

## DIURNAL AND SEASONAL VARIATIONS OF OESTROUS BEHAVIOUR IN EGYPTIAN BUFFALOES

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### ABSTRACT

Thirty five cyclic buffalo cows (20 in the cold season and 15 in the hot season) 3-9 years in age and 1-7 in parity were used in this study. Oestrous behaviour was monitored 24 hrs per day, using a closed TV circuit and night illumination, beside direct visual observations. Teaser bull was absent in the first half and present with buffalo cows in the second half of each season. Blood samples were withdrawn from each animal twice a week, for plasma progesterone determination by radioimmunoassay in order to detect false oestrus, ovulation and quiet ovulation. The most frequent sign of oestrous behaviour was restlessness (87.90%), followed by walking along side the wall (81.45%) and bellowing (78.23%). The least frequent sign was vulvar swelling (6.29%), followed by boating and vaginal mucus discharge (17.74% for each). Signs of the mutual behaviour with male recorded frequency higher than those of self oestrous behaviour and ranged from 83.94 to 87.27%.

About half the number of oestrus signs commenced and terminated within 6-14 hrs. A moderate intensity of oestrus (exhibiting 7-14 signs) represented 67.83% of total oestrus cases, while strong or weak oestrus represented only 15-17% of total oestrus cases. Incidence of oestrous behaviour increased in the cold season during day and night / night period, while that in the hot season increased during night and night / day period, indicating that season may modify diurnal variation of the oestrous behaviour in buffaloes. Percentages of quiet ovulation and false oestrus were 1.25 and 14.13 in the cold season versus 3.70 and 10.34 in the hot season, respectively. Mean length of oestrus period was 11.18 hrs, while the estrous cycle length was 24.32 days, and both traits were not significantly influenced by season.

**Keywords:** Buffalo, oestrous behaviour, seasonal variation.

### INTRODUCTION

Oestrus detection in buffaloes has always been difficult as compared to cattle (Kanai and Shimizu, 1983; Aboul-Ela, 1988; Hafez, 1993). High frequency of quiet ovulation is considered one of the main reproductive problems in buffaloes, particularly during the post-partum period, resulting in long days open and consequently prolonged calving intervals (Abdalla, 2002).

Oestrus signs are characteristic for animal species, but there are individual, seasonal and diurnal variations occur in oestrus manifestations (Fraser, 1980). However, seasonal and diurnal variations of oestrous behaviour in buffaloes were not adequately studied. The present study was carried out to investigate the oestrous behaviour in Egyptian buffalo cows under intensive daily observation. The influences of year season on frequency, intensity and diurnal variation of the different oestrus signs were investigated. In addition, length of estrous cycle and oestrus period were also investigated.

## MATERIALS AND METHODS

This study was carried out at Mehallet Mousa Buffalo Experimental Station, Kafr El-Sheikh Governorate, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. Thirty five post-partum cyclic buffalo cows, 3-9 years in age and between the 1<sup>st</sup> and the 7<sup>th</sup> parity, were used in this study. Buffaloes were housed loosely in open shady yards under the ordinary herd management of the farm. Animals were hand milked twice daily during the lactation period. They were fed a concentrate mixture according to their live body weight and milk production. During the cold season (Nov. to Apr.), animals were fed *ad libitum* Egyptian berseem (*Trifolium alexandrinum*) along with rice straw and concentrate mixture, while during the hot season (May to Oct.) berseem hay was offered, beside the other roughage and concentrate rations. Average minimum and maximum ambient temperature were 6.7 and 21.2°C in the cold season, and 18.1 and 32.1°C in the hot season, respectively. Corresponding values for mean relative humidity were 41.9 and 72.5%, and 42.1 and 82.5%.

Animals were divided randomly into two groups: cold-season group (n=20) and hot-season group (n=15). A teaser bull (vasectomized bull) was absent in the first half and present in the second half of each season to detect the mutual behaviour during oestrus with male. Buffaloes in each season were observed for 24 hours daily to record all oestrous behaviour signs, using a closed television circuit beside visual observations. A closed TV circuit was consisted of 21 inch color TV, and constant and mobile video cameras provided with zoom and focus controlled lenses and can operate in partial darkness and bright light conditions. Time lapse video - cassette recorder was continuously used for up to 24 hours daily, and an ordinary video cassette recorder was used as displayer. All buffaloes were numbered using a white paint on both sides on the shoulder and rump to facilitate the identification with the aid of night illumination (150 lux) of the open yard.

Quiet ovulation and false oestrus were detected according to the visual observation of oestrus signs and the changes in plasma progesterone concentration. Ovulation time was determined as the day of oestrus, which was followed by an increase in plasma progesterone level to more than 1.0 ng/ml and remained at this level for at least two consecutive samples (Abdalla, 2002). For progesterone determination, blood samples were withdrawn from jugular vein by a venepuncture technique twice a week at 3 to 4 days intervals. Blood plasma was separated and kept frozen at -20°C until the hormone determination by radio-immunoassay technique.

The recorded signs of oestrous behaviour were classified into three groups :

- 1- female self behaviour

- 2- mutual behaviour among females (homosexual behaviour)

- 3- mutual behaviour with teaser bull. Intensity of oestrus was evaluated according to the number of oestrus signs, being strong (>14), moderate (7-14) and weak (<7 signs). Interval lasted between two successive true oestruses represented estrous cycle length, while oestrus period was

determined as the duration between the beginning and the termination of standing behaviour. Length of estrous cycle and oestrus period were statistically analyzed using SAS program (1990).

## RESULTS AND DISCUSSION

### 1- Oestrous behaviour signs

Frequency distribution (%) and duration (hrs) of different oestrous behaviour signs are presented in Table 1:

#### a. Self oestrous behaviour signs

The highest percentage (87.90%) was recorded for restlessness, followed by walking along side the wall (81.45%) and bellowing (78.23%), while the lowest (6.29%) was detected for vulvar swelling, followed by boating and vaginal mucus discharge (17.74 for each). Percentage of restlessness in buffaloes was found to be 55% (El-Wardani, 1990) and 59% (Aboul-Ela, 1993), while percentage of bellowing was found to be 80% (Singh *et al.*, 1984) and 70% (Barkawi *et al.*, 1993). Aboul-Ela (1988) reported that 98% of small farmers in eight villages of the Nile Delta region use frequent bellowing as an indicator for the onset of oestrus in buffaloes, and it is usually accompanied by restlessness. However, bellowing as a sign of oestrus may be less in buffalo heifers than in buffalo cows (Rao and Kodagali, 1983; Nemat Ullah and Usmani, 1985).

In accordance with the present results, Singh *et al.* (1984) recorded 83.69% of Murrah buffalo heifers showing wall walking (segregation) during oestrus. This phenomenon may indicate restlessness and nerviness during oestrus in buffaloes. The least frequent signs of oestrous behaviour were vulvar swelling, boating and vaginal mucus discharge. In agreement, Gill *et al.* (1973) reported vaginal mucus discharge in only 16.9% of a total of 162 oestrus periods in Murrah buffaloes. While, Barkawi *et al.* (1993) reported 30% of oestrus periods displayed mucus discharge in Egyptian buffaloes. Hafez (1954) showed that the vaginal secretions in buffalo cows were very scanty at the different phases of oestrus, but were recorded during metoestrus and prior to the onset of oestrus.

As reported in the literature, self oestrous behaviour in farm animals varies among species, breeds and individuals within breed. For example, in buffaloes there is a considerable difference in oestrous behaviour signs among Indian, Chinese and Egyptian buffaloes. Moreover, there are great differences in the pattern of oestrous behaviour according to system of management (Abdalla, 2002), method and intensity of heat detection, physiological status of animals (heifers or cows) and climatic conditions (Bearden and Fuquay, 1992).

#### b. Female homosexual behaviour signs

More frequent homosexual behaviour signs were sniffing vulva and following behaviour, while the least frequent was mounting behaviour (Table 1). Inconsistent results were obtained by El-Wardani (1990), who showed that percentage of oestrus periods in which oestral buffalo cows sniffed vulva of

other cows was 20%, while the reverse condition represented 30%. Homosexual behaviour in buffalo cows was also observed through mounting behaviour, but it recorded a lower percentage. Comparable results were obtained earlier by Randhawa (1980) and Nemat Ullah and Usmani (1985) who reported a mutual mounting incidence about 30%. El-Wardani (1990) and Barkawi *et al.* (1993) have reported that incidence of mutual mounting in Egyptian buffalo cows was unreliable sign of oestrus, which recorded only 5 to 10% in their studies.

**Table (1): Seasonal and total frequency and duration of oestrous behaviour signs of cyclic buffalo cows**

Oestrous behaviour signs	Frequency (%) <sup>*</sup>			Duration <sup>**</sup> (hrs)
	Cold season	Hot season	Total	
<b>a. Self oestrous behaviour</b>				
1- Bellowing	86.67	65.31	78.23	15.37±1.54
2- Tail raising	62.67	32.65	50.81	10.81±0.13
3- Restlessness	85.33	91.84	87.90	18.27±1.44
4- Isolation	69.33	77.55	72.58	9.02±1.20
5- Walking along side the wall	78.67	87.76	81.45	18.52±1.58
6- Boating	18.67	16.33	17.74	2.00±0.32
7- Frequent urination	70.67	42.86	59.68	7.11±1.50
8- Vaginal mucus discharge	24.00	8.16	17.74	2.68±1.05
9- Vulvar swelling	33.33	40.82	6.29	3.67±0.20
10- Vulvar hyperaemia (red vulva)	36.00	40.82	37.90	3.53±0.18
11- Spontaneous let-down of milk	26.67	6.12	18.55	1.30±0.30
<b>b. Mutual behaviour among females</b>				
12- Sniffing vulva by oestrous female	58.67	63.27	60.48	11.29±1.77
13- Sniffing vulva of oestrous female by others	66.67	67.35	66.94	7.83±1.51
14- Following other females	64.00	44.90	56.45	5.97±1.08
15- Following of oestrous female by others	56.00	38.78	53.23	6.79±1.26
16- Mounting others	28.00	32.65	30.65	7.97±2.55
17- Mounting of oestrous female by others	30.67	30.61	30.65	3.53±1.27
<b>c. Mutual behaviour with bull</b>				
18- Sniffing vulva by the bull	78.79	95.45	85.45	17.55±2.30
19- Following the female by the bull	81.82	95.45	87.27	20.17±2.21
20- Mounting the female by the bull	81.82	100.0	87.27	12.52±1.66
21- Standing behaviour	75.76	95.45	83.94	11.18±1.14

\* Frequency of oestrus signs appearance as a percentage of total oestrus periods.

\*\* Mean ±SE for the duration lasted from the beginning to the termination of each sign during the oestrus period.

**c. Mutual behaviour with male**

Percentages of mutual behaviour signs with male (Table 1) ranged between 83.94 and 87.27%, which were higher than those of the other oestral signs, except for restlessness. El-Wardani (1990) and Barkawi *et al.* (1993) reported that percentage of male mounting signs ranged between 90 and 100% and found that standing behaviour was the most frequent sign of oestrus, but signs of oestrus started to appear 1-2 days before the onset of standing behaviour in Egyptian buffaloes. It could be concluded from the present study that restlessness, bellowing, standing behaviour and other mutual behaviour signs with male are considered the most reliable signs of oestrus in buffalo cows.

## 2- Duration of oestrous behaviour signs

Durations of oestrus signs (Table 1) were different and could be classified as follow : signs observed within long duration ( $\geq 15$  hrs), signs observed within moderate duration (6-14 hrs) and signs observed within short duration ( $< 6$  hrs). Data in Table 1 indicate that about half the number of oestrus signs (10 signs) occurred within moderate durations, while few number (5-6 signs) occurred within either long or short durations.

## 3- Intensity of oestrous behaviour

Present study indicated that among 115 cases of oestrus there were 18 (15.65%) with strong, 78 (67.83%) with moderate and 19 (16.52%) with weak intensity (Table 2). These results show that the majority of oestrus cases were moderate (7-14 signs) in intensity. Comparable results were obtained by El-Wardani (1990) in Egyptian buffalo cows. El-Sheikh and El-Fouly (1971) found that 19.35, 16.13 and 58.06% of oestrus cases were weak, intermediate and strong in oestrous behaviour intensity, respectively. These differences with the present results are due to the method of intensity evaluation. Those authors considered expression of oestrus was strong when it was accompanied by glairy vaginal discharge, mating desire and restlessness, while oestrus with only one of these three symptoms was considered weak.

**Table (2): Quiet ovulation, false and true oestrus, intensity of oestrous behaviour, and length of oestrus period and estrous cycle as influenced by the year season**

Reproductive parameters	Cold season	Hot season	Total
	% (n)	% (n)	% (n)
1. Ovulation			
a. Ovulatory oestrus	98.75 (79)	96.30 (52)	97.76 (131)
b. Quiet ovulation	1.25 ( 1)	3.70 ( 2)	2.24 ( 3)
2. Oestrus			
a. False oestrus	14.13 (13)	10.34 (6)	12.67 (19)
b. True oestrus	85.87 (79)	89.66 (52)	87.33 (131)
3. Intensity of oestrous behaviour			
a. Strong	19.40 (13)	10.42 (5)	15.65 (18)
b. Moderate	68.66 (46)	66.67 (32)	67.83 (78)
c. Weak	11.94 ( 8)	22.91 (11)	16.52 (19)
	$\bar{X} \pm SE$	$\bar{X} \pm SE$	$\bar{X} \pm SE$
4. Oestrus period length (hrs)	11.38 $\pm$ 1.44	10.94 $\pm$ 1.88	11.18 $\pm$ 1.41
5. Estrous cycle length (days)			
a. < 20 days	18.00 $\pm$ 0.00	18.75 $\pm$ 0.25	18.60 $\pm$ 0.24
b. 20-24 days	22.17 $\pm$ 0.19	21.89 $\pm$ 0.76	21.91 $\pm$ 0.15
c. > 24 days	30.36 $\pm$ 0.82	28.67 $\pm$ 0.53	29.91 $\pm$ 0.83
d. Total	24.77 $\pm$ 0.61	23.06 $\pm$ 0.83	24.32 $\pm$ 0.47

n = Number of oestrus periods.

## 4- Quiet ovulation and false oestrus

Present results showed that percentage of quiet ovulation was 2.24% as detected from 134 ovulations, while that of false oestrus was 12.67% as detected from 150 oestrus cases (Table 2). The percentage of quiet ovulation

is much lower than that reported by El-Sheikh and El-Fouly (1971), Khattab *et al.* (1988), Barkawi *et al.* (1998) and El-Wardani and El-Asheeri (2000). High frequency of quiet ovulation is considered one of the main reproductive problems in buffaloes, particularly during the post-partum period (Mohamed and El-Sheikh, 1983; Abdalla, 2002). Small percentage of quiet ovulation in this study is mainly attributed to the continuous and careful observation of the oestrus signs using a closed TV circuit beside visual observations.

#### **5- Oestrus period length**

Mean length of oestrus period was found to be 11.18 hrs (Table 2), ranging between 3 and 35 hrs, as determined from 38 oestrus periods, depending on the standing behaviour. About 63% of the total periods ranged from 6-23 hrs in length. Average length of oestrus period in Egyptian buffaloes ranged from 11 to 24 hrs (Barkawi, 1981; Shafie *et al.*, 1982; Hamam, 1987 and Tarek, 1989). Barkawi (1981) reported that about 75% of oestrus periods in Egyptian buffaloes ranged between 10 and 20 hrs in length. In agreement with the present results, El-Wardani (1995) reported that average length of oestrus period was 11.6 with range 6-24 hrs in Egyptian buffalo cows. Differences in the length of oestrus period may be due to the differences in oestrus signs have been used by different authors to measure that trait.

#### **6- Estrous cycle length**

Average length of 106 estrous cycles was  $24.32 \pm 0.47$  days (Table 2), with a range from 18 to 46 days. Cycles were classified into short (18-19 days), medium (20-24 days) and long (25-46 days), with frequency distribution of 4.7, 63.2 and 32.1%, respectively. Mean length of estrous cycle was 18.6, 21.91 and 29.91 days for short, medium and long cycles, respectively. In accordance with the present results, mean length of estrous cycle in Egyptian buffaloes was reported to be 21.14 days (Ibraheim, 1991), 24.70 days (Youssef, 1992) and 23.78 days (Hashem, 1996).

#### **7- Diurnal variation of oestrous behaviour**

Intensive observation of oestrus (24 hrs / day) revealed that out of 124 oestrus cases, 12 (9.68%) occurred during day, 14 (11.29%) during night, 52 (41.93%) commenced during day and terminated during night, and 46 (37.1%) commenced during night and terminated during day (Table 3). These results clearly show that about 90% of oestrus periods fell totally or partially in the night, which is defined as the duration from sunset to sunrise. Previously, Hafez (1954) showed in Egyptian buffalo cows that the onset of oestrous behaviour took place from 6.00 pm to 6.00 am in 84% of oestrus cases. El-Wardani and El-Asheeri (2000) revealed that oestrus incidence showed two peaks, the first in the early morning (3.00 am to 9.00 am) and the second in the evening (3.00 pm to 9.00 pm), while the lower percentage (12%) started during the period from 9.00 am to 3.00 pm.

**Table (3): Diurnal variation of oestrous behaviour in buffalo cows as influenced by year season**

Time of oestrus period	Cold season		Hot season		Total	
	%	n	%	n	%	n
Day	14.66	11	2.03	1	9.68	12
Night	9.34	7	14.29	7	11.29	14
Day / Night	44.00	33	38.78	19	41.93	52
Night / day	32.00	24	44.90	22	37.10	46

Day : Duration from sunrise to sunset.  
 Night : Duration from sunset to sunrise.  
 Day / Night : Oestrus commenced in day and terminated in night.  
 Night / Day : Oestrus commenced in night and terminated in day.  
 n : Number of oestrus periods.

### 8- Seasonal variation of oestrous behaviour

As shown in Table 1, all signs of self oestrous behaviour were observed in the two seasons, however some of them tended to increase in the cold season particularly bellowing, tail raising, frequent urination, vaginal mucus discharge and spontaneous let-down of milk. The other signs of self oestrous behaviour and female homosexual behaviour as well were approximately similar in both seasons. In accordance with the present results, Barkawi *et al.* (1993) reported that behavioural signs of oestrus in Egyptian buffalo cows were more pronounced during the cold season than in the hot season. It is worth to note that mutual behaviour with a teaser bull was more frequent in the hot season, this may indicate that teaser bull was sexually more active during the moderate hot season. Beg and Totey (1999) concluded that degree of expression of oestrus are affected by various factors, such as climate, photoperiod, temperature and nutrition.

Percentage of oestrus cases with strong intensity increased in the cold season as compared to the hot season (19.40 vs 10.42%), while that with weak intensity was higher in the hot season than in the cold season (22.91 vs 11.94%) (Table 2). This shows that high ambient temperature and relative humidity during the hot season may weaken the expression of oestrous behaviour. These results were in agreement with those reported by Shafie *et al.* (1982) and El-Wardani (1990) in Egyptian buffaloes. Table 3 also revealed that season may influence the diurnal variation of oestrous behaviour, since its incidence increased during day and day / night in the cold season and during night and night / day in the hot season. This indicates that animals express their oestrous behaviour during the time of day at which ambient temperature is relatively more convenient. Similar results were obtained by El-Wardani (1995) and El-Wardani and El-Asheeri (2000).

Statistical analysis indicated that season had no significant effect on the length of oestrus period, which tended to be shorter in the hot than in the cold season (10.94 vs 11.38 hrs). Similar results were obtained for the estrous cycle length (Table 2). Similarly, Gill and Rurki (1985) showed that season did not affect the length of oestrus period in water buffaloes, while Gill *et al.* (1973) showed that high ambient temperature shortened oestrus period in buffaloes. However, Hafez (1954), Salama *et al.* (1967) and Barkawi

(1981) reported that the hot season was associated with longer estrous cycle in Egyptian buffaloes. In the present study, estrous cycle length tended to decrease in the hot season (23.06 vs 24.77 days) and this may be due to the early regression of corpora lutea, probably due to a decrease in the luteotrophic hormone secretion. Similarly in cattle, Wolfenson *et al.* (1988) showed that heat stress shortened estrous cycle length as indicated by the length of the luteal phase.

In conclusion, oestrous behavioural activity in Egyptian buffaloes is nonseasonal in nature, since it was observed throughout the year. However, frequency, intensity and diurnal variation of oestrous behaviour may be influenced by the year season. Restlessness, bellowing and the mutual behaviour with male could be considered as the most reliable symptoms for oestrus detection in buffalo cows. In addition, this study also indicates that most animals show oestrous behaviour around sunset and sunrise. Female homosexual behaviour is also evident in buffaloes, but it was detected with less frequency. Continuous observation of oestrous behaviour using a closed TV circuit and visual observations with the presence of teaser bull could facilitate oestrus detection and overcome the problem of quiet ovulation in buffaloes.

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**التغيرات اليومية والموسمية للسلوك الشبقي في الجاموس المصري**  
**فاروق عبد الله خليل<sup>١</sup> ، عصمت بكرى عبدالله<sup>١</sup> ، محمد عبد العزيز الوردانى<sup>٢</sup> ، منى حسن محمد<sup>٢</sup>**

- 1- قسم الإنتاج الحيوانى - كلية الزراعة - جامعة عين شمس - القاهرة - مصر**  
**2- معهد بحوث الإنتاج الحيوانى - الجزيرة - مصر**

استخدم في هذه الدراسة خمسة وثلاثون جاموسة منتظمة الشبق (٢٠ في الموسم البارد و١٥ في الموسم الحار) عمر ٣-٩ سنوات وتقع في موسم الولادة الأول إلى السابع. تم ملاحظة السلوك الشبقي خلال ٢٤ ساعة يومياً باستخدام الدائرة التليفزيونية المغلقة والإضاءة الليلية ، بمساعدة الملاحظات البصرية المباشرة ، وذلك مع غياب الطلقة الكاشف للشباع في النصف الأول ووجوده مع إناث الجاموس في النصف الثانى من كل موسم. أخذت عينات الدم من كل حيوان مرتين أسبوعياً لتقدير البروجستيرون في بلازما الدم باستخدام طريقة المناعة الإشعاعية بغرض تحديد الشباع الكاذب وموعد ونوع التبويض. كانت أكثر علامات الشبق تكراراً بين فترات الشبق هي ظاهرة القلق (٨٧,٩%) تبعها السير بجوار الحائط (٨١,٤٥%) والتنعير (٧٨,٢٣%). وكانت أقل العلامات تكراراً علامة الانتفاخ الشفري (٦,٢٩%) تليها ظاهرة تقعر الظهر وإفراز المخاط المهبلى (١٧,٧٤% لكل منهما). سجلت علامات السلوك التبادلى مع الذكر تكراراً أعلى من نظيره للسلوك الشبقي الذاتى للأنثى حيث تراوحت بين ٨٣,٩٤ و ٨٧,٢٧%.

بدأت نصف عدد علامات الشبق تقريباً وانتهى ظهورها علي الحيوان أثناء الشبق خلال ٦-١٤ ساعة. مثلت حالات الشبق المتوسطة الشدة (٧-١٤ علامة) ٨٣,٦٧% من حالات الشبق الكلية ، بينما مثلت حالات الشبق القوية أو الضعيفة الشدة ١٥-١٧%. في الموسم البارد زادت حالات الشبق التى بدأت وانتهت أثناء النهار أو بدأت أثناء النهار وانتهت أثناء الليل. بينما في الموسم الحار زادت حالات الشبق التى بدأت وانتهت أثناء الليل أو بدأت أثناء الليل وانتهت أثناء النهار ، مما يدل علي أن موسم السنة قد يغير من التباين اليومي للسلوك الشبقي. كانت نسب التبويض الهادئ والشبق الكاذب ١,٢٥ و ١٤,١٣ في الموسم البارد و ٣,٧ و ١٠,٣٤ في الموسم الحار ، علي التوالي. كان متوسط طول فترة الشبق ١١,١٨ ساعة ومتوسط طول دورة الشبق ٢٤,٣٢ يوماً ولم تتأثر كلا الصفتين معنوياً بموسم السنة. ١