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## EARLY PREGNANCY DIAGNOSIS IN BUFFALOES FROM SERUM PROGESTERONE CONCENTRATION

(With Two Tables)

By

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(Received at 30/3/1995)

التشخيص المبكر للحمل في الجاموس بقياس تركيز هرمون البروجستيرون في مصل الدم

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أجريت هذه الدراسة على عدد ١٩ جاموسة غير عشار حلابة ومنظمة في دورة الشبق وفي حالة صحية جيدة. تراوح عمر هذه الحيوانات بين ٥-٨ سنوات وأعطت من ٣-٦ ولادات. أخذت عينات دم من هذه الحيوانات أثناء الشيع (اليوم صفر) ثم في اليوم الثامن بعد الشيع وكذلك اليوم ٢١ بعد التوثيب الطبيعي ، وتم فصل مصل الدم لتحديد تركيز هرمون البروجستيرون بواسطة طريقة إختبار المناعة الأشعاعية. عند ظهور علامات الشيع على أي من هذه الحيوانات عرضت في الوقت المناسب للتوثيب الطبيعي من طلوقة جاموسي سليم وخصب وتم عزلها بعد ذلك لمدة ٦٠ يوم لتشخيص الحمل بالجنس عن طريق المستقيم. كانت دقة إختبار البروجستيرون في مصل الدم عند اليوم ٢١ بعد التوثيب الطبيعي في تشخيص الحالات غير العشار مرتفعة (٨٥٪). كانت الدقة الكلية لإختبار البروجستيرون في مصل الدم عند اليوم ٢١ بعد التوثيب الطبيعي لتشخيص الحالات العشار (٧٠٪).

### SUMMARY

The present study was carried out on 19 non pregnant regular cycling lactating and healthy buffalo-cows. They aged 5-8 years and gave from 3-6 births. During estrus (day 0), day 8 and day 21 after estrus blood samples

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were taken from each buffalo-cow and serum progesterone was determined by radioimmuno-assay. Each buffalo-cow manifested estrus was subjected to natural service at the suitable time by a sound good fertile buffalo bull and isolated for 60 days till rectal palpation was performed to confirm pregnancy. Non pregnancy can be predicted with a high degree of accuracy (85.7%) at 21 days after service. The overall accuracy of predicting pregnancy by serum progesterone test in buffaloes was 70%.

**Keywords:** Early, pregnancy diagnosis, buffaloes, serum progesterone.

### INTRODUCTION

In cattle the most wide spread method of pregnancy diagnosis have depended on non-return to estrus and subsequent confirmation by rectal palpation, then it became evident that the concentration of progesterone in blood (ROBERTSON and SARDA, 1971) or milk (HEAP *et al.*, 1973) at 20 to 24 days after service can be used as an early indicator of pregnancy in cattle.

In the buffaloes where the returns to estrus are often unobserved due to poor expression of estrus signs, a reliable test for early pregnancy diagnosis would be particularly useful (PERERA *et al.*, 1977). ARORA *et al.* (1980) found that visual detection of estrus in buffaloes has become less reliable as the animals manifest estrus weakly and

therefore the common methods used for pregnancy diagnosis in buffaloes were the non return to estrus or reproductive tract palpation by skilled personnel. The disadvantages of these methods are the inability of the herdsmen to detect estrus and a delay of 50-60 days for rectal palpation. PERERA *et al.*, (1980) concluded that the determination of plasma progesterone concentration 21 days after insemination was an accurate method of predicting non pregnancy in buffaloes and a progesterone concentrations more than 1.0 ng/ml were predicted pregnant while concentrations less than 0.7 ng/ml were predicted non pregnant and values within the range 0.7 to 1.0 ng/ml were doubtful. The present study has been undertaken to determine the reliability of serum progester-

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one for diagnosing pregnancy or non pregnancy in buffaloes.

#### MATERIAL and METHODS

A total of 19 non pregnant regular cycling lactating healthy buffalo-cows were included in the present work. The buffalo-cows aged 5-8 years weighed 500-600 kg and gave from 3-6 births. They were selected from a herd kept at the farm of the Faculty of Veterinary Medicine. The selected buffaloes were kept under the same nutritional and managerial conditions during the course of the experiment. Each buffalo-cow was gynaecologically examined according to ZEMJANIS (1970). The principles recommended by ROBERTS (1982) to confirm the absence of cervicitis and endometritis were taken. Blood samples were collected from the jugular vein of each buffalo-cow during day 0 (day of estrus), day 8 post ovulation (to confirm that the animal in a true estrus) and day 21 after natural service (to predict pregnant and non pregnant animals) and allowed to clot and cen-

trifugated at 3000 r.p.m for 15 minutes for separation of serum. The separated serum was stored at -20 °C till processed further. Each buffalo-cow manifesting estrus (confirmed by receptivity toward the male, appearance of estrus signs and a palpable Graafian follicle and turgid uterus by rectal palpation) was subjected to natural mating by a sound good fertile buffalo bull common to the farm. The animals were kept away from buffalo bulls from the time of mating till 60 days when they were confirmed for pregnancy by rectal palpation. Progesterone in serum was determined by radioimmunoassay technique according to ABRAHAM *et al.*, (1971).

The obtained data concerning the mean  $\pm$  standard error were obtained according to SNEDECOR and COCHRAN (1967).

#### RESULTS

Due to the great variation in progesterone concentration at day 21 after natural mating for animals predicted pregnant therefore they were classified into 3 groups a,b

and c (Table 1 and 2). Group a: included four buffalo-cows predicted pregnant from their mean progesterone concentration at day 21 post service ( $3.61 \pm 0.9$  ng/ml), Group b: included three buffaloes predicted pregnant from their mean progesterone concentration at day 21 after natural service ( $4.63 \pm 1.85$  ng/ml) and Group c: included also three buffalo-cows predicted pregnant from their mean progesterone concentration at day 21 after service ( $6.84 \pm 1.01$  ng/ml).

As shown in table (1) all predicted animals as pregnant, non pregnant and doubtful manifested true estrus from their progesterone concentration at days 0 and 8 after natural service.

According to PERERA *et al.* (1980) at day 21 after fixed time artificial insemination progesterone concentration more than 1.0 ng/ml serum was predicted pregnant and less than 0.7 ng/ml serum was predicted non pregnant and values within the range 0.7 to 1.0 ng/ml serum were doubtful. From table (2) 10 buffaloes were predicted pregnant, however, 7 of them were confirmed pregnant by rectal pal-

pation 60 days after natural mating with an overall accuracy 70%. 7 buffalo-cows were predicted non pregnant 6 of them were confirmed by rectal palpation with an accuracy 85.7%. Two buffaloes were doubtful and were proved pregnant by rectal palpation.

## DISCUSSION

In the present study (Table, 2) the mean serum progesterone concentrations of pregnant buffaloes at 21 days after service vary from  $3.61 \pm 0.9$  ng/ml to  $6.84 \pm 1.01$  ng/ml. These obtained data were higher than those obtained by PERERA *et al.* (1980). However the mean serum progesterone concentration for non pregnant animals at 21 days after service ( $0.4 \pm 0.07$  ng/ml) was nearly similar to those obtained by PERERA *et al.* (1980).

The accuracy of predicting non pregnancy on the basis of low progesterone levels at 21 days was found to be high (85.7%) and compare favourably with the results obtained using plasma or milk in cattle (WISHART *et al.*, 1975 and HEAP *et al.*, 1976). The single animal that was found pregnant in

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spite of a progesterone concentration less than 0.7 ng/ml at 21 days after service remain an enigma. Similar cases have been reported in cattle (ROCHE *et al.*, 1978 and PENGELLY, 1979). It is possible that this reflect a true physiological occurrence, there is also the possibility of changes in hormone level after the sample was collected.

The overall accuracy of predicting pregnancy on the basis of high progesterone level at 21 days after service was only 70%. Similar results was reported in buffaloes (PERERA *et al.*, 1980). it is lower than that reported in cattle where an accuracy of 73% (PENNINGTON *et al.*, 1976), 83% (WISHART *et al.*, 1975) can be expected. The lower accuracy of predicting pregnancy was thought to be due mainly to embryonic death occurring in the interval between sampling and rectal palpation (POPE *et al.*, 1976).

As a conclusion, non pregnancy can be predicted in buffaloes with a high degree of accuracy when serum progesterone concentrations are below 0.7 ng/ml at 21 days af-

ter service according to Perera *et al.*, (1980). it was also possible to detect at 21 days almost one third of the animals which were found to be non pregnant at 60 days.

This is particularly useful in buffaloes where poor external manifestation of estrus (ARORA *et al.*, 1980) prevent identification of a high proportion of those returning to heat.

The low accuracy of serum progesterone test in predicting pregnancy makes rectal palpation at a later stage essential for detecting animals giving false positive results.

The early identification of non pregnant animals by the progesterone test reduces the number of animals that need to be examined per rectum for routine pregnancy diagnosis.

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**Table 1: Mean ( $\pm$  S.E.) Serum progesterone concentration (ng/ml) for predicted pregnant, non pregnant and doubtful buffaloes at days 0, 8 and 21 respectively.**

			n	Predicted buffaloes by prog. test
21	8	0		
3.61 $\pm$ 0.9	2.87 $\pm$ 0.3	0.44 $\pm$ 0.12	4	Pregnant
4.63 $\pm$ 1.85	2.9 $\pm$ 0.26	0.43 $\pm$ 0.08	3	Group a:
6.84 $\pm$ 1.01	3.8 $\pm$ 0.34	0.82 $\pm$ 0.09	3	Group b:
0.4 $\pm$ 0.07	1.9 $\pm$ 0.2	0.43 $\pm$ 0.1	7	Group c:
0.82	1.94	0.34	2	Non pregnant
(0.813-0.828)	(1.853-2.021)	(0.317-0.369)		Doubtful

N.B.: (n) for non pregnant buffaloes at day 0 & 8 was 6 for each

Table 2: Accuracy of predicting pregnancy and non pregnancy in buffaloes from serum progesterone concentration at day 21 after service.

Confirmation by rectal palpation				Mean prog. (ng/ml)	n	Predicted buffaloes by prog. test
Non pregnant		Pregnant				
%	No.	%	No.			
25	1	75	3	3.61 ± 0.9	4	Pregnant
33.3	1	66.7	2	4.63 ± 1.85	3	Group a:
33.3	1	66.7	2	6.84 ± 1.01	3	Group b:
30	3	70	7	4.89	10	Group c:
-	-	100	2	0.82	2	Subtotal
85.7	6	14.3	1	0.4 ± 0.07	7	Doubtful
47.4	9	52.6	10	-	19	Non pregnant
						Total

**N.B.:**

This classification was according to Perera *et al.* (1980).

Progesterone value at day 21 for pregnant cow in the non pregnant group was 0.542 ng/ml.