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INFLUENCE OF SEASON AND NUMBER OF HEAT CHECKS ON DETECTING OF OVULATORY ESTRUS IN EGYPTIAN BUFFALOES

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SUMMARY

Post- partum 57 buffaloes were divided into two groups according to their season of calving: cold (Nov. - April, n=29) and hot (May - October, n= 28). Buffaloes were checked for heat four times daily, in the presence of a teaser, at 6 h intervals. Standing behavior was considered the true sign of heat regardless of the appearance of other symptoms. Blood samples were collected regularly, at 3-4 day intervals, for progesterone (P_4) determination to monitor ovarian activity throughout the experimental period (6 months). Day of estrus was defined by the appearance of standing behavior followed by an increase in P_4 concentration to ≥1.0 ng/ml in at least two consecutive samples through the next seven days. If P_4 level increased to the pre-mentioned level without detecting standing behavior throughout the previous seven days, the ovulation was counted quiet. Data were deduced to test the effect of regime (one, two, three and four times daily) and time of heat detection (possible combinations within regimes, n= 15 scenarios) on the efficiency of estrous recognition.

Hot season calvers had lower percentage of detected ovulatory estrus compared to cold season ones. Increasing frequency of heat checks improved the efficiency of heat detection. Checking estrus once daily resulted in detecting a maximum of 60% of estrous cases vs. 87.8, 95.3 and 100 % for two, three and four checks / day, respectively. Within any regime, season affected the order of scenarios. In the hot season, buffaloes displayed their sexual behavior whenever the ambient temperature was low (06:00 and 24:00 h), while they displayed estrous symptoms during the moderate temperature times (06:00 and 12:00 h) of the day in the cold season.

Percentage of detected estrus was almost similar when heat was checked three or four times daily. Detecting estrus twice daily at 06:00 and 18:00 h showed reasonable percentage of detected estrus similar to most of scenarios obtained if three times of heat detection were conducted.

Keywords: Egyptian buffalo, heat detection efficiency, regime, scenarios, season.

INTRODUCTION

High frequency (up to 86%) of quiet ovulation is considered one of the main reproductive failures in buffaloes, particularly during the post-partum period (Mohamed and El-Sheikh, 1983, Barkawi *et al.*, 1986, and Aboul-Ela *et al.*, 1987). Quiet ovulation was reported to be more frequent in hot season (Khattab *et al.*, 1990) resulting in longer days open and consequently longer calving intervals (El-Sheikh and Mohamed, 1976; El-Fouly *et al.*, 1977 and Metry *et al.*, 1994).

The common practice in buffalo farms is to perform estrous detection twice daily at morning (06:00-08:00 h) and afternoon (12:00 -16:00 h). However, increasing frequency of heat detection per day and / or the interval between the heat detection rounds were reported to decrease the percentage of quiet ovulation (El-Sheikh and El-Fouly, 1971, Khattab *et al.*,1988 and Barkawi *et al.*, 1998). However, no data are available suggesting the proper frequency and timing of estrous detection rounds.

In this study, different regimes of heat detection (one, two, three or four times daily) with all timing combinations within regimes were tested to define the optimum efficient system of heat detection in relation to season of calving.

MATERIALS AND METHODS

Animals and management

A total of 57 normally calved buffaloes, belonging to Mehallet Mousa Experimental Station, Animal Production Research Institute, Ministry of Agriculture, Egypt, was used in this study. Buffaloes were between the 2 *nd* and the 8 *th* parity and were diagnosed as free from reproductive diseases at the beginning of the experiment. Buffaloes were kept loose under open shed yards throughout the experimental period, which lasted for six months post- partum per each season, cold and hot.

Buffaloes were fed according to their live body weight and milk production level on concentrate mixture and rice straw. Egyptian clover (*Trifolium alexandrinum*) or its hay were offered when available. Buffaloes were hand-milked twice daily at 8:00 and 15:00 h starting on day seven post-partum.

Experimental procedure

To study the effect of season, cold (November-April) and hot (May - October) seasons were defined. The cold season was characterized with availability of green fodder, low ambient temperature (14.9°C) and short day-light length (11.2 h). The hot season was characterized with unavailability of green fodder, high ambient temperature (24.6°C) and long day-light length (13.1 h). Buffaloes were divided into two groups as calving in cold or hot seasons, 29 and 28, respectively.

Buffaloes were checked regularly for estrus four times daily, at 6 h intervals (6:00, 12:00, 18:00 and 24:00 h). Detection was conducted in presence of a buffalo teaser, which was allowed to run with the females for 30 min at each round of heat detection.

Regularly, blood samples were collected every 3-4 days for progesterone (P_4) determination starting seven days post-partum and continued up to day 120. Blood samples were centrifuged for 15 min at 3000 rpm for serum separation. Serum

samples were stored at -20° C till time of assay. Direct radioimmunoassay technique was performed according to the procedure outlined by the manufacturer "Orion Diagnostic, Finland". The cross- reaction of P_4 antibody was reported to be 100% with P_4, 3.9% with pregnanolone and 0.9 % with each of the other steroids. The standard curve ranged between 0.0 and 31 ng /ml. The sensitivity value is reported to be 0.06-0.09 ng /ml. Intra and inter assay coefficients of variation were 9.7% and 8.0%, respectively.

Studied traits and calculations

Estrus was accounted only when buffaloes showed standing behavior, regardless of the appearance of any other estrous behavioral signs. If the cow did not display standing behavior in the next round, even though they displayed other signs, heat period was considered terminated. Day of estrus was considered as the day of ovulation, if the standing behavior date coincided with P_4 value of < 1.0 ng/ml, followed by an increase in P_4 concentration to ≥1.0 ng /ml and continued at that level in at least the two consecutive samples throughout the next seven days. If P_4 level increased to the pre-mentioned levels without detecting standing behavior throughout the previous seven days, ovulation was counted quiet. Date of quiet ovulation was determined by subtracting three days from the date at which P_4 reached the level characterizing ovulation.

Frequency of estrous incidence under different regimes (once, R1; twice, R2; thrice, R3 and four rounds, R4, daily) was calculated from the data collected under four times of heat detection. Possible time combinations within each of the studied regimes (scenarios, n=15) were deduced according to the manifestation of standing behavior. Times of studied scenarios are shown in table 1.

To compare among the proposed regimes, the total detected ovulatory estrus under four times heat checks was considered as 100 % and the detected ovulatory estrouses in the other regimes and scenarios within each season were calculated as a proportion of those of four times.

Statistical analysis

Data were analyzed using the general linear model procedure (SAS, 1990). Effects of season (S_i); scenario (C_j) and the interaction between season and scenario (SC_{ij}) were included in the following model:

$$Y_{ijk} = \mu + S_i + C_j + SC_{ij} + e_{ijk}$$

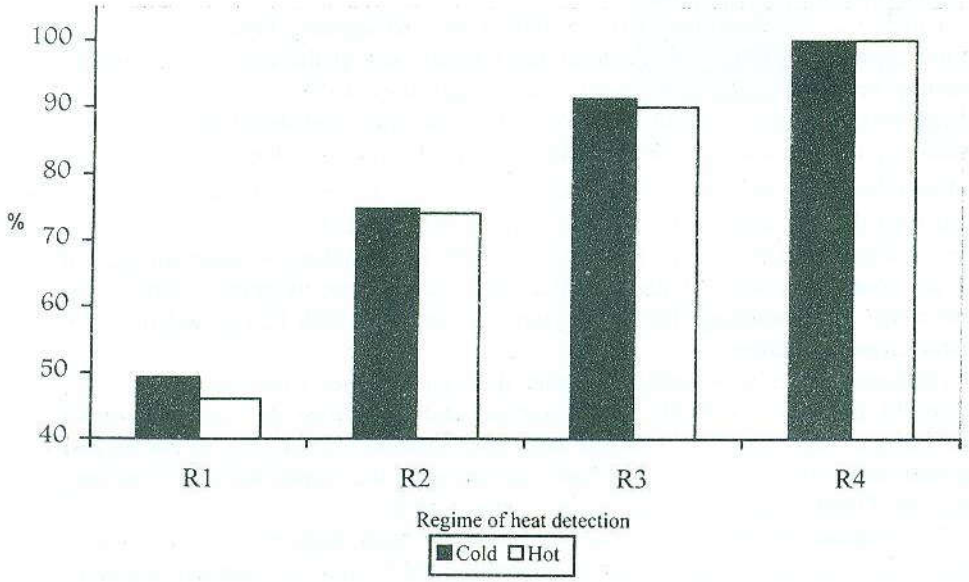
Means were compared using Duncan's Multiple Range Test (Duncan, 1955). Probability values ≤ 5% were considered significant. Chi square was used to test the significance between percentages. The overall mean of any regime was calculated as average of scenarios within regimes.

RESULTS

Under four times of heat detection, only 88 % out of the total ovulations were diagnosed as ovulatory estrus. The rest were not accompanied by standing behavior thus recorded as quiet ovulations. Percentage of detected ovulatory estrus was

influenced by season of calving, where calvers of the hot season tended to have lower percentage (86.1 %) as compared to cold ones (90.6 %).

In both seasons, increasing heat detection rounds per day improved ($P< 0.01$) the percentage of recorded estrus (Figure 1). Time of heat detection (scenarios) had great bearing on estrous detection efficiency. Regardless of scenario no. 15 (four times



R1= Once daily *R2= Twice daily*
R3= Thrice daily *R4= Four times daily*

Figure 1. Percentage of detected estrus in Egyptian buffaloes as affected by regimes of heat detection in the cold and hot seasons, calculated as proportional to R4.

daily), which was the best for detecting estrus, scenario no. 1 within R1 (06:00 h), no. 6 within R2 (06:00 and 18:00 h) and no. 13 within R3 (06:00, 18:00 & 24:00 h) showed the best efficiency of heat detection. Season affected the order of options. While the best scenarios in the hot season in R1, R2 and R3 were 1, 6 and 13, the corresponded scenarios were 2, 6 and 11 in the cold season (Table 1).

Comparison among scenarios indicated that percentage of detected estrus in scenario no. 14 (R4) was similar to 11, 12 and 13 (R3). Scenario no. 6 (R2) showed the highest percentage of detected estrus. This scenario was statistically similar to all the scenarios of R3, which means that checking estrus two times daily at 06:00 and 18:00 h (scenario no.6) could be applied with similar efficiency to three times daily.

It is very interesting to note that in scenarios at which rounds of heat detection of morning (06:00 h) and / or evening (18:00 h) were absent, percentage of detecting estrus tended to decrease. Meanwhile, the best scenarios were those including one of or both these two times. This trend was very clear in R2 and R3 (Table 1).

Table 1. Percentage of cows detected in estrus under different regimes of heat detection in relation to season of the year, proportional to those detected four times per day observation

Scenario	Regime of heat detection	Season		Overall
		Cold	Hot	%
	Once daily (R1)			
1	06:00 h	54.7 ^{fgh}	65.3 ^{ef}	60.0 ^{f}
2	12:00 h	56.0 ^{fg}	28.0 ^{f}	42.0 ^{g}
3	18:00 h	46.0 ^{gh}	41.7 ^{h}	43.8 ^{g}
4	24:00 h	41.0 ^{h}	49.7 ^{gh}	45.3 ^{g}
	Twice daily (R2)			
5	06:00&12:00 h	69.3 ^{def}	73.0 ^{de}	71.2 ^{e}
6	06:00&18:00 h	83.7 ^{bcd}	92.0 ^{ab}	87.8 ^{bc}
7	06:00&24:00 h	72.3 ^{de}	83.3 ^{bc}	77.8 ^{cde}
8	12:00&18:00 h	77.0 ^{cde}	57.7 ^{fg}	67.3 ^{ef}
9	12:00&24:00 h	79.7 ^{bcde}	70.7 ^{de}	75.2 ^{de}
10	18:00&24:00 h	66.7 ^{ef}	68.0 ^{de}	67.3 ^{ef}
	Thrice daily (R3)			
11	06:00&12:00&18:00 h	93.0 ^{ab}	93.3 ^{ab}	93.2 ^{ab}
12	06:00&12:00&24:00 h	89.0 ^{abc}	90.0 ^{ab}	89.5 ^{ab}
13	06:00&18:00&24:00 h	92.0 ^{abc}	98.7 ^{a}	95.3 ^{ab}
14	12:00&18:00&24:00 h	91.3 ^{abc}	78.0 ^{cd}	84.7 ^{bcd}
	Four times daily (R4)			
15	06:00&12:00&18:00&24:00h	100.0 ^{a}	100.0 ^{a}	100.0 ^{a}

^{abcdef} Means with different superscripts within raw or columns are different ($P<0.05$)

The interaction between season and scenarios was statistically insignificant. However, in the hot season buffaloes given to display their sexual behavior whenever the ambient temperature was low (at 06:00 h and 24:00 h, scenarios no. 1, 4, 6, 7, 11 and 13), while the least was recorded at noon (scenarios no. 2, 8 and 14). In the cold season, scenarios included 06:00 and 12:00 h showed the best percentage of detected estrus.

DISCUSSION

Results obtained on quiet ovulation indicated that even under four times of heat detection, buffaloes still show quiet ovulations, where neither the teaser nor the herdsman could recognize ovulation. However, the percentage of recorded quiet ovulation is lower than that reported previously by El-Shiekh and El-Fouly (1971) under similar regime of heat detection. The trend of decrease of recorded quiet ovulations by increasing the frequency of heat detection per day is in agreement with the results of El-Shiekh and El-Fouly (1971) and Khattab *et al.* (1990).

Increasing incidence of quiet ovulation during the hot season (13.9 %) compared to 9.4 % in the cold one agrees with the findings of Barkawi *et al.* (1986) and Barkawi (1993). This is, most probably, attributed to the effect of heat stress. Heat stress has a negative effect on the pituitary function (Aboul-Ela and Barkawi, 1988) as well as on ovarian activity of buffaloes. The poor sexual behavior during the hot season may be due to the low circulating level of estrogen (Shafie *et al.*, 1982). Such low level may not reach the threshold needed to activate the sex centers in the hypothalamus. Meanwhile, hot season might reduce the sensitivity of the sexual centers and decrease ability of the buffaloes to express their sexual desire (Hafez, 1987).

In the hot season, it is clear that buffaloes display the most remarkable sexual activity in the morning (06:00 h) and at mid-night (24:00 h), better than at noon time (12:00 h). This seems to avoid times of the day having high ambient temperature. On the other hand, in the cold season, where the ambient temperature goes extremely down, particularly at night (24:00 h), buffaloes might modulate their physiological function to display most of their sexual behavior at the time of the day where the ambient temperature is moderate. Egyptian buffaloes conform to the statement of Fraser and Broom (1990) on cattle that low temperature affects negatively the estrous behavior as the high temperature does.

High efficiency of heat detection in scenario no. six (R2) clearly shows that at these two times (06:00 h and 18:00 h); which are so close to the times of sun rise and sun set; buffaloes express obvious heat signs better than the other times of the day. This trend agrees with those reported previously by Hafez (1954) and Barkawi *et al.* (1997) reporting two peaks for heat incidence at dawn and sun set times. Meanwhile, about 60% of heat periods of buffaloes start and terminate during the period from 15:00 h to 09:00 h of the next day (Barkawi *et al.*, 1997). Sexual behavior response to ambient temperature needs more studies to clarify the mechanism(s) controlling sexual activity under different ambient temperatures.

In conclusion, applying one time of estrous check per day should be avoided since it achieves the lowest heat detection efficiency. Frequency of four times of heat detection achieve the best efficiency of heat detection, however, it is faced by many managerial obstacles (labor, light availability, insemination, ...etc) and increases the cost of the farm as well. Checking estrus twice a day at 06:00 and 18:00 h seems to be the best scenario at the farm level, from practical point of view due to its lower cost of heat detection with similar efficiency compared to three times daily. Economic evaluation of application of regimes and scenarios need more studies to verify their impact on the farm profitability.

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تأثير فصل السنة و تكرار كشف الشياع فى التعرف على الشبق فى الجاموس المصرى

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استخدم فى هذه الدراسة ٥٧ جاموسة قسمت إلى مجموعتين تبعاً لموسم الولادة ، حيث تم تقسيم العام إلى فصلين : بارد (نوفمبر - أبريل ،٢٩ جاموسة) وحار (مايو - أكتوبر ، ٢٨ جاموسة). تم اجراء عمليات التشميم للتعرف على الحيوانات فى دور الشبق بمعدل أربع مرات يومياً فى السادسة صباحاً ، الثانية عشرة ظهراً ، السادسة مساءاً ، الثانية عشرة عند منتصف الليل فى وجود ذكر كشاف. وتم اعتبار ثبات الأنثى للذكر هو المظهر الأساسى للشياع بغض النظر عن ظهور الأعراض الأخرى. تم جمع عينات الدم بانتظام بمعدل مرة كل ٣-٤ أيام لتقدير هرمون البروجستيرون لمتابعة النشاط المبيضى خلال فترة التجربة التى امتدت لمدة ٦ شهور بعد الولادة . تم استخدام النتائج المتحصل عليها من اكتشاف الشبق أربع مرات يومياالاختبار كفاءة اكتشاف الشبق إذا تمت مراقبته مرة أو مرتين أو ثلاث مرات .كما تمت دراسة كل الاحتمالات الناتجة من تباديل أوقات مراقبة الشياع (عددها ١٥) لتحديد أكثرها كفاءة.

وأشارت النتائج إلى أن :

١) متابعة النشاط المبيضى تحت نظام مراقبة الشياع أربعة مرات/ اليوم أتاح كشف ٨٨% فقط من جملة االتبويضات المصحوبة بمظاهر الشبق .

٢) زيادة تكرار ملاحظة الشياع يُحسن معنوياً (٠١. %) من نسبة حالات الشياع المكتشفة

٣) كان الحد الأقصي لحالات إكتشاف الشبق عند مراقبة الشياع مرة واحدة فى اليوم هو٦٠% مقابل ٨٧,٨، ٩٥,٨ ، ١٠٠ % بالنسبة لمراقبة الشبق مرتين أو ثلاثة أو أربع مرات على التوالى.

٤) كان لفصل السنة تأثير على الوقت الأمثل لمراقبة الشياع ، ففى الفصل الحار أظهرت الحيوانات السلوك الجنسى فى الساعة ٦ صباحاً و الساعة١٢ ليلاً بينما فى الفصل البارد أظهرت الحيوانات نشاطها الشبقى فى الساعة ٦ صباحا ة الساعة ١٢ ظهراً.

٥) مراقبة الشياع مرتين يومياً في السادسة صباحاً و السادسة مساءاً كان لها كفاءة مماثلة لتلك المتحصل عليها عند مراقبة الشياع ثلاث مرات يومياً ، مما يجعلها الأمثل للتطبيق فى مزارع الجاموس.