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DOSE TIME OF BREEDING AFFECT THE INCIDENCE OF EARLY EMBRYONIC MORTALITY AND REPRODUCTIVE PERFORMANCE IN BUFFALO?

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ABSTRACT

This work aimed at studying the relationship between time of breeding and early embryonic losses in buffalo. The Effect of this time on some reproductive traits was also studied. For that, 32 clinically normal buffalo calvers were used. All buffalo cows calved normally and no cases of retained placenta were recorded. Heat detection was performed throughout the 24-hours of the day-round using a TV closed circuit unit. Heat detection was started from the 10th day post-partum. A voluntary waiting period of 40 days was allowed to the dams before breeding. Blood samples were collected from the jagular vein at 5-days intervals for the determination of P_4 concentration. An additional blood sample was obtained on the day of estrus to confirm the occurrence of heat. In addition, a vasectomized buffalo bull was used as an aid for heat detection. The buffalo cows were randomly assigned into 4 groups according to the time of breeding. The dams of the comparable groups were bred at 3.0±0.4 hrs (G_1, n=9); 11.6±0.5 hrs (G_2, n=8); 16.2±0.9 hrs (G_3, n=7) and 24.7±1.1 hrs (G_4, n=8) after the beginning of standing heat using a fertile buffalo bull. Rectal palpation was performed on the same days of blood sampling. Pregnancy was diagnosed per-rectum 45-60 days post service. The incidence of early embryonic losses was assessed on the basis of both rectal palpation results and P_4 concentration (when its level was sustained above 1ng/ml for 45 days) after a successful service, then abrupt decline to ≤ 0.5 ng did occur. The Effect of time of breeding on the days-open, number heats to-conception, number of services/conception, conception rate and calving interval was also studied.

It has been shown that the incidence of early embryonic losses of this study was 9.4%. All cases of early embryonic losses were confined to G_1 as the breeding were performed at an early stage of heat (after 3.0±0.4 hrs from the onset of standing estrus). This constituted 33.3% of the dams belonging to G_1. No cases of embryonic losses were detected in G_2, G_3 and G_4. The corresponding conception rates to the 1st service were 44.4, 100, 85.7 and 75% for the comparable groups, respectively. Too late breeding in G_4 resulted in the highest days-open and longest calving interval.

It could be concluded that breeding buffalo too early during estrus may result in a higher incidence of early embryonic losses and inferior conception rate. Too late breeding may result in high days-open and long calving interval. Such that, in buffalo too early or too late breeding should be avoided. Optimum reproductive performance of buffalo could be achieved when breeding is to be applied 11-16 hrs after the beginning of estrus.

INTRODUCTION

It has been early reported by Hafez (1952) that the high frequency of too long estrous cycles in buffalo (up-to 3-5 months) may be due to early embryonic mortality. In another study, El-Fouly (1966) recorded an embryonic mortality rate of 13.1% during the first 4- months post-coitus in buffalo heifers.

Ten percent embryonic losses was also recorded by El-Sheikh and El-Fouly (1971) for the same animal. They reported that about 5% of the total-time loss from 1st to fertile service was attributed to embryonic mortality. It was also shown that about 15% of long ovulatory cycles (more than 35 days) was due to early embryonic mortality (Mohamed, 1974). In a more recent study, El-Shafie et al (1983) found that 34% of loss in time between 1st to fertile service resulted from early embryonic mortality which occurred at a rate of 19%.

Barkawi (1984) indicated that low progesterone (P_4) levels may lead to embryonic atrophy during the 1st stage of pregnancy. It was also reported that 8.3% out of 60 buffalo cows showed P_4 levels higher than 1ng/ml for 46 days post service then sudden decline to its basal level (< 1.0 ng/ml) was reached (El-Wardani, 1995). It has been suggested that such continuation of high P_4 level may reflect the occurrence of pregnancy, which was followed by embryonic loss.

It is the authors concept that the increased incidence of early embryonic losses in buffalo may be a hidden reason responsible for the too long estrous cycles reported for this animal in the pervious literature. In addition, factors responsible for losses of early embryos are many and interrelated.

This paper aimed at answering the questions that: Does time of breeding affect the viability and subsequent survival of buffalo embryos during the early stages of pregnancy? and to what extent does time of breeding affect the reproductive performance in this animal?

MATERIAL AND METHODS

This work was conducted at Mehallet Moussa Research Station, Kafr El-Sheikh Governorate. The farm belonges to Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture.

Experimental Animals and Management:

Thirty two post-partum buffalo cows were used. They ranged between 5-12 years old and 600-700 kg body weight. All buffalo cows calved normally and no cases of retained placenta were recorded. Two weeks after calving, the dams were examined per-rectum at weekly intervals until pregnancy was confirmed (45-60 days after breeding).

The animals were fed according to their body weight and milk production (APRI, 1997). Fresh drinking water was available three times daily (at 7.00 a.m., 1.30 p.m. and 4.00 p.m.). The buffalo cows were kept free in a 20 × 15 m yard-barn with counter-slope asbestos sheds of 4 meters height.

Reproductive Management:

1- Observation of estrus:

Starting from the 10th day post-partum, buffalo cows were checked for estrus signs throughout the 24 hours of the day–round using a TV closed circuit unit. In addition, a vasectomized buffalo bull was introduced to the female group at six hrs. intervals (i.e., at 6.00 a.m., 12.00 noon, 6.00 p.m. and 12.00 midnight) as a heat detection aid. The vasectomized buffalo bull stayed within the females for an hour per each detection round.

2- Time of breeding

The buffalo cows were allowed to a- 40 days post calving voluntary waiting period. Then, they were randomly assigned into 4 groups according to the time of breeding (Table 1).

Table 1: Mean time of breeding (hr.) for the different female groups.

Group No.	No. of females	Mean time of breeding "hr." (After the onset of standing heat)
1	9	3.0±0.4
2	8	11.6±0.5
3	7	16.2±0.9
4	8	24.7±1.1

3- Clinical examination of the female reproductive tract:

Starting from the 3rd week post-partum, rectal palpation of the female reproductive tract was performed at weekly intervals. The uterine tone, size and location were evaluated. In addition, the ovarian structures including: follicles, corpora lutea and cysts were diagnosed.

Pregnancy was diagnosed 45-60 days post-service depending on the presence of one or more of the positive signs of pregnancy at this stage of gestation.

Blood sampling:

A total of 458 blood samples were collected from the jugular vein throughout the period of study and analyzed for progesterone (P4) concentration. Each blood sample (5 ml) was collected in a glass tube containing EDTA as an anticoagulant at 5-days intervals. Additional blood sample was obtained on the day of heat. Immediately the samples were transferred to the lab and centrifuged at 3000 rpm for 5 min. then, the plasma was separated, packaged in 5-ml glass vials and stored at -20° C in the deep freezer until the time of analysis.

Determination of progesterone (P4) concentration:

Direct radio– immunoassay technique was performed for plasma P4 using ready antibody coated tubes kit "Diagnostic systems labs Inc., Webster, Texas, U.S.A.".

Assessment of the reproductive traits:

The number of days-open (time interval between parturition and the day of conception); number of heats-to fertile service/animal; conception rates-to 1st service and calving interval (CI) were recorded for each comparable group.

The incidence of early embryonic mortality (EEM) was determined by P_4 concentration when its level was sustained above 1 ng/ml for > 45 days after service, then, followed by an abrupt decline in this level to< 0.1 ng/ml. The results of P_4 profiles were matched with those of the rectal palpation and field observations to confirm the findings of all traits studied.

Statistical analysis:

The data were subjected to statistical analysis using GLM Procedure/SAS Program (1998). The actual means as well as the ANOVA between the comparable groups for each trait were computed. The χ^2 test was used to determine the statistical significance between the traits expressed as percentages. Duncan's Multiple Range Test was performed for the traits showing statistical significance.

RESULTS AND DISCUSSION

The effect of time of breeding on the number of heats to-fertile service/animal; number of services/conception; number of days-open; conception rate to- the 1st service and calving interval is shown in Table 2.

Table 2: Impact of time of breeding on some reproductive traits (means ± SE) in Egyptian buffalo cows.

Group No.	Time of breeding (hrs)	No. heats to - fertile service	No.services/ conception	No. days-open	Conception rate- to the 1st service	Calving interval (days)
1	3.0± 0.4 (9)	2.2 ± 0.3 (20)	1.4 ± 0.2 (13)	70.4 ± 8.5 (9)	44.4 % ^{C}	384.7 ± 7.3
2	11.6 ±0.5 (8)	1.9 ±0.4 (15)	1.0± 0.0 (8)	66 .1± 8.0 (8)	100 % ^{A}	383.4±8.2
3	16.2 ±0.5 (7)	1.8 ± 0.3 (13)	1.1 ± 0.1 (8)	54.4 ±5.6 (7)	85.7% ^{A}	377.3 ± 4.2
4	24.7 ±1.1 (8)	2.1 ± 0.3 (17)	1.2 ± 0.2 (10)	72.1 ±9.7 (8)	75.0% ^{B}	389. 1± 8.6
Overall mean ± SE		2.03 ± 0.2 (65)	1.2 ± 0.1 (39/32)	66.3 ± 4.1 (32)	75.0 %	383.6 ±3.6

Figures in parenthesis indicate the number of observations.
Values bearing different superscripts in the same column differ significantly (P<0.05).

It could be seen that G_2 and G_3 were superior to G_1 and G_4 in all reproductive traits studied. These results came in agreement with those of El-Hariri (1973) who found that breeding buffalo too early or too late during estrus resulted in a lower conception rate and an increased number of services/conception. Regardless of the significance magnitude, G_3 had the lowest number of heats to-conception (1.8±0.3), shortest time of days open (54.4±5.6) and subsequently, the shortest calving interval (377.3±4.2 d). These results showed good harmony with the findings of Afifi (1988) who obtained the highest conception rate (57.2%) when inseminations were performed between 14-18 hr after the beginning of heat. Similar trends were also reported by Singh and Mishra (1983) for Indian buffalo.

The P_4 profile showed that 50% (16 out of the total 32 buffalo cows used in this study) had a short duration-low peak of P_4 prior to the onset of regular cyclicity of the animal (Fig. 1). This what is termed " P_4 hump" had a P_4 level of ≥1.0 ng/ml and a duration of 7-14 days. This finding is in complete agreement with El-Wardani (1995). It has been postulated that the source of this P_4 spikes could be the adrenal gland or the lutein cells in the ovary

(Corah *et al.*, 1974 and Kaur and Arora, 1982). This minor elevation of P_4 may be responsible for sensitizing the central nervous system to the follicular estrogens of the ovary to facilitate estrus manifestation (Kaur and Arora, 1982). The other probability is that these P_4 spikes may play an important role in the initiation of ovarian cycles (Peters and Lamming, 1984).

The present results showed that the P_4 concentration reached its minimum level (0.14±0.02 ng/ml) during estrus. This concentration ranged from a non-detectable level- to 0.52 ng/ml during this phase. The P_4 peak of mid luteal phase ranged from 2.2±0.5 to 3.5±0.7 ng/ml with an overall mean of 3.1±0.3 ng/ml. The P_4 peak during the 1st 2 months of pregnancy ranged from 2.0-9.5 ng/ml with an overall mean of 4.8±0.4 ng/ml. These results agreed with those reported by Kaur and Arora (1982) in Indian buffalo and El-Wardani (1995) in Egyptian buffalo.

Early embryonic mortality (EEM):

The incidence of EEM as determined by the blood P_4 profiles and confirmed by rectal palpation was 9.4% (3 out of the 32 buffalo cows). Interestingly, all these cases belonged to G_1 (animals No. 4, 5 and 9). In these cases, the buffalo cows showed true (ovulatory) heats, bred by the fertile bull and then followed-up for pregnancy confirmation (Per-rectum 45-days post service). All of them were diagnosed pregnant (the result which coincided with the P_4 profiles). The P_4 continued at a high level (the peak ranged from 2.5-4.3 ng/ml) for 47-53 days post service when the P_4 abruptly declined to its basal level (ranging from non-detectable level to 0.2 ng/ml) and eventually the buffalo cows got in heat (Figures 3, 4 and 5).

These results are almost similar with those reported by El-Wardani (1995) who recorded an incidence of 8.3% for EEM in buffalo. On the other hand, the current results were superior to the early findings of Hafez (1952) who ascribed the occurrence of high frequency of long estrous cycles (between 91-98 days) to embryonic losses and El-Sheikh and El-Fouly (1971) who reported 10% embryonic losses in buffalo heifers. The present incidence of EEM is also lower than those of Mohamed (1974) and El-Shafie at al., (1983) who recorded percentages of 15.4% and 19% for this case, respectively.

It should be mentioned that the probable reasons responsible for EEM are many and interrelated. Of them, are the nutritional status of the dam, high ambient temperature, fever of the dam and impaired estrogen/progesterone ratio. One or more of these factors would result in a retarded growth of the conceptus (less than 15 mm before day 15-17 after fertilization), leading to decreased secretion of bovine trophoblast protein (the luteotrophic factor secreted by the conceptus and responsible for its survival) by preventing CI regression during that critical phase up-to 35-45 days of pregnancy (Bazer *et al.*, 1991).

In the present study, the too early breeding (during estrus) in G_1 may be responsible for aging of spermatozoa during the transit time in the female reproductive tract until the ovulation occurs. Hence, even if the fertilization occurs, the unhealthy retarded embryo may not have the ability to survive and continue its early life.

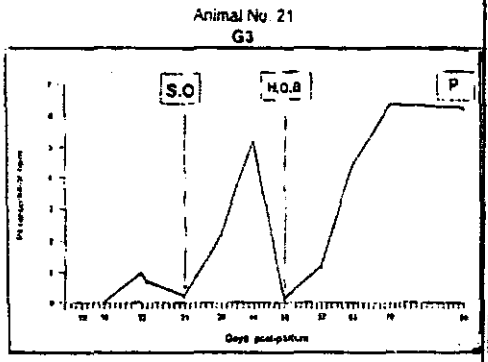

Figure 1: Plasma P4 profile showing a P4 hump prior to resumption of normal cyclicity.

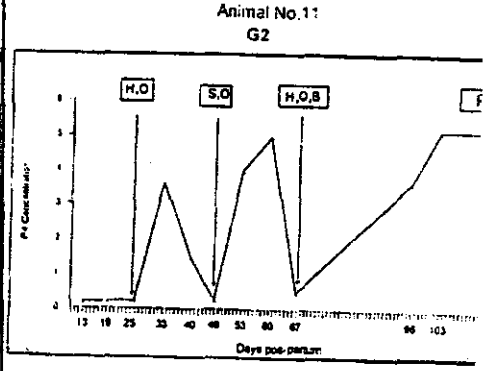

Figure 2: Plasma P4 profile showing normal cyclicity of a buffalo cow prior to conception.

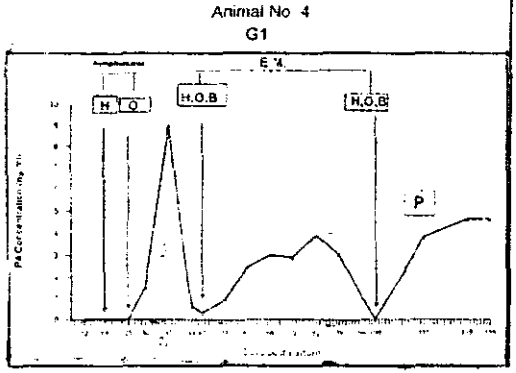

Figure 3: Plasma P4 profile showing embryonic mortality after 55 days of gestation.

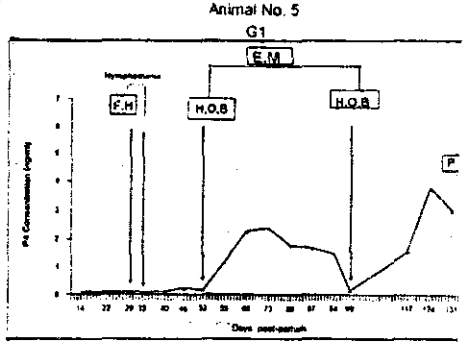

Figure 4: Plasma P4 profile showing embryonic mortality after 47 days of gestation.

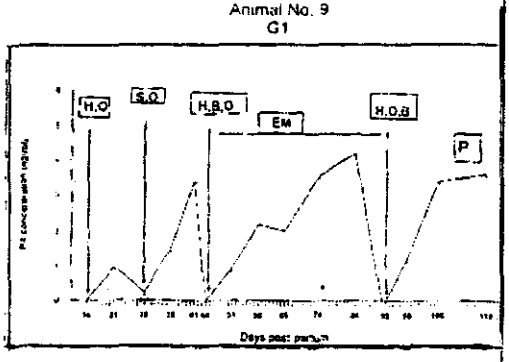

Figure 5: Plasma P4 profile showing embryonic mortality after 48 days of gestation.

H = Heat

O = Ovulation

S.O.= Silent ovulation

B = Breeding

E.M.= Embryonic mortality

P = Pregnancy

It may be expected that the current incidence of EEM (9.4%) is lower than the real figure (if the altra-sonography as a more precise tool for diagnosing the case, was used). Thus, specific further studies in this respect are urgently needed.

It could be concluded that breeding buffalo cows 11-16 hrs after the onset of heat is suitable for this animal. Too early or too late breeding should be avoided. This would contribute to normal cyclicity of the dams, short days-open, best conception rates, low incidence of EEM and finally short calving intervals.

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تأثير ميعاد التلقيح على معدلات النفوق المبكر للأجنة، والأداء التناسلى للجاموس خلال فترة ما بعد الوضع

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أجريت هذه الدراسة بمحطة بحوث الإنتاج الحيواني بمحلة موسى / كفر الشيخ - التابعة لمعهد بحوث الإنتاج الحيواني - مركز البحوث الزراعية - وزارة الزراعه. وقد استهدف البحث دراسة تأثير ميعاد التلقيح على معدلات النفوق المبكر للأجنة ، وعلاقة ذلك بالأداء التناسلي للجاموس خلال فترة ما بعد الوضع. وقد استخدم فى هذه الدراسة عدد ٣٢ جاموسة سليمة من الناحية الصحية، وأعطت ولادات طبيعية، ولم تسجل لها أى من حالات احتباس المشيمة. وقد استمرت الدراسه خلال الفتره من سبتمبر/ ١٩٩٧ حتى يونيه/ ١٩٩٨ . هذا، وقد حصلت هذه الأمهات على مقرراتها الغذائيه تبعا لتوصيات معهد بحوث الانتاج الحيوانى (١٩٩٧). كذلك فقد تم مراقبة الشياع لهذه الإناث على مدار الـ ٢٤ ساعة باستخدام كاميرا متصله بدائره تليفزيونيه مغلقه، وقد بدأت أعمال مراقبة الشياع من اليوم العاشر بعد الولاده ، كذلك فقد تم منح هذه الإناث فترة انتظار قدرها ٤٠ يوما قبل التلقيح. وتم جمع عينات دم من الوريد الوداجى بمعدل مره كل خمسة أيام مع الحصول على عينه اضافيه اثناء فترة الشياع وذلك لتقدير مستوى هرمون البروجسترون. كذلك فقد تم الاستعانه بطلوقه جاموسى مقطوع الوعاء الناقل للمساعده فى التيقن من حدوث الشياع.

وقد تم تقسيم الجاموس الى ٤ مجموعات مقارنه تبعا لميعاد التلقيح ، حيث تم تلقيح جاموس المجموعة الأولى (م١: ن=٩) بعد ٣,٠ ± ٠,٤ ساعه ، والمجموعه الثانيه (م٢: ن =٨) بعد ١١,٦ ± ٥. ٠ ساعه ، والمجموعه الثالثه (م٣: ن= ٧) بعد ١٦,٢ ± ٠,٩ ساعه، والمجموعه الرابعه (ع٤: ن= ٨) بعد ٢٤,٧ ± ١,١ ساعه من بداية فترة الشياع باستخدام طلوقه صحيح تناسليا. وقد تم اجراء عملية الفحص التناسلى عن طريق المستقيم فى نفس اليوم الذى تم فيه الحصول على عينات الدم، وقد تم تشخيص الحمل بعد ٤٥ الى ٦٠ يوم من التلقيح، كذلك فقد قدرت معدلات موت الأجنه على أساس كل من نتائج الفحص المستقيمى، وكذلك مستويات هرمون البروجسترون (عند وصول تركيزه الى أكثر من ١٠٠ نانو جرام / مل لمدة ٤٥ يوما من التلقيح- يكون متبوعا بانخفاض فجائى ليصل الى أقل من ٠,٥ نانو جرام / مل). كذلك فقد تم دراسة تأثير وقت التلقيح على كل من عدد الأيام المفتوحه، عدد مرات الشياع حتى الاخصاب، عدد التلقيحات اللازمه للإخصاب، معدل الأخصاب، الفترة بين ولادتين.

وقد أظهرت النتائج أن المعدل الكلى لفقد الأجنة فى الدراسة هو ٩,٤ % ، وكانت جميع هذه الحالات مقصورة على حيوانات المجموعة الأولى- حيث تم تلقيح هذه الحيوانات فى مرحله مبكرة من بداية فترة الشياع ، وشكل هذا ٣٣,٣ % من الجاموس الذى ينتمى لهذه المجموعة. هذا، ولم تسجل أى حالات لفقد الأجنة في المجاميع الثلاثة الأخرى. وكانت معدلات الإخصاب الناتجة من أول تلقيحه للمجموعات الأربعة هي ٤٤,١ ، ١٠٠ ، ٨٥,٧ ، و٧٥% - على الترتيب. وقد أوضحت النتائج أن التلقيح المتأخر فى المجموعة الرابعة أدى الى استطالة كل من فترة الأيام المفتوحة، وكذا الفترة بين ولادتين.

وهكذا، فانه يمكن استنتاج أن التلقيح المبكر أكثر من اللازم، يؤدى الى زيادة معدلات فقد الأجنة، وخفض معدلات الإخصاب في الجاموس، كما أن التلقيح المتأخر بدرجه أكثر من اللازم قد يؤدى الى زيادة فترة الأيام المفتوحة، واستطالة الفترة بين ولادتين. وبالتالي، فلابد من تلافى التلقيح المبكر أو المتأخر بدرجه أكثر من اللازم. كذلك فان الأداء التناسلي الأفضل يمكن الحصول عليه عند تلقيح الجاموس بعد ١١ - ١٦ ساعة من بداية فترة الشياع.