RELATIONSHIP BETWEEN DETECTED OESTROUS SIGNS, PROGESTERONE PROFILE IN MILK FAT AND CONCEPTION IN EGYPTIAN BUFFALOES UNDER FIELD CONDITIONS

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ABSTRACT

Ovulation cases and oestrous cycles of ten Egyptian buffalo cows during 120 days postpartum were investigated to study the relationship between oestrous signs, milk fat progesterone (P_4) concentrations and conception. Buffalo cows were between the 1st and 5th parity and from 3 to 9 years old. One investigator routinely recorded the detected oestrus signs and buffalo cows were checked for heat four times daily by a teaser bull. Milk samples were individually collected every three days for P_4 assay (RIA) in milk fat, and P_4 was determined on days 6 and 3 before ovulation, day of ovulation and days 1, 3 and 6 after ovulation. Results revealed that overall concentrations of P_4 after each of the 1st, 2nd, 3rd and 4th ovulation were 18.2, 41.6, 69.3 and 99.5 ng/ml, respectively, the differences were significant. In ovulatory oestrous cases, frequency of oestrous signs was the highest in term of sniffing of vulva and mounting by bull (100%), followed by response to placing hand on rump (76.2%), bellowing and tail raising (66.7% for each), response to slight message of vulva lips (57.1%) and hyperemia of vulva mucous membrane and teat tight (47.6% for each). Teat tight as oestrous signs was more frequent (P<0.05) in ovulatory anoestrous (63.6%) than in ovulatory oestrous cases (47.6%). Overall concentration of P_4 did not differ significantly between ovulatory oestrous and ovulatory anoestrous cases. Intensity of oestrous signs including sniffing of vulva and mounting by bull, hyperemia of vulva mucous membrane and response to slight message of vulva lips increased (P<0.05) only between 1st and 2nd oestrus, while those involving bellowing, response to placing hand on rump, tail raising and teat tight increased (P<0.05) by increasing sequence of oestrus up to 3rd oestrus. Overall concentration of P_4 increased (P<0.05) by increasing sequence of oestrus, being 36.1, 54.6 and 104.4 ng/ml for the 1st, 2nd and 3rd oestrus, respectively. In ovulatory oestrus and served cases, intensity of all oestrous signs was higher (P<0.01) in cases ended with conception than those not conceived, except for sniffing of vulva and mount-

ing, which was more frequent (100%) in conceived and non conceived cases. Also, overall concentration of P_4 was higher (P<0.05) in conceived than nonconceived cases (82.2 vs. 27.1 ng/ml). All oestrous signs were significantly higher in cold than hot season, except for sniffing of vulva and mounting by bull, which was highly expressed (100%) in both seasons. Overall P_4 concentration did not differ significantly between both seasons. In light on the foregoing results, the current study may conclude that intensity of oestrous signs was affected by sequence of oestrus. Increasing intensity of detected oestrous signs may consider as good indicator for conception in buffalo cows. Also, cold season was characterized by higher intensity of oestrous signs than hot season. Generally, oestrous sign intensity may be associated with P_4 concentration, in particular on days prior to oestrus incidence. Consequently, this results indicated strong relationship between intensity of oestrous signs, P_4 concentration and conception.

Keywords: Egyptian buffaloes; oestrous signs; progesterone; conception.

INTRODUCTION

Oestrus signs are characteristic for animal species, but there are individual, seasonal and diurnal variations occur in oestrus manifestations **(Fraser, 1980)**. Behavioural signs of oestrus are not nearly as obvious in buffaloes as in the bovine **(El-Sheikh and El-Fouly, 1971; Harinadharao et al., 1979; Kamonpatana et al., 1979; Kanai and Shimizu, 1983; Rao, 1985 and Sarma, 1987)** and oestrus detection in buffaloes has always been difficult as compared to cattle **(Kanai and Shimizu, 1983; Aboul-Ela, 1988)**.

High frequency of quiet ovulation is considered one of the main reproductive problems in buffaloes, particularly during the postpartum period, resulting in long days open and consequently prolonged calving interval **(Abdalla, 2003)**.

Under practical farm conditions in Egypt, a considerable number of buffalo cows were kept in small herds (without bulls) and even in tie barns **(Mostageer et al., 1981; El-Khaschab et al., 1984 and El-Moghazy, 2003)**. In these buffalo cow herds, oestrous signs often remain undetectable because these cows are not able to display their typical breeding behaviour **(Gill et al., 1973; Janakiraman, 1981 and Singh et al., 1984)**. Therefore measuring progesterone (P_4) in milk fat is an appropriate method to monitor the postpartum ovarian activity **(Batra et al., 1979; Arora et al., 1980 and Kamonpatana et al., 1983)**.

It was concluded that P_4 monitoring offers an objective and accurate method for assessing typical and atypical ovarian function in postpartum cows **(Lamming and Darwash, 1998)**

and in buffaloes **(Perera et al., 1981; Jainudeen et al., 1983 and Kamonpatana et al., 1983)**.

Milk is preferred over plasma for P_4 assay under field conditions because collection of blood samples **(Barkawi et al., 1998)** from dairy animals is more difficult by farmers, while in P_4 assay in skim milk **(El-Moghazy, 2003)** or milk fat **(Claus et al.,1985)** samples, no problem of this nature is faced. In buffaloes, following the first postpartum ovulation, plasma P_4 levels were found to increase and remain above 0.7 ng/ml for about 10 days, and then it declined to below 0.25 ng/ml at the next oestrus **(Perera et al., 1981; Jainudeen et al., 1983 and Kamonpatana et al., 1983)**. In Nili-Ravi buffaloes, assay of P_4 was successfully used in oestrus detection **(Qureshi et al., 1989)** and in the assessment of ovarian status **(Qureshi et al., 1992). Usmani et al. (1983, 1984 and 1990)** found that 86% of buffaloes showed at least one short luteal phase (8 to 13 days) before the first postpartum oestrus. **Jain and Pandey (1985)** reported that plasma P_4 concentrations in buffalo heifers were significantly affected by the season and weaning.

The objective of this study was to investigate relationship between detected oestrous signs, P_4 concentration in milk fat and conception throughout 120 days postpartum in Egyptian buffaloes kept under field conditions.

MATERIALS AND METHODS

This study was carried out under the field condition of a medium size holding in Al-Talaba village, Dakahlia governorate, where animals were checked for 120 days postpartum during cold (November-April) and hot (May-October) seasons throughout an experimental period lasted from end of May 2004 till July 2005. lasted.

Animals and management:

Ten Egyptian buffaloes were kept with fertile buffalo bull at the same conditions. The bull was used for mating and as a teaser. All buffalo cows ranged between 3-9 years old and 1^{st} - 5^{th} parity. Buffaloes were hand milked in the absence of their calves twice daily at 6:0 a.m. and 6:0 p.m. throughout the lactation period. All animals normally calved and diagnosed as free of reproductive diseases at the beginning of this field study.

Buffalo cows were housed in a tiestall fact out all the year round with daily short period of exercise. Animals were kept under the regular systems of feeding and management adopted by the Egyptian farmers. All animals were fed, according to their body weight, reproductive status and

milk production, on a concentrate mixture (16% crud protein, 2% fat and 16.5% crude fiber) and roughages. Roughages in dry season were green maize (darawa) in June and July, Egyptian clover hay in Augusts and September and rice straw in October and November, however in green season roughages were only fresh Egyptian clover (Berseem, Trifolium alexandrinum) during the period from December to May. Concentrate feed mixture was offered for all animals at the time of milking, while roughages were ad. libitum.

Oestrus detection:

Oestrus signs were observed routinely by herdsman. Moreover, the buffalo cows were checked for heat by introducing teaser bull four times daily at 5:0 a.m., 11:0 a.m., 5:0 p.m., and 11:0 p.m. During the same times, buffalo cows were observed for external signs of oestrus by the investigator. As soon as considered in heat, the buffalo cows were observed continuously during the oestrus period. External signs of oestrus such as frequent sniffing of vulva and mounting by the bull, bellowing, hyperemia of vulva mucous membrane, response to placing hand on rump, response to slight massage of vulva lips and tail raising were checked and individually recorded. Occurrence of behavioural parameters was recorded as being present or absent in each buffalo cow. The total number of occasions in which behavioural parameters were present was expressed as a percentage of the total number of observed animals.

Milk sampling :

Milk samples (bulk milk) were collected every three days for each buffalo cow from calving until two weeks of established pregnancy and additionally on the day of oestrus and mating as well as day after oestrus. Whole milk samples (20 ml) were collected in test tube containing about 0.5 g of anticoagulant (potassum dichromat) and kept frozen at -20°C until P_4 assay.

Progesterone radioimmunoassay (RIA):

Individual P_4 profiles were obtained from each buffalo cow and P_4 concentration was determined by radioimmunoassay technique (RIA) in milk fat as described by **Claus and Rattenberger (1979) and Claus et al. (1985)**. The assay procedure was carried out as previously described by **Hoffmann et al. (1973) and Claus and Rattenberger (1979)**. Incubation periods of 15 min at 37°C and 60 min at 4°C are used. Bound/free separation is achieved by dextran-coated charcoal. The whole test procedure takes about 2 h for 100 samples.

Reproductive parametors:

During the experimental period, postpartum first ovulation interval (PPFVI), and number of ovulations per buffalo cow were recorded according to the individual P_4 concentration in milk fat. Also, postpartum first oestrus and service intervals (PPFOI & PPFSI), days open (DO), service period (SP, as an interval from 1st service to conceived service), number of service per conception (NSC) and calving interval (CI) were recorded. Average length of ovulatory cycles (cycles with ovulation, regardless oestrus), total number of ovulations, quiet ovulation (ovulation without any signs of oestrus) were determined. A case of quiet ovulation was identified if a period of low P_4 concentration in milk fat of <22.2 ng /ml without detection of any of the oestrous signs, was followed by an increase in milk fat P_4 concentration to >23.0 ng /ml maintained for at least two consecutive milk samples. This was based on the finding of changes in P_4 concentration in milk samples collected daily from buffaloes in relation to oestrus and ovulation as reported by **Shemesh et al. (1978), Heckman et al. (1979) and Kamonpatana et al. (1983)**.

Reproductive performance, ovulation parameters and ovarian activity of experimental animals are summarized in tables (1, 2 and 3, respectively).

Statistical analysis :

Data were analyzed by one-way analysis of variance (GLM) SAS program (2005). Chi-square was conducted to test the differences in percentages values. The significance differences was set at P<0.05 using Duncan multiple range test (1955).

RESULTS AND DISCUSSION

Progesterone profile at consecutive ovulations:

Results in table (4) reveal that overall P_4 concentration for all sampling days showed gradual increase (P<0.05) with increasing ovulation sequence, being the highest at the 4^{th} ovulation and the lowest at the 1^{st} ovulation

As affected by day of oestrous cycle, P4 concentration on days before ovulation showed marked reduction towards day of oestrus incidence; thereafter it profoundly showed gradual increase on days after ovulation (Table 4). Similar trends were reported by **Muhammad et al. (2000)** using milk P_4 level in Pakistan buffaloes, **Perera et al. (1981)** in buffaloes of **Sirlanka and Jainudeen et al. (1983)** in Swamp buffaloes.

Also, this trend was found for P_4 in blood plasma of buffaloes (**Kaur and Arora, 1984**) and in

serum P_4 of Surti buffaloes **(Sarvaiya and Pathak, 1992)**. Such results concerning the trend of change in P_4 level in milk and blood (plasma and serum) were indicated also for change in P_4 level in milk fat as obtained in our study.

Continuously with increasing ovulation sequence, overall P_4 concentration in milk fat for all days studied significantly ($P<0.05$) increased. This trend was observed on days 6 and 3 before ovulation and days 1, 3 and 6 after ovulation. But on day of ovulation, P_4 concentration did not differ significantly being 12.6, 14.6, 16.8 and 16.7 ng/ml at the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} ovulations, respectively (Table 4). The observed gradual increase in P_4 concentration by sequence of ovulation may be due to gradual increase in the GnRH secretion from hypothalamus that stimulate the synthesis and secretion of LH and FSH **(Gordon, 1996)**. Increasing LH-GnRH, in turn, promotes gradual steroids (progesterone) from granulose luteal cells of CL **(Sealfon and Millar, 1995)**.

Oestrous signs and P_4 profile at consecutive oestrus cases:

Results in table (5) show that intensity of oestrous behaviour in term of bellowing, response to placing hand on rump, tail raising and teat tight increased significantly ($P<0.05$) by increasing sequence of oestrus up to 3^{rd} oestrus. However, other signs such as sniffing of vulva and mounting by bull, hyperemia of vulva mucous membrane and response to slight message of vulva lips increased significantly ($P<0.05$) only between 1^{st} and 2^{nd} oestrus.

It is of interest to note that oestrous signs including frequent sniffing of vulva and mounting by bull, bellowing, response to placing hand on rump, tail raising and teat tight were frequent in all buffalo cows (100%) following incidence of the 3^{rd} oestrus (Table 5). Such trend in oestrus intensity may be associated with gradual increase in pulsatile release of GnRH from hypothalamus, which stimulate release of gonadotrophines (FSH and LH) from the anterior pituitary, and in turn increase oestradiol secretion from the ovarian follicles **(Gordon, 1996)**.

With increasing oestrus sequence, overall P_4 concentration for all days studied in milk fat significantly ($P<0.05$) increased. This trend was observed on days 6 and 3 before oestrus and days 1 and 3 after oestrus. While on day of oestrus, P_4 concentration did not differ significantly being 14.7, 15.0 and 14.7 ng/ml at the 1^{st}, 2^{nd} and 3^{rd} oestrus, respectively (Table 5).

The present results indicated that the observed increase ($P<0.05$) in intensity of oestrous activity of buffalo cows with oestrus sequence was associated with significant ($P<0.05$) increase in P4 concentration on days pre- and post-oestrus incidence (Table 6). **Aboul-Ela et al. (1987)** suggested that P_4 has a priming effect on onset of postpartum oestrous activity.

Oestrous signs and P_4 profile at ovulatory oestrous and anoestrous cases:

In ovulatory oestrous cases (Table 6 and Figure 1), frequency of sniffing of vulva and mounting by bull was the highest (100%), followed by response to placing hand on rump (76.2%), bellowing and tail raising (66.7% for each), response to slight massage of vulva lips (57.1%), hyperemia of vulva mucous membrane and teat tight (47.6% for each).

It is worthy noting that, teat tight as an oestrous sign was observed in buffalo cows exhibiting oestrous behaviour and some animals showing quiet ovulation as proved from P_4 profile (See materials and methods chapter). This sign was significantly ($P<0.05$) more frequent in ovulatory anoestrous (63.6%) than in ovulatory oestrous cases (47.6%).

In nearly similarity with the present results, **El-Wardani (1990)** reported that percentage of male mounting signs ranged between 90 and 100% and 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.

However, the frequent of bellowing shown in this study was less than reported by several investigators. Percentage of bellowing was found to be 98% **(Aboul-Ela, 1988)**, 80% **(Singh et al., 1984)**, 78.2% **(Khalil et al., 2002)** and 70% **(Barkawi et al., 1993)**. The less frequent of bellowing recorded in this study may be related to age variation of buffalo cows. **Rao and Kodagali (1983) and Nemat Ullah and Usmani (1985)** indicated that bellowing as a sign of oestrus may be less in buffalo heifers than in buffalo cows. On the other hand, lower percentage of vaginal mucus discharge (17.74%) was reported by **Khalil et al. (2002)** in buffaloes.

Several investigators found that bellowing has been reported to be the most reliable sign of oestrus behaviour in Egyptian buffaloes under field conditions of small holders **(Aboul-Ela, 1988 & 1993 and Aboul-Ela et al., 2000)** or in large herds in absence of bull **(Barkawi et al., 1992)**. In Egyptian buffaloes under field conditions, bellowing was the most oestrous sign (97.5-100%) used by breeders **(Aboul-Ela, 1993; Aboul-Ela et al. 2000 and El-Moghazy, 2003)**. The present study may suggest that mounting by bull and other mutual behaviour signs with male, response to placing hand on rump, beside bellowing are considered the most reliable signs of oestrus incidence in buffalo cows.

Concentration of P_4 on most sampling days studied tended to be higher in ovulatory oestrous than in ovulatory anoestrous cases, but the differences were not significant (Figure 1). Generally, concentration of P_4 was higher on days 6 and 3 pre- and 1 post- ovulatory oestrous than ovulatory anoestrous cases. While, it was nearly similar on days 3 and 6 post-ovulation for both cases. However, the trend of change in P_4 concentration in both oestrous and ovulatory anoestrous cases was the same on all sampling days (Figure 1).

Ovulatory oestrous cases of conceived and non-conceived buffalo cows:

Intensity of all oestrous signs was significantly (P<0.05) higher in ovulatory oestrous cases ended with conception than those which did not conceived, except for sniffing of vulva and mounting, which was frequent in all conceived and non conceived cases (Table 7). The highest magnitude of differences was observed for hyperemia of vulva mucous membrane, response to slight message of vulva lips and teat tight (14.3 vs. 90%, for each). While the lowest magnitute of differences was found in bellowing and response to placing hand on rump (57.1 vs 100%, for each).

Concentration of P_4 on pre- and post- oestrous days was significantly (P<0.05) higher in conceived than non-conceived cases, which reflected in significantly (P<0.05) higher overall concentration of P_4 during pre- and post- oestrous days in conceived than non-conceived cases (82.2 vs. 27.1 ng/ml, Figure 2).

In this respect, **El-Moghazy et al. (2006)** found that P_4 peak was nearly the same and interval to P_4 peak in conceived was longer than in non-conceived prior to ovulatory oestrous incidence in buffalo cows under field conditions, which may prove the role of P_4 in preparation to appear higher intensity of oestrous signs and incidence of fertilization.

Ovulatory oestrous cases in cold and hot season:

As shown in table (8), all detected oestrous behaviour were observed in the two seasons, being significantly higher in cold than in hot season, except for sniffing of vulva and mounting, which was observed on all buffalo cows (100%) in the two 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 cold than hot season.

It is worthy noting that mutual behaviour with a teaser bull was sexually more active during hot season, whereas the difference in incidence of bellowing was more pronounced between both seasons (100% in cold vs. 46.2% in hot season). **Khalil et al. (2002)** found that some signs of self oestrous behaviour in Egyptian buffalo cows increased in cold than in hot season, particularly bellowing, tail raising and vaginal mucus discharge (86.67, 62.67 and 24.0% vs. 65.31, 32.65 and 8.16%, respectively).

Generally, the higher degree of oestrus expression in cold than hot season may be attributed to various factors, such as climate, photoperiod, temperature and nutrition (**Beg and Totey 1999**), system of management (**Abdalla, 2003**), method of heat detection and climatic conditions (**Bearden and Fuquay, 1992**) and energy intake and nutritional deficiency (**Qureshi, 1998**).

Concerning concentration of P_4 in milk fat on days pre- and post-oestrous during both seasons (Figure 3), it was significantly ($P<0.05$) higher in cold than hot season only on day 6 post-oestrus (101.6 vs. 56.7 ng/ml). However, the tendency of increasing P_4 concentration on days 6 and 3 pre-oestrus and day 3 post-oestrus in cold than hot season reflected in significantly ($P<0.01$) higher overall P_4 concentration in cold season than in hot season (60.5 vs. 40.9 ng/ml). This confirms the findings of **Madau (1994)**, who attributed the low reproductive efficiency of buffaloes in summer to low luteal activities, indicated by low P_4 levels. **Tan and Chen (1992)** reported that a cow experienced four oestrus cycles, three of which were behavioral during a 2-months period. The missing cycle and the low values of plasma and fecal P_4 during the luteal phase from May to June implied a weak seasonal effect in Taiwan.

In light on the foregoing results, the current study may conclude that intensity of oestrous signs was affected by sequence of oestrus. Increasing intensity of detected oestrous signs may consider as good indicator for conception in buffalo cows. Also, cold season was characterized by higher intensity of oestrous signs than hot season. Generally, oestrous sign intensity may be associated with P_4 concentration, in particular on days prior to oestrus incidence. Consequently, this results indicated strong relationship between intensity of oestrous signs, P_4 concentration and conception.

Table (1): Means and standard errors of reproductive performance of experimental buffaloes.

Reproductive parameter	Mean±SE
Number of animals	10
Postpartum 1st ovulation interval (PPFVI, day)	23.2±4.20
Average P_4 conc. (ng/ml) during PPFVI	13.9±1.03
Postpartum 1st oestrous interval (PPFOI, days)	41.7±9.48
Postpartum first service interval (PPFSI, days)	45.6±9.67
Days open (DO, days)	71.0±7.90
Service period (SP, days)	25.4±7.28
Number Service per conception (NSC)	2.0±0.26
Calving interval (CI, days)	388.5±7.63

Table (2): Means and standard errors of ovulation parameters of buffalo cows throughout 120 days postpartum.

Item		Mean±SE
Total ovulations	Total number	32
	Ovulations/animal	3.2±0.20
	Ovulatory oestrous cases/animal*	2.1±0.16
Quiet ovulations	Relative to total ovulations (%)	34.4
	Quiet ovulations/animal	1.1± 0.30
	At 1st ovulation (%)	44.4
	At 2nd ovulation (%)	30.0
	At 3rd ovulation (%)	42.9
	At 4th ovulation (%)	20.0

* cases with ovulation and oestrous activity.

Table (3): Means and standard errors of ovarian activity of buffalo cows throughout 120 days postpartum.

Item		Mean±SE
Total ovulatory cycles (cycles with ovulation, regardless oestrus)	Total number	19
	Average length (day)	16.4±1.23
	P_4 peak (ng/ml)	114.3±24.56
	Interval to P_4 peak (day)	10.5±1.46
Ovulatory oestrus cycles (cycles with ovulation and oestrous activity)	Frequency distribution (%)	73.7
	Average length (day)	15.6±1.46
	P_4 peak (ng/ml)	126.2±30.81
	Interval to P_4 peak (day)	10.7±1.88
Ovulatory anoestrus cycles (cycles with ovulation and without oestrous activity)	Frequency distribution (%)	26.3
	Average length (day)	18.6±2.23
	P_4 peak (ng/ml)	81.0±36.05
	Interval to P_4 peak (day)	9.8±2.03
Short ovulatory cycles (<19 days)	Frequency distribution (%)	47.4
	Average length (day)	11.4±1.03
	P_4 peak (ng/ml)	60.1±15.65
	Interval to P_4 peak (day)	5.3±0.83
Normal ovulatory cycles (19-23 d)	Frequency distribution (%)	52.6
	Average length (day)	20.9±0.48
	P_4 peak (ng/ml)	163.1±39.21
	Interval to P_4 peak (day)	15.1±1.59

Table (4): Average P4 concentration (ng/ml) in milk fat at different days of ovulatory cycle at consecutive ovulations of buffalo cows.

Item	Ovulation sequence				
	1st ovulation	2nd ovulation	3rd ovulation	4th ovulation	±MSE
No. of ovulation	10	10	7	5	
Progesterone concentration (ng/ml):					
-6 day	15.9 ^{c}	43.9 ^{bc}	106.5 ^{b}	220.6 ^{a}	26.95
-3 day	12.4 ^{b}	42.7 ^{b}	63.6 ^{b}	155.8 ^{a}	19.37
Day of oestrus	12.6	14.6	16.8	16.7	1.82
+1 day	18.0 ^{b}	23.4 ^{ab}	26.1 ^{ab}	37.5 ^{a}	5.52
+3 day	24.4 ^{b}	45.9 ^{ab}	61.3 ^{a}	58.5 ^{a}	9.36
+6 day	25.4 ^{b}	75.4 ^{a}	123.1 ^{a}	95.6 ^{a}	15.79
Overall mean	18.2 ^{d}	41.6 ^{c}	69.3 ^{b}	99.5 ^{a}	13.14

^{a,b,c,d} Means in the same row with different superscript are significantly different (P ≤0.05).

Table (5): Frequency of detected oestrous signs (%) and means and standard errors of P4 concentration (ng/ml) in milk fat on consecutive days of the 1st, 2nd and 3rd oestrus.

Item	Sequence of oestrus			
	1st Oestrus	2nd Oestrus	3rd Oestrus	±MSE
Number of oestrous cases	10	8	3	
Frequency of mutual behaviour with bull (%):				
Sniffing of vulva and mounting by bull	90.0 ^{b}	100.0 ^{a}	100.0 ^{a}	
Frequency of oestrus signs (%):				
Bellowing	50.0 ^{c}	75.0 ^{b}	100.0 ^{a}	
Hyperemia of vulva mucous membrane	20.0 ^{b}	75.0 ^{a}	66.7 ^{a}	
Response to placing hand on rump	60.0 ^{c}	87.5 ^{b}	100.0 ^{a}	
Response to slight message of vulva lips	40.0 ^{b}	75.0 ^{a}	66.7 ^{a}	
Tail raising	40.0 ^{c}	87.5 ^{b}	100.0 ^{a}	
Teat tight	20.0 ^{c}	75.0 ^{b}	100.0 ^{a}	
Progesterone concentration (ng/ml):				
-6 day	53.5 ^{b}	67.8 ^{b}	224.5 ^{a}	38.61
-3 day	37.3 ^{b}	73.4 ^{ab}	177.3 ^{a}	28.33
Day of oestrus	14.7	15.0	14.7	2.22
+1 day	18.9 ^{b}	23.4 ^{b}	44.4 ^{a}	4.43
+3 day	35.1 ^{b}	53.4 ^{ab}	74.9 ^{a}	12.87
+6 day	55.2	94.4	90.4	23.55
Overall mean	36.1 ^{c}	54.6 ^{b}	104.4 ^{a}	18.33

^{a,b,c} Means in the same row with different superscript are significantly different (P ≤0.05).

Table (6): Frequency of detected oestrous signs (%) of ovulatory oestrous and ovulatory anoestrus cases of buffalo cows.

Item	Ovulatory cases	
	Ovulatory oestrous [1]	Ovulatory oestrous [1]
Number of cases	21	11
Frequency of mutual behaviour with bull (%):		
Sniffing of vulva and mounting by bull	100	-
Frequency of oestrus signs (%):		
Bellowing	66.7	-
Hyperemia of vulva mucous membrane	47.6	-
Response to placing hand on rump	76.2	-
Response to slight message of vulva lips	57.1	-
Tail raising	66.7	-
Teat tight*	47.6 [b]	63.6 [a]

[1] Ovulatory oestrous: Ovulation cases accompanied with oestrus signs.
[2] Ovulatory anoestrous: Ovulation cases determined by P_4 profile without oestrous signs.
[a,b] Means in the same raw with different superscript are significantly different ($P \leq 0.05$).
* This sign was observed in cases detected with quiet ovulations.

Table (7): Frequency of detected oestrous signs (%) of ovulatory oestrus cases ending with conception and non-conceived buffalo cows.

Item	Ovulatory oestrus case		Sig.
	Conceived	Non-conceived	
Number of cases	10	7	
Frequency of mutual behaviour with bull (%):			
Sniffing of vulva and mounting by bull	100	100	NS
Frequency of oestrus signs (%):			
Bellowing	80.0	57.1	**
Hyperemia of vulva mucous membrane	90.0	14.3	**
Response to placing hand on rump	100.0	57.1	**
Response to slight message of vulva lips	90.0	14.3	**
Tail raising	100.0	42.9	**
Teat tight	90.0	14.3	**

NS: Not significant ** Significant difference at $P<0.01$

Table (8): Frequency of detected oestrous signs (%) of ovulatory oestrus cases during cold and hot season.

Item	Season of oestrus detection		Sig.
	Cold season	Hot season	
Number of ovulatory oestrous cases	8	13	
Frequency of mutual behaviour with bull (%):			
Sniffing of vulva and mounting by bull	100.0	100.0	NS
Frequency of oestrus signs (%):			
Bellowing	100.0	46.2	**
Hyperemia of vulva mucous membrane	75.0	30.8	**
Response to placing hand on rump	87.5	69.2	*
Response to slight message of vulva lips	75.0	46.2	**
Tail raising	75.0	61.5	*
Teat tight	75.0	30.8	**

NS: Not significant * Significant difference at P<0.05 ** Significant difference at P<0.01

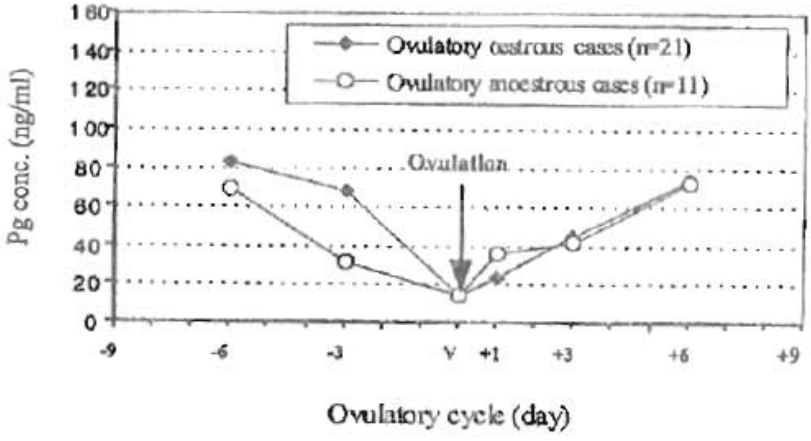

Figure (1): Means of P4 profile (ng/ml) in milk fat at different days of ovulatory oestrous and ovulatory anoestrous cases.

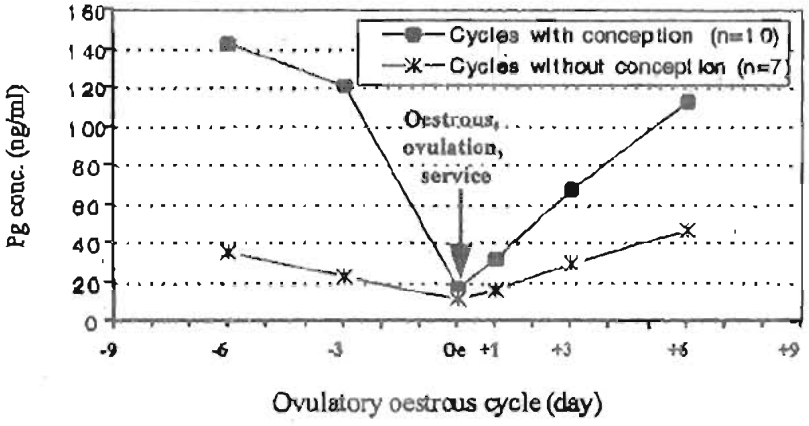

Figure (2): Means of P4 profile (ng/ml) in milk fat on conceived days of ovulatory oestrus cases ending with conception and non-conceived buffaloes.

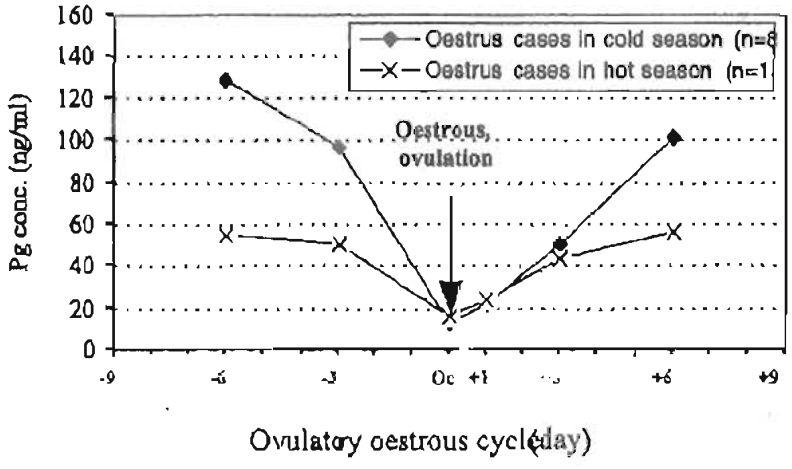

Figure (3): Means of P4 profile (ng/ml) in milk fat on conceived days of ovulatory oestrus cases during cold and hot season.

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الملخص العربى

العلاقة بين علامات إكتشاف الشياع وتركيز هرمون البروجستيرون فى دهن اللبن والخصوبة فى الجاموس المصرى تحت الظروف الحقلية

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أجريت هذه الدراسة على حالات التبويض ودورات الشياع فى ١٠ حيوانات من الجاموس المصرى خلال ١٢٠ يوم بعد الولادة وكانت بهدف معرفة العلاقة بين ظهور علامات إكتشاف الشياع وتركيز هرمون البروجستيرون (P_4) فى دهن اللبن والخصوبة، وكانت الحيوانات بين الموسم الأول والخامس بعمر يتراوح بين ٣-٩ سنوات، جمعت عينات اللبن كل ثلاث أيام وتم تحليل هرمون البروجستيرون فى دهن اللبن بطريقة المناعة الإشعاعية خلال الأيام ٦ و ٣ قبل الشياع ويوم حدوث الشياع والأيام ١ و٣ و٦ بعد الشياع، أوضحت النتائج وجود زيادة معنوية ($P<0.05$) فى المتوسط الكلى لتركيز هرمون البروجستيرون عند كلاً من التبويض الأول والثانى والثالث والرابع (٢ر١٨ و ٦ر٤١ و ٣ر٦٩ و ٥ر٩٩ نجم / ملیلتر على التوالى)، فى حالات التبويض المصاحب بشياع كانت نسبة حدوث شم الطلوقة لفرج الأنثى وأيضاً إعتلاء لها (١٠٠٪)، يليها الاستجابة لوضع اليد على الردف (٢ر٧٦٪)، وإصدار صوت عالى وأيضاً رفع الذليل (٧ر٦٦٪)، والاستجابة لتدليك شفرى الفرج (١ر٥٧٪) وزيادة إفراز السائل المخاطى من الفرج، وكان تصلب الحلمات كعلامة من علامات الشياع أكثر تكراراً (٦ر٦٣٪) فى حالات التبويض غير المصاحب بظهور علامات الشياع (التبويض الصامت) عنها فى حالة التبويض المصاحب بظهور علامات الشياع (١ر٤٧٪) وكانت الفروق معنوية ($P<0.05$). وكان المتوسط الكلى لتركيز هرمون البروجستيرون أعلى فى حالة التبويض المصاحب بشياع عن حالة التبويض غير المصاحب بظهور علامات الشياع (التبويض الصامت) وكانت الفروق غير معنوية. وزادت معنوياً ($P<0.05$) نسبة حدوث شم الطلوقة لفرج الأنثى وأيضاً إعتلاء لها، وزيادة إفراز السائل المخاطى من الفرج، والاستجابة لتدليك شفرى الفرج بين الشياع الأول والثانى فقط، بينما زادت معنوياً ($P<0.05$) نسبة حدوث إصدار صوت عالى، والاستجابة لوضع اليد على الردف ورفع الذيل وتصلب الحلمات بزيادة تكرار حدوث الشياع حتى الشياع الثالث. وكانت هناك زيادة معنوية ($P<0.05$) فى المتوسط الكلى لتركيز هرمون البروجستيرون بزيادة تكرار حدوث الشياع وكان ١ر٣٦ و ٦ر٥٤ و ٤ر١٠٤ نجم/ملليتر لكل من الشياع الأول والثانى والثالث على التوالى، فى حالة التبويض المصحوب بشياع وحدوث التلقيح كان ظهور كل علامات الشياع أعلى معنوياً ($P<0.01$) فى الحالات التى إنتهت بحدوث إخصاب عن الحالات التى إنتهت بعدم حدوث إخصاب، ماعدا نسبة حدوث شم الطلوقة لفرج الأنثى وأيضاً إعتلاء لها والتى كانت أكثر تكراراً (١٠٠٪) فى الحالتين، أيضاً كان المتوسط الكلى لتركيز هرمون البروجستيرون أعلى معنوياً ($P<0.05$) فى الحالات التى أخصبت عن الحالات التى لم تخصب (٢ر٨٢ مقابل ١ر٢٧نجم/ملیلتر)، ظهور كل علامات الشياع كانت أعلى معنوياً ($P<0.05$) فى

الموسم البارد عن الموسم الحار، ماعدا حدوث شم الطلوقة لفرج الأنثى وأيضاً إعتلاءه لها والتى كانت أكثرتكراراً (١٠٠٪) فى كلاً الموسمين، على أية حال، الاختلافات فى المتوسط الكلى لتركيز هرمون البروجستيرون كانت غير معنوية بين كلاً الموسمين.

فى ضوء النتائج السابقة تستخلص من هذه الدراسة أن كثافة ظهور علامات الشياع تأثرت بتكرار مرات حدوث الشياع، وأن زيادة ظهور علامات الشياع تأثرت بتكرار مرات حدوث الشياع، وأن زيادة ظهور علامات الشياع المكتشف ربما تكون مؤشر جيد للحمل فى الجاموس، أيضاً، تميز الموسم البارد بزيادة كثافة ظهور علامات الشياع عن الموسم الحار، وعموماً فإن كثافة ظهور علامات الشياع ربما تعزى إلى تركيز هرمون البروجستيرون، خاصة فى الأيام التى تسبق حدوث الشياع، وبناء على ذلك تشير هذه النتائج إلى وجود علاقة قوية بين كثافة ظهور علامات الشياع وتركيز هرمون البروجستيرون والخصوبة فى الجاموس.