Patterns of Oestrus Onsets and Behavioural Signs in Holstein-Friesian Dairy Cows in the Peri-urban Khartoum City (Sudan).

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1 ABSTRACT

The objective of this study was to determine the patterns of oestrus onsets and behavioural signs in Holstein – Friesian dairy cows. One hundred and Forty six (146) cows from the control group and 92 cows from the prostaglandin $F_{2\alpha}$ treated group were visually observed for oestrus onsets and other behavioural signs in group pens and open exercise yards. The observers recorded the time when oestrus behaviour first became evident, noted and registered the identity of the cow exhibiting the behavioural interactions associated with oestrus. Fifty three (53) % of the onsets of oestrus in both control and treated cows were associated with night hours, 18.00 pm to 06.00 am, and most frequently between 18.00 and 20.00 hours. Forty three (43), 56 and 63% of the oestrus onsets occurred at night in winter, hot dry summer, and hot wet summer respectively. Forty six (46) % of the cows at parities 1 and 2, and 62% of those at parities 3 and over showed onsets of oestrus at night. Onsets of oestrus occurred mostly at night in cows at 41-60, 61-80 and 101 and over days postpartum as compared to cows at 81-100 days postpartum. Onsets of mounting activity occurred mostly during daylight hours in winter and hot dry summer as compared to the hot wet summer. Onsets of mucus discharge occurred mostly at night in winter as compared to the summer seasons. Onsets of mounting activity and mucus discharge were low at night for cows of all parities. Cows at 41-60 days postpartum had the highest mounting activity at night as compared to cows at 61-80, 81-100, and 101 and over days postpartum. Onsets of mucus discharge were low at night for all cows at 41 and over days postpartum. These results suggest that onsets of standing when a cow is mounted by the herd mate occur at night. Behavioural signs of mounting and mucus discharge are associated with daylight hours. Season of the year, parity and days postpartum of the cow interfere with patterns of oestrus onsets and behavioural signs.

2 INTRODUCTION

Oestrus is characterized as the period of sexual receptivity for the female (Senger, 1997). It is one of the four phases of the oestrous cycle. Proestrus and oestrus make up the follicular phase, and metoestrus and dioestrus are in the luteal phase. The follicular phase is the shorter of the two phases in the cow's oestrous cycle, and starts with proestrus with a decline of progesterone resulting from luteolysis. Proestrus lasts approximately 2 – 5 days and is the transition from progesterone dominance to oestrogen dominance (Senger, 1997). Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) are responsible for this transition during proestrus as the reproductive tract is preparing for the onset of oestrus and mating.

The importance of oestrus is that this is the period of time in the oestrous cycle that the female is fertile and able to conceive when mating. In cattle the oestrous cycle ranges from 18 – 24 days and oestrus is considered day zero of the cycle. This is because it is the day that outward signs of the cycle are apparent. On average, the cow in oestrus is mounted 9 times for approximately 3 seconds per mount (Sanders, 2000). This means visual oestrus detection is 27 seconds per oestrus period. This stresses the importance of proper oestrus detection required to successfully catch a large percentage of animals in oestrus. Breeding records of an individual cow such as animal number, calving date, pre – breeding dates, and first and repeat service dates make oestrus detection easy and efficient (Diskin and Sreenan, 2000), but oestrus onset and behavioural signs seem to follow distinct patterns. Van Vliet and Van Eerdenburg, 1996; Pinheiro *et al*, 1998 observed this pattern of oestrus onsets to occur more frequently during nocturnal period from 18.00 and 06.00 hours. Several researchers reported the distribution of oestrus onset in two peaks from 6.00 to 10.30 am and 14.00 to 18.30 pm during daylight hours (Cardozo et al, 1994 and Neguise, 2002). In contrast, (Xu *et al,* 1998), found no variation in the onset of oestrous activities. Environmental temperatures also interfere with the pattern of oestrus onsets and other behavioural signs. Sonmez *et al*, 2005; and Bulbul and Ataman, 2009 reported reduced intensity of oestrus, reduced oestrus length and anoestrus in lactating cows during hot daylight hours in summer. Due to conflicting reports from different workers from different environments, the present study was undertaken to determine the patterns of oestrus onsets in Holstein – Friesian dairy cows and the proportion of those cows that showed standing, mounting activity and mucus discharge associated with oestrus under tropical conditions in the peri–urban Khartoum City (Sudan).

3 MATERIALS AND METHODS

3.1 The study Area: This study was conducted at the dairy unit of the Arab company for agricultural production and processing (Sudan) limited. The unit is located in the area of Bagier on the western bank of Blue Nile River, about 30 Km south of Khartoum. Its location is approximately at latitude 15°36 ´N, longitude 32° 33 ´E and altitude 380 metres above sea level. The area has three marked seasons, namely winter (November to February), hot dry summer (March to June) and hot wet summer (July to October). Average annual rainfall is 121.4 mm, and average minimum and maximum temperatures of 17.0 and 34.4 C° respectively.

3.2 The study herd: The herd, which was studied, consisted of only Holstein – Friesian stock. The herd numbered 559 head at the time of this study and no bulls ran with cows. From the reproductive records kept on the farm, the herd was segregated into reproductive management groups as outlined by Britts (1977) as follows:

Group	Animals in group
(1)	• Early post-partum cows; pre-breeding • Breeding group • Problem cows; open more than 120 days
(2)	• Pregnant cows; lactating
(3)	• Pregnant cows; Non lactating

After the classification scheme, reproductive records for each of the cows in group one (1) were checked and evaluated, and cows with reproductive health problems were removed from the population. Samples for this study were randomly selected from the group of cows without reproductive problems. The selected cows were then identified with clearly numbered plastic ear tags. After identification and evaluation of records, the whole herd was sprayed with anti-parasitic drugs in order to keep the herd free from external parasites.

3.3 Housing of the Dairy herd: The herd was housed in a loose housing system with concrete floor. The building was partitioned into 10 group pens, and each group pen was constructed to accommodate 48 cows, but at the time of this study, it accommodated a maximum of 32 cows. The cows were grouped according to milk yield, with the high yielders in pen one and the other yield groups followed till pen seven. Pen eight till ten accommodated the dry pregnant cows. Each group pen had a roofed resting and feeding area of 7.7 m^2/cow and free access to open exercise yard of 4.3 m^2/cow. Cows from two adjacent group pens were fed from a single feed passage, and each group pen along the feed passage was provided with a self-locking feed fence. The resting area for each group was provided with a line of 26 raised cubicles with a central passage. Each of the group pens was equipped with two water troughs, each with a width of 38.5 cm, length of 3.5 m, and depth of 40 cm. The building's lights, together with some supplementary lights were kept on throughout the hours of darkness.

3.4 The experimental Cows: The dairy cows not inseminated by 45 days postpartum because of unobserved oestrus were presented for rectal examination. Those cows with a palpable corpus luteum were assigned randomly to the prostaglandin $F_2\alpha$ treated group and the control group. Those cows with unobserved oestrus after insemination and diagnosed open at pregnancy examination were also assigned randomly to the treated and control groups if the corpus luteum was palpable. Cows assigned to the treated group received an intramuscular injection of 25 mg prostaglandin $F_2\alpha$ between 08.00 and 10.00 hours. Each control group of cows were visually observed for the onset of oestrus throughout the day and night for a period of 30 days. Each treated group was also continuously observed for 168 hours following the first dose of prostaglandin $F_2\alpha$ injection. If onset of oestrus was not detected in any of the cows from the treated group up to the eleventh day after the first dose of prostaglandin $F_2\alpha$, she was given a second dose of 25 mg, and continuously observed for the onset of oestrus again. 146 cows from the control group and 92 from the treated group were used during the oestrus detection process.

3.5 Oestrus Detection: A team of two trained oestrus detectors, one A.I technician, and the senior author visually observed cows. Continuous observation was maintained throughout the day and night for a period of one year. The experimental cows were observed when they were in the group pens and open exercise yards. The observers recorded the time when oestrus behaviour first became evident, and then noted and registered the identity of the cow exhibiting behavioural interactions associated with oestrus. To facilitate observations, the day was divided as follows:

(i) Morning from 06.00 to 12.00 hours
(ii) Afternoon from 12.00 to 18.00 hours
(iii) Night from 18.00 to 0.6.00 hours.

During the observation period the criteria for determining oestrus were:

(i) Bellowing with greater frequency than would normally be encountered.
(ii) Increased activity by the cow, and especially when walking the fence line.
(iii) Head butts – when one cow strikes another cow with the head.
(iv) Head rubbing – when one cow rubs her head on the body of another cow.
(v) Sniffing the anogenital area of another cow and display of the flehmen posture.
(vi) One cow follows another cow in a linear fashion.
(vii) One cow licks another cow on the front or back half of the body.
(viii) One cow positions her body in preparation to mount another cow, but does not attempt the mount.
(ix) Chin resting – one cow rests her chin on the back line of another cow.
(x) One cow runs towards another cow without initiating.

When any of above behaviours was noticed, the cow was watched closely for the onset of oestrus. First, when one cow mounted or attempted to mount another cow, the time when the mount or attempted mount was initiated, the observer recorded it. The identity of the cow initiating the mount or attempted mount was also recorded. Second, when one cow was noticed standing immobile when mounted by another cow, the time when standing was initiated and identity of the cow initiating the standing were recorded by the observer. When a clear mucus discharge from the vagina, which looked and had the consistency of egg white was noticed from one cow, the time when mucus was first observed and identity of the cow that had mucus discharge were recorded. The following physical signs were also recorded in some cows:

(i) Roughened tail head or loss of hair and presence of abrasions about the tail head.

(ii) Metoestrus bleeding.

Statistical Analysis: Data were analysed using MINITAB ® for windows, Inc. personal computer release 11.12 32 Bit (1996). Data on the distribution of onset of oestrus, mounting activity and mucus discharge were analysed by using Chi – squared test, which compared the observed numbers in each of the four categories in the contingency table with the numbers expected if there were no difference in the time of onset between night and day in the control and treated cows. Data on the factors influencing time of onset of oestrus and behavioural signs of oestrus were analysed by using chi – squared test, which first analysed the 2x2 subsets of the data separately, and combining the subsets by Mantel – Haenszel Chi – Squared test as recommended by Kirkwood (1988).

4 RESULTS

4.1 Time of onset of oestrus:

Time of onset of oestrus: Onset of oestrus was assessed as the first mount received during an oestrus period. At that instant, the cow made no effort to escape when mounted by the herdmate. The frequency distribution of true oestrus onsets during two – hour periods over 24 hours is shown in figure 1.1.

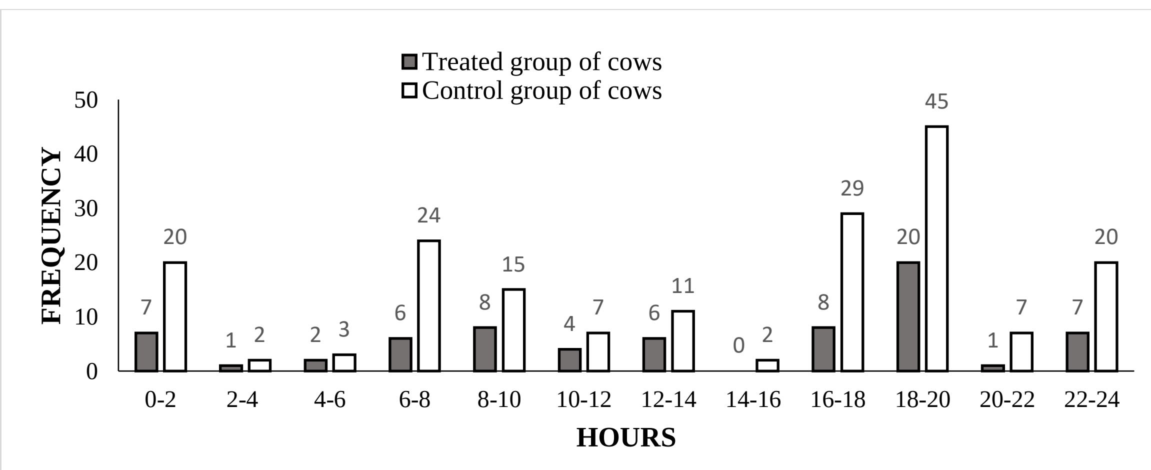

Fig. 1: Frequency Distribution of True Oestrus Onset during a Day (24h)

Onset of oestrus was associated with night hours, 18.00 to 06.00 hours. This occurred most frequently from 18.00 to 20.00 hours in both control and treated cows. Least onsets of oestrus occurred between 02.00 and 04.00 hours at night and between 14.00 and 16.00 hours during the day. Onsets of oestrus at night were 52% in the control group, 54% in the treated cows and 53% overall as shown in table 1

Table 1: Distribution of onset of oestrus in the control and treated cows.

TREATMENT GROUP	ONSET OF OESTRUS		TOTAL
	NIGHT	DAY	
CONTROL (N)	97	88	185
%	52%	48%	
TREATED (N)	38	32	70
%	54%	46%	
TOTAL (N)	135	120	255
%	53%	47%	

Chi – squared comparison showed no significant association (P>0.5) between onset of oestrus at night and day in the control and treated cows. Therefore, under this study onset of oestrus occurred more frequently during night than day. Overall results for observation of behavioural signs indicated that this pattern had failed to detect 18% of the cows in standing oestrus. 91% of the cows were observed mounting other cows, and only 9% did not contribute to the mounting activity (table 2). Mucus was noticed in only 43% of the cows, and this indicates that a large proportion of the cows would have been missed if this sign alone were used for the detection of oestrus.

Table 2: Distribution of behavioural sign of oestrus.

TREATMENT GROUP	BEHAVIOURAL SIGNS OF OESTRUS					
	STANDING		MOUNTING		MUCUS	
	YES	NO	YES	NO	YES	NO
CONTROL	185	27	192	20	87	125
TREATED	70	28	91	7	47	51
TOTAL	255	55	283	27	134	176
%	82	18	91	9	43	57

4.2 Mounting Activity: The time when a mount or attempted mount was initiated by a cow was recorded as the first display of mounting activity. Peaks of mounting activity can be distinguished in the daily frequency distribution shown in figure 1.2. Mounting activity had an estimated peak between 18.00 and 20.00 hours in the treated cows, while the peaking of mounting activity was between 16.00 and 18.00 hours in the control group of cows. This indicates that the treated cows needed a very short period of mounting before standing to be mounted by herd mates because mounting activity and onset of oestrus occurred within the same time interval.

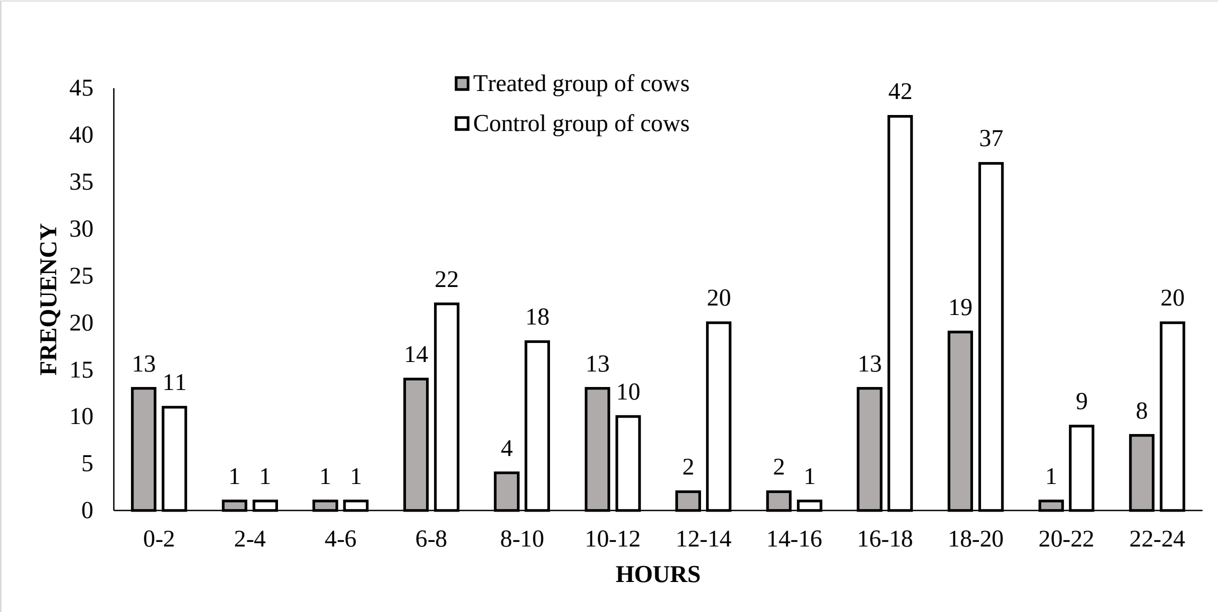

Fig. 2: Frequency of Distribution of Mounting Activity during an Oestrus Day (24 h).

Onsets of mounting activity during the day were 59% in the control group of cow, 53% in the treated cows, and 57% overall as shown in table 3. Chi–squared comparison showed no significant association (P>0.25) between onset of mounting activity at night and day in the control and treated cows. Therefore, onset of mounting activity occurred more frequently during the day.

Table 3: Distribution of onset of mounting activity in the control and treated cows.

TREATMENT GROUP		MOUNTING ACTIVITY		TOTAL
		NIGHT	DAY	
CONTROL	(N)	79	113	192
	%	41%	59%	
TREATED	(N)	43	48	91
	%	47%	53%	
TOTAL	(N)	122	161	283
	%	43%	57%	

4.3 Mucus Discharge: The time when a clear mucus discharge from the vagina was first noticed was recorded as the time of onset of mucus discharge. Onsets of mucus discharge during the day were 57% in the control group of cows 53% in the treated cows and 56% overall as shown in table 4. Chi–squared comparison showed no significant association (P>0.5) between onset of mucus discharge at night and day in the control and treated cows. Therefore, this study confirms the occurrence of mucus discharge at daytime than night.

Table 4: Distribution of onset of mucus discharge in the control and treated cows.

TREATMENT GROUP	MUCUS DISCHARGE		TOTAL
	NIGHT	DAY	
CONTROL (N)	37	50	87
%	43%	57%	
TREATED (N)	22	25	47
%	47%	53%	
TOTAL (N)	59	75	134
%	44%	56%	

5 Factors influencing the time of onset of oestrus and behavioural signs of oestrus.

5.1 Factors influencing the time of oestrus onset: Onsets of oestrus at night in winter were 43% in the control group of cows, 41% in the treated cows, and 43% overall (table 5). Onsets of oestrus at night in the hot dry summer were 55% in the control group of cows, 59% in the treated cows, and 56% overall. Onsets of oestrus at night in the hot wet summer were 62% in the control group of cows, 67% in the treated cows, and 63% overall. Chi – squared comparison for each of the subsets and all the subsets together showed no significant association (P>0.5) between onset of oestrus at night and day during the three seasons. Therefore, under this study onset of oestrus occurred more frequently during the night than day in summer.

Table 5: Influence of season on the time of onset of oestrus in Holstein – Friesian cows.

Season	Treatment Group	Time of onset of oestrus		Total	% onset of oestrus at Night	X^2 (I d.f.)
		Night	Day			
Winter	Control	29	38	67	43%	
	Treated	12	17	29	41%	
	Total	41	55	96	43%	0.030
Hot dry summer	Control	39	32	71	55%	
	Treated	10	7	17	59%	
	Total	49	39	88	56%	0.084
Hot wet summer	Control	29	18	47	62%	
	Treated	16	8	24	67%	
	Total	45	26	71	63%	0.169

Onsets of oestrus at night in cows of parities one and two were 44% in the control group of cow, 51% in the treated cows and 46% overall (table 6). Chi – squared comparison showed no significant association (P>0.25) between onset of oestrus at night and day in cows of parities one and two. Onsets of oestrus at night in cows of parities three and over were 63% in the control group of cows, 58% in the treated cows and 62% overall. Chi – squared comparison showed no significant association (P>0.5) between onsets of oestrus at night and day in cows of parities three and over. Chi – squared comparison combining the two subsets showed no significant association (P>0.5) between onsets of oestrus at night in cows of different parities. Therefore, under this study, there existed a difference in the time of onset of oestrus in cows of different parities.

Table 6: Influence of parity on the time of onset of oestrus in Holstein – Friesian cows.

Parity	Treatment Group	Time of onset of oestrus		Total	% onset of oestrus at night	X^2 (1.d.f.)
		Night	Day			
1 and 2	Control	47	59	106	44%	
	Treated	19	18	37	51%	
	Total	66	77	143	46%	0.543
3 and over	Control	50	29	79	63%	
	Treated	19	14	33	58%	
	Total	69	43	112	62%	0.321

Onsets of oestrus at night in cows at 41 – 60 days postpartum were 58% in the control group of cows, 50% in the treated cows and 55% overall (table 7). Chi – squared comparison showed no significant association (P>0.5) between onsets of oestrus at night and day. Onsets of oestrus at night in cows at 61 – 80 days postpartum were 68% in the control group of cows, 55% in the treated cows, and 62% overall. Chi – squared comparison showed no significant association (P>0.25) between onsets of oestrus at night and day. Onsets of oestrus at night in cows at 81 – 100 days postpartum were 38% in the control group of cows 50% in the treated cows, and 42% overall. Chi –squared comparison showed no significant association (P>0.5) between onsets of oestrus at night and day. Onsets of oestrus at night in cows at 101 and over day postpartum were 51% in the control group of cows, 60% in the treated cows, and 52% overall. Chi – squared comparison showed no significant association (P>0.5) between onsets of oestrus at night and day. Chi – squared comparison combing the four subsets also showed no significant association (P>0.5) between onsets of oestrus at night and day. Therefore, this study showed a variation in the time of onset of oestrus at different days postpartum.

Table 7: Influence of days postpartum on the time of onset of oestrus in Holstein – Friesian cows.

Days postpartum	Treatment group	Time of onset of oestrus	Total	% onset of oestrus at night	X^2 (1.d.f.)	
		Night	Day			
41 – 60	Control	15	11	26	58%	
	Treated	8	8	16	50%	
	Total	23	19	42	55%	0.237
61 – 80	Control	17	8	25	68%	
	Treated	11	9	20	55%	
	Total	28	17	45	62%	0.799
81 – 100	Control	10	16	26	38%	
	Treated	7	7	14	50%	
	Total	17	23	40	42%	0.496
101 and over	Control	55	53	108	51%	
	Treated	12	8	20	60%	
	Total	67	61	128	52%	0.557

5.2 Factors influencing the onset mounting activity: Onset of mounting activity during night in winter were 32% in the control group of cows, 39% in the treated cows and 35% overall (table 8). Chi – squared comparison showed no significant association (P>0.25) between onsets of mounting activity at night and day. Onsets of mounting activity at night in the hot dry summer were 38% in the control group of cows, 63% in the treated cows and 43% overall. Chi- squared comparison showed a significant association (P>0.05) between onsets of mounting activity at night and day. Onsets of mounting activity at night in the hot wet summer were 58% in the control of cows, 48% in the treated cows, and 54% overall. Chi – squared comparison showed no significant association (P>0.5) between onsets of mounting activity at night and day. Chi –squared comparison combining the seasons showed no significant association (P>0.25) between onsets of mounting activity at night and day. Therefore, this study showed a variation in the time of onset of mounting activity in the three seasons.

Table 8: Influence of season on the time of onset of mounting activity in Holstein – Friesian cows.

Days postpartum	**Treatment group**	**Time of onset of mounting activity**		**Total**	**% onset of mounting activity at night**	**X^2 (1.d.f.)**
		Night	**Day**			
Winter	Control	21	45	66	32%	
	Treated	16	25	41	39%	
	Total	37	70	107	33%	0.581
Hot dry summer	Control	29	47	76	38%	
	Treated	12	7	19	63%	
	Total	41	54	95	43%	3.873
Hot wet summer	Control	29	21	50	58%	
	Treated	15	16	31	48%	
	Total	44	37	81	54%	0.713

Onsets of mounting activity at night in cows of parities 1 and 2 were 41% in the control group of cows, 50% in the treated cows and 43% of overall (table 9). Chi – squared comparison showed no significant association (P>0.25) between onset of mounting activity at night and day. Onsets of mounting activity at night in cows of parities 3 and over were 42% in the control group of cows, 45% in the treated cows and 43% overall. Chi-squared comparison showed no significant association (P>0.5) between onset of mounting activity at night and day. Chi -squared comparison combining the two parity groups showed no significant association (P>0.25) between onsets of mounting activity at night and day. Therefore, onsets of mounting activity were associated with day light hours in both parity groups.

Table 9. Influence of parity on the time of onset of mounting activity in Holstein – Friesian cows.

Parity	**Treatment Group**	**Time of onset of mounting activity**		**Total**	**% onset of mounting activity at night**	**X^2 (1.d.f.)**
		Night	**Day**			
1 and 2	Control	45	66	111	41%	
	Treated	22	22	44	50%	
	Total	67	88	155	43%	1.149
3 and over	Control	34	47	81	42%	
	Treated	21	26	47	45%	
	Total	55	73	128	43%	0.089

Onsets of mounting activity at night in cows at 41-60 days postpartum were 52% in the control group of cows, 55% in the treated cows, and 53% overall (table 10). Chi-squared comparison showed no significant association (P>0.5) between onsets of mounting activity at night and day. Onsets of mounting activity at night in cows at 61 – 80 days postpartum were 64% in the control group of cows, 32% in the treated cows, and 47% overall. Chi –squared comparison showed a significant association (P>0.01) between onset of mounting activity at night and day. Onsets of mounting activity at night in cows at 81 – 100 days postpartum were 41% in the control group of cows, 53% in the treated cows and 46% overall. Chi – squared comparison showed no significant association (P>0.25) between onset of mounting activity at night and day. Onsets of mounting activity at night in cows at 101 and over days postpartum were 33% in the control group of cows, 55% in the treated cows, and 37% overall. Chi – squared comparison showed no significant association (P>0.05) between onset of mounting activity at night and day. Therefore, this study showed a variation in the time of mounting activity at different days postpartum.

Table 10. Influence of days postpartum on the time of onset of mounting activity.

Days postpartum	Treatment group	Time of onset of mounting activity		Total	% onset of mounting activity at night	X^2 (1.d.f.)
		Night	Day			
41 – 60	Control	14	13	27	52%	
	Treated	12	10	22	55%	
	Total	26	23	49	53%	0.035
61 – 80	Control	16	9	25	64%	
	Treated	9	19	28	32%	
	Total	25	28	53	47%	5.379
81 – 100	Control	12	17	29	41%	
	Treated	10	9	19	53%	
	Total	22	26	48	46%	0.0585
101 and over	Control	37	74	111	33%	
	Treated	12	10	22	55%	
	Total	49	84	133	37%	3.541

5.3 Factors influencing the onset of mucus discharge : Onsets of mucus discharge at night in winter were 66% in the control group of cows, 42% in the treated cows and 56% overall (table 11). Chi–squared comparison showed no significant association (P>0.05) between onsets of mucus discharge at night and day. Onset of mucus discharge in summer were 24% in the control group of cows, 52% in the treated cows, and 33% overall. Chi – squared comparison showed a significant association (P>0.01) between onsets of mucus discharge at night and day. Chi – squared comparison combining the two subsets showed no significant association (P>0.5) between onsets of mucus discharge at night and day. Therefore, this study showed a variation in the onsets of mucus discharge between control and treated cows in winter and summer.

Table 11: Influence of season on the time of onset of mucus discharge in Holstein – Friesian cows.

Parity	Treatment Group	Time of onset of mucus discharge		Total	% onset of mucus discharge at night	X^2 (1.d.f.)
		Night	Day			
Winter	Control Treated	25 10	13 14	38 24	66% 42%	
	Total	35	27	62	56%	3.482
Summer	Control Treated	12 12	37 11	49 23	24% 52%	
	Total	24	48	72	33%	5.398

Onsets of mucus discharge at night in cows of parities one and two were 38% in the control group of cows, 48% in the treated cows, and 42% overall (table 12). Chi–squared comparison showed no significant association (P>0.25) between onsets of mucus discharge at night and day. Onsets of mucus discharge at night in cows of parities three and over were 49% in the control group of cows, 45% in the treated cows, and 47% overall. Chi – squared comparison showed no significant association (P>0.5) between onsets of mucus discharge at night and day. Chi –squared comparison combining the two subsets showed no significant association (P>0.5) between onsets of mucus discharge at night and day. Therefore, this study showed that onsets of mucus discharge were associated with daylight hours in cows of all parties.

Table 12: Influence of parity on the time of onset of mucus discharge in Holstein – Friesian cow.

Parity	Treatment Group	Time of onset of mucus discharge		Total	% onset of mucus discharge at night	X^2 (1.d.f.)
		Night	Day			
1 and 2	Control Treated	19 13	31 14	50 27	38% 48%	
	Total	32	45	77	42%	0.743
3 and over	Control Treated	18 9	19 11	37 20	49% 45%	
	Total	27	30	57	47%	0.069

Onsets of mucus discharge in cows at 41 – 80 days postpartum were 50% in the control group of cows, 40% in the treated cows, and 44% overall (table 13). Chi –squared comparison showed no significant association (P>0.5) between onsets of mucus discharge at night and day. Onsets of mucus discharge in cows at 81 and over days postpartum were 40% in the control group of cows, 50% in the treated cows, and 43% overall. Chi – squared comparison showed no significant association (P>0.25) between onset of mucus discharge at night and day. Chi –squared comparison combining the two subsets showed no significant association (P>0.5) between onsets of mucus discharge at night and day. Therefore, this study showed a variation in the time of onset of mucus discharge in the control and treated cows at different days postpartum.

Table 13: Influence of days postpartum on the time of mucus discharge in Holstein – Friesian cows.

Days	Treatment Group	Time of onset mucus discharge		Total	% onset of mucus discharge at night	X^2 (1.d.f.)
		Night	Day			
41 – 80	Control	10	10	20	50%	
	Treated	10	15	25	40%	
	Total	20	25	45	44%	0.450
81 and over	Control	27	40	67	40%	
	Treated	11	11	22	50%	
	Total	38	51	89	43%	0.637

6 DISCUSSION

In the present study, 53% of the oestrus onsets were associated with night hours, 18.00 to 06.00 hours (table 1). A pooled 2-hour interval taken from the distribution of oestrus onset over a 24 – hour day (fig 1) demonstrated the highest frequency between 18.00 and 20.00 hours. Similar observations were reported by Van Vliet and Van Eerdenburg., 1996; Pinheiro *et al.*, 1998; Quezada Casasala, A., 2015. Other reports claimed onset of oestrus during the day (Cardozo *et al.,* 1994*;* Neguissie *et al.,* 2002*;* Lovendahl and Chagunda*.,* 2010*;* Kwang-Soo *et al.*, 2013). Other workers failed to find a relationship between time of true oestrus onset and time of the day (Dransfield., 1998; Xu *et al.*, 1998). The percentage of onsets of mounting activity during the day was 57% in both treated and control group of cows (table 2). Onset of mounting activity had an estimated peak between 18.00 and 20.00 hours in the treated cows, and between 16.00 and 18.00 hours in the control group of cows (fig 2). Similar observations were reported by Redden *et al.*, 1993. Conversely, Nebel *et al.*, 2000 noticed that total mounting activity did not parallel the hourly distribution of osterus onsets, but was more evenly distributed across the day despite a trend toward more mounting activities in the afternoon. Mucus was observed in 43% of the cows and 56% of the onsets of mucus discharge were during the day. Conversely, Madkar *et al.*, 2015 reported 100% of the cows that had mucus discharge with 83.34% intense degree of expression, but failed to indicate the time of onset of mucus discharge. Unalan., 2016 reported high percentage (48.4%) of mucus discharge during oestrus in winter and low percentage (6.0%) in Summer. The factors that influence the onset of oestrus and behavioural signs of oestrus in a tropical environment are of a complex nature, but high ambient temperatures, management conditions, and managerial factors may be the predominant ones. In the present study, 43, 56 and 63% of oestrus onsets occurred at night during the winter, hot dry summer and hot wet summer respectively. These results showed that high environmental temperatures during the day in summer might have depressed the onset of oestrus. Our findings agree with those of Hussian *et al.,* 1992; Jordan., 2003; Sonmez *et al.*, 2005; Bulbul and Ataman., 2009. These workers reported reduced intensity of oestrus, reduced oestrus length and anoestrus in lactating cows during the hot daytime hours in summer. They found that the peripheral heat receptors on the skin acted protectively to inhibit behavioural activities which increased body heat production. Parity appeared to be important in this investigation too. From the results it must be concluded that 46% of the cows in parities 1 and 2 showed onset of oestrus at night compared to 62% of the cows in parities 3 and over. Our findings concur with those of Rocha *et al.*, 2001 who noticed increased oestrus detection rates in cows of parities 3 and 4 (54.7% and 60.5%) compared to cows of parities 1 and 2 (47.5% and 50.6%). These workers failed to indicate the time of the day when cows of parities 1,2,3 and over showed onset of oestrus.

In the present investigation, onset of oestrus occurred mostly at night in cows at 41-60, 61-80, and 101 and over days postpartum compared to cows at 81-100 days postpartum which showed onset of oestrus at daytime. No information to justify these results was obtained in the literature, but Elmetwally, *et al.,* 2016 found that parity numbers, season and month have significant effects on postpartum fertility parameters. With regards to factors which influence the onset of mounting activity and mucus discharge, season of the year, parity of the cow, and days postpartum have been studied by a few investigators. Most of them found the effects of season, but no effects of parity or days postpartum were reported. The results of the present work point to a distinct influence of season. In winter and hot dry summer low onset of mounting activity took place at night, while in the hot wet summer the highest onsets of mounting activity were at night. In winter the highest onsets of mucus discharge were at night compared to onsets of mucus discharge in summer. Hot daytime heat in summer was reported to reduce oestrous behaviour in lactating cows (Jordan, 2003; Sonmez *et al.,* 2005; and Unalan, 2015). At the same time the investigation showed that onset of mounting activity and mucus discharge were lowest at night in cows of parities 1and 2, and 3 and over. Melendez *et al*, 2008 reported no effect of parity on the onset of mounting activity or mucus discharge, but they noticed the similarities in the heat detection rate among parities. Onset of mounting activity was found to be highest at night in cows at 41-60 days postpartum compared to those at 61-80, 81-100 and 101 and over days postpartum. Onset of mucus discharge was lowest at night in cows at 41 and over days postpartum. Melendez *et al*, 2008 found no effect of days postpartum on the time of mounting activity or mucus discharge, but they found oestrus detection risk to be lower for cows at 50-70 days postpartum than those of 71-91 and 92-112 days postpartum. The present study confirms standing as the most important visual signs of oestrus, but showed the importance of the time of observation on the detection rate of oestrus by this sign. The frequent occurrence of the onset of oestrus between 18.00 and 06.00 hours suggestions that direct observation during this period or the use of an efficacious aid to detection of oestrus is warranted. The results presented here are the production of a one-year period of observation in a single herd of cows. It is known that many factors are likely to affect the onset of oestrus and other overt signs. Clearly, the authors do not know the extent to which these results and conclusions can be generalized to other herds with other management routines and systems. It would seem from the positive benefit likely to accrue from such observations, however, that a more widespread study of the organization of oestrous behaviour within herds should be worthwhile.

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