	3	2	66.6
6	2	1	50.0
7	1	1	100.0*
8	2	2	100*

* Values differ at $p < 0.05$

Heat sign score (HS): Heat sign score of the cows also affected CR (Table 6). A significantly greater ($p<0.05$) CR was achieved by inseminating cows which had >9 heat sign score at insemination. Heat sign score, which reflects the intensity of expression of heat signs, depends on circulating estrogen: progesterone ratio. While the relative concentration of these two hormones in circulation is altered under thermal stress, it affects the blood flow to the reproductive tract and its micro-environment, controls gamete transport, capacitation, fertilization and development of the embryo. All these factors determine the conception rate (Thatcher *et al*., 1985). Although the hormonal concentrations were not monitored in this experiment, the observed decline in HS with ET (equation 3), and decline in CR with reduction in HS are explained by the above phenomenon.

Rectal temperature (RT): The relationship between RT and CR is given in Fig. 3. Conception rate declined ($p < 0.05$) by 3.9% with each 0.1 °C increase in RT at the time of insemination, while RT, which reflects the body core temperature of the animals, increased by 0.06 °C with every 1 °C increase in ET (equation 1). Inseminations, performed when RT was below 38.2 °C at insemination, had a significantly greater CR compared to inseminations, conducted when RT was above 38.6 °C (Fig. 3). This agrees with the findings of Thatcher *et al* (1985), who reported a decline in CR due to an inability to maintain normal body temperatures in lactating cows under thermal stress. Elevated body temperatures have been reported to exert negative influence on secretion of proteins and prostaglandins by the uterine endometrium, embryonic

viability and production of embryonic signals for the establishment of pregnancy (Shearer and Beede, 1990). The observed decline in CR with elevated body temperature can be explained by the above phenomenon

Table 6. Changes in conception rate with heat sign score of the cow at the time of insemination

Heat sign score	Number of cows inseminated	Number of cows conceived	Conception rate %
3	4	2	50.0
4	4	2	50.0
5	3	2	66.7
6	20	11	55.0
7	16	9	56.3
8	25	16	64.0
9	12	10	83.3 [a]
10	9	8	88.9 [a]
11	6	6	100 [a]
12	2	2	100 [a]

[a] Values differ at $p < 0.05$

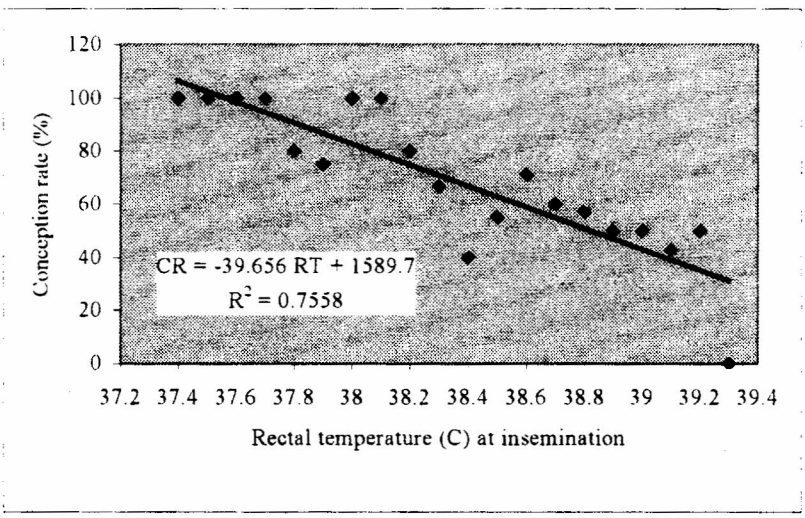

Fig. 3. Relationship between conception rate and rectal temperature at the time of insemination

Vaginal temperature: Conception rate declined by 5.2% with every 0.1° C increase in VT (Fig. 4). Inseminations performed when VT was below 39.7 ° C had significantly ($p<0.05$) lower CR than inseminations conducted when VT was above 39.3 ° C (Fig. 4). Vaginal temperature, which reflects the temperature within the reproductive tract, increased by 0.5 ° C with each 1 ° C increase in RT (Fig. 1) in this experiment. Elevated reproductive tract temperatures near time of insemination is detrimental to conception rate (Thatcher *et al.*, 1985). Elevated reproductive tract temperatures are caused by lowered blood flow to the reproductive tract, due to increased blood flow to the periphery in the animal's attempt to accelerate heat dissipation to maintain body temperature. Lowered blood flow to the reproductive tract in turn creates a particularly hostile micro-environment for fertilization (Shearer and Beede, 1990). Observed decline in CR with increasing VT can be due to such changes.

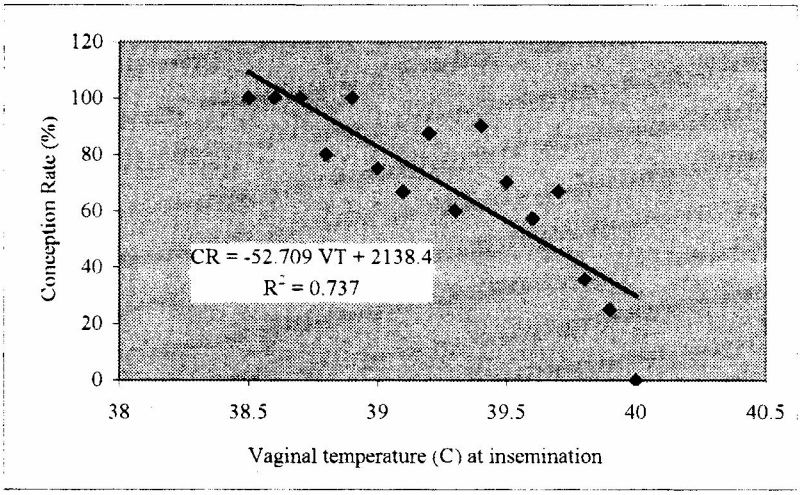

Fig. 4. Relationship between conception rate and vaginal temperature at insemination

c) Changes in conception rate with management parameters: Conception rate has been reported to change due to several management parameters such as the skill of the AI technician (Alexander *et al.*, 1998), time lapse from detection of heat to insemination (McMillan and Watson, 1975) and days postpartum (Malven, 1984). The relationship of these parameters to CR of the cows in this experiment is discussed below.

Inseminating technician: Conception rates achieved through inseminations performed by the two technicians were not different ($p > 0.05$; 64.8% *versus* 66.8%) resulting in a mean conception rate of 65.4±1.2 % for the two technicians. This suggests that both the AI technicians used in this study were equally skillful.

Days postpartum (DPP): Conception rate varied with DPP (Table 7). A superior CR ($p<0.05$) was achieved from inseminations performed in cows that are between 61 and 210 DPP. It appears that inseminations performed before 60 DPP were unsuccessful, even if the postpartum cow comes into estrus, while considerably a minority came into estrus before 60 DPP (Table 7.). Factors such as suckling, under-nutrition and negative energy balance, which are common among early postpartum cows, exert negative influence on resumption of postpartum ovarian activity and estrus (Smith *et al.*, 1981; Butler and Smith, 1989). These factors would have prevented large number of cows in this study from coming to estrus before 60 DPP. Although the CR was superior even up to 240 DPP, such a long open period to insemination would adversely affect the overall life-time reproductive efficiency of the cow. Therefore, it is advisable to inseminate cows within 61-150 DPP to achieve higher CR and reproductive efficiency.

Table 7. Changes in conception rate of cows with days postpartum to insemination

Days Postpartum to insemination	Number of cows inseminated	Number of cows conceived	Conception rate %
<60	1	0	0
61-90	5	4	80.0*
91-120	6	5	83.3*
121-150	6	5	83.3*
151-180	14	10	71.4*
181-210	7	6	85.4*
211-240	6	4	66.7
>241	15	9	60

* $p < 0.05$

Interval from detection of heat to insemination (IDI): Conception rate declined by 4.9% with every hour delay in time from 6 hr of detection of heat to insemination, while inseminations performed after 19 hr lapse from detection of heat were not successful (Fig. 4). Conception rate obtained from inseminations performed within 13 hr of detection of heat was superior ($p<0.05$). This is in agreement with the findings of McMillan and Watson, 1975). Circulating estrogen: progesterone ratio, which influences the transport of gametes to the site of fertilization, changes with IDI (Shearer and Beede, 1990), while the released ovum also becomes aged and unsuitable for fertilization with increasing IDI. These factors would have contributed to the observed decline in CR with increasing IDI beyond 13 hr.

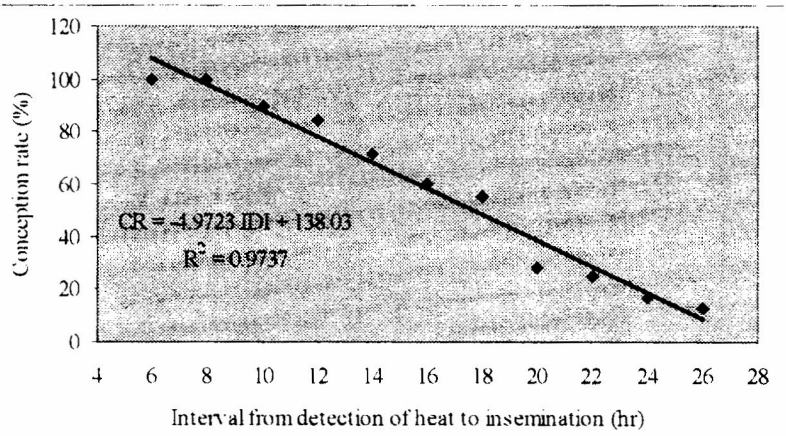

Figure 5. Relationship between conception rate and time interval from heat detection to insemination

Fig. 5. Changes in conception rate with time interval from detection of heat to insemination

CONCLUSIONS

The results suggest that the CR in Exotic (European) * Indigenous cross bred cows in the study was affected by ET, RT, VT, BCS, parity, DPP and IDI. Highest CR was resulted from inseminating cows having BCS of 2-3, parity of 3, between 61 to 150 DPP, within 13 hours of detection of heat, and when ET, RT, VT at insemination were below 30.9 °C, 38.2 °C, and 39.3 °C respectively.

ACKNOWLEDGEMENTS

The authors acknowledge the cooperation extended by the Provincial Director, North Western Province, Department of Animal Production & Health by permitting to conduct this study in the Pannala Veterinary Range, the two Livestock Development Instructors by performing AI, and the farmers by allowing the use of their animals.

REFERENCES

1. Alexander, P.A.B.D., Abeygunawardena, H., Perera, B.M.A.O., and I.S. Abeygunawardena. 1998. Reproductive performance and factors affecting the success rate of artificial insemination of cattle in up-country multiplier farms in Sri Lanka. *Tropical Agricultural Research*, **10,** 356-366.

2. Butler, W.R., and R.D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *Journal of Dairy Science*, **72,** 767-783.

3. De Silva, A.W.M.V., Anderson, G.W., Gwazdauskas, F.C., McGilliard, M.L., and J.A. Lineweaver. 1981. Interrelationship with estrus behaviour and conception in dairy cattle. *Journal of Dairy Science,* **64,** 2409-2415.

4. Domecq, J.J., Skidmore, A.L., Lloyd, J.W., and J.B. Kaneene. 1997. Relationship between body condition score and conception at 1st AI in large herd of Holstein cows. *Journal of Dairy Science,* **80,** 113-121.

5. Dunlap, S.E. and C.K. Vincent. 1971. Influence of post-breeding thermal stress on conception rate of beef cattle. *Journal of Animal Science,* **32,** 1216 – 1220.

6. Gwazdauskas, F.C., Thatcher, W.W., and C.J. Wilcox. 1973. Physiological, environmental and hormonal factors at insemination which may affect conception. *Journal of Dairy Science,* **56**, 873-877.

7. Ingraham, R.H., Gillette, D.D., and W.C. Wagner. 1974. Relationship of temperature and humidity to conception rate of Holstein cows in Hawaii. *Journal of Dairy Science,* **59,** 2086-2094.

8. Jones, G.M., Wildman, E.E., Wagner, P.E., Bowman, R.L., Lesch, T.N. and H.F. Trout. 1982. A dairy cow body conditio scoring system and its relationship to selected production characteristics. *Journal of Dairy Science,* **65**: 495 – 499.

9. Malven, P.V. 1984. Pathophysiology of the puerperium: definition of the problem. *Proceedings of the 10th International Congress on Animal Reproduction and Artificial Insemination* **10,**1.

10. McMillan, , K.L., and J.D. Watson. 1975. Fertility differences between groups of sires relative to the stage of estrus of the time of insemination. *Animal Production*, **21,** 243-250.

RARA03

11. Moss, G.E., Parfet, J.R., Marvin, C.A., Alrich, R.D. and M.A. Diekman. 1985. Pituitary concentrations of gonadotropins and receptors for GnRH in suckled beef cows at various intervals after calving. *Journal of Animal Science*, **60,** 285-292.

12. Munida, C.M., and S. Yamamoto. 1996. Day-night variation of thermoregulatory responses of heifers exposed to high environmental temperatures. *Journal of Agricultural Science*, **129,** 199-204.

13. Shearer, J.K., and D.K. Beede. 1990. Thermoregulation and physiological responses of dairy cattle in hot weather. *Agri-practice*, **11(4),** 5-17.

14. Stott, G.H. and R.J. Williams 1962. Causes of low breeding efficiency of dairy cattle associated with seasonal high temperatures. *Journal of Dairy Science*, **45,** 1369-1375.

15. Smith, J.F., Payne, E., Tewit, H.R., McGowan, L.T., Fairclough, R., Kilgour, R. and P.G. Goold. 1981. The effect of suckling upon the endocrine changes associated with anoestrous in identical twin dairy cows. *Journal of Reproduction and Fertility (Supplement)*, **30,** 241–249.

16. Thatcher, W.W., Collier, R.J., Beede, D.K., and C.J. Wilcox. 1985. Interaction of environment and reproductive processes in cattle. *Proceedings of IAEA* on "Nuclear and related techniques for improving productivity of indigenous animals in harsh environments" **IAEA-SR-115/104**, 61-73.