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USE OF MILK PROGESTERONE ASSAY FOR MONITORING OESTRUS AND EARLY PREGNANCY IN NILI-RAVI BUFFALOES

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ABSTRACT

In this study, the use of milk progesterone assay for oestrus detection and early pregnancy diagnosis in Nili-Ravi buffaloes was investigated. For this purpose, 100 milking buffaloes with clinically normal reproductive tract were used. Oestrus was detected on the basis of visual signs and uterine changes observed through rectal palpation. These buffaloes were then inseminated on day of oestrus. Three milk samples were taken from each buffalo, one each on day of insemination and then on 10-12 and 21-22 days post insemination. These animals were rectally examined for pregnancy 60 days after insemination. The milk samples were analyzed for progesterone concentrations through RIA. Out of 100 buffaloes observed to be in oestrus on the basis of visual signs and rectal examination, 94% showed the milk progesterone concentrations ranging from 0.01 to 0.90 ng/ml, whereas in only 6% animals, milk progesterone concentrations ranged from 3.31 to 7.44 ng/ml. Thus, 6% buffaloes seemed to have been inseminated during luteal phase. On day 21-22, out of 84 buffaloes examined, 50(59.52%) showed the milk progesterone concentrations >2.0 ng/ml and were assumed to be pregnant. However, when 41 out of these 50 animals were rectally examined 60 days post insemination, 36(87.81%) were found pregnant. Perhaps there was early embryonic death in the remaining 5(12.19%) buffaloes. It was concluded that milk progesterone assay may be used to evaluate the accuracy of clinically diagnosed oestrus, as well as for early pregnancy diagnosis in Nili-Ravi buffaloes.

Key words: Milk progesterone, oestrus, early pregnancy, buffaloes.

INTRODUCTION

The most common reproductive problem affecting reproductive efficiency of the buffalo is silent/sub-oestrus (Singh and Chandan, 1987) and its incidence in Pakistan was reported to be 33.30% (Samad *et al*., 1987). In buffaloes kept under field conditions, Qureshi (1998) recorded 51.1% cases of silent oestrus. Among these, 29.4% cases occurred in cows calved during peak breeding season, while 70.6% were recorded in those calved during low breeding season. Because of poor manifestation of behavioral oestrus signs (Kanai and Shimizu 1983), oestrus detection remains a problem in many females of this species.

Milk progesterone level has been used for oestrus detection, early pregnancy diagnosis and determination of luteal activity (Heap *et al*., 1976). Shah *et al*. (1990) reported 85% conception rates in buffaloes monitored for oestrus through determining milk progesterone levels compared with 52% in buffaloes inseminated on the basis of clinical signs of heat alone. Embryonic mortality usually takes place during first 14 days of pregnancy and length of oestrus cycle remains unaffected (Ayalon, 1978). If a viable embryo is present beyond this time, the life span of corpus luteum is prolonged and subsequent embryonic losses can be monitored by measuring peripheral progesterone levels.

The present work was executed to evaluate the accuracy of oestrus detection and pregnancy diagnosis on the basis of clinical examination and through milk progesterone levels.

MATERIALS AND METHODS

Experimental animals

For this study, 100 lactating Nili-Ravi buffaloes, brought for artificial insemination to the clinic of the Animal Reproduction Department, University of Agriculture, Faisalabad, were selected. In order to confirm their clinical status, these animals were examined for visual signs of heat i.e. bellowing, mucus discharge and swelling of vulva. Oestrus was confirmed through rectal examination of uterus and ovaries. Only buffaloes showing signs of true oestrus with clinically normal reproductive tract were included in the study.

Collection of milk samples

After heat detection, experimental animals were inseminated intrauterine using good quality liquid semen diluted in milk-egg yolk extender. About 5 ml of

milk sample was collected from each animal at the time of insemination (day 0 of oestrus cycle) in a sterilized sampling tube containing sodium azide as a presservative. Likewise, milk samples were also collected on days 10-12 (n=94) and 21-22 (n=84) post-insemination. Experimental animals were examined for pregnancy through rectal palpation 60 days post-insemination.

Immediately after collection, milk samples were centrifuged at 2000 rpm for 10 minutes, stored at 4ºC for 15 minutes and the fat was removed. These fat-free milk samples were stored at -20ºC until used for the determination of progesterone levels through radioimmunoassay (RIA).

Determination of milk progesterone

The progesterone levels in milk samples were determined through RIA, using the kit method. For this purpose, polypropylene tubes coated with progesterone antibodies provided with the RIA kit, were used. The standards used in the analysis covered the physiological milk progesterone concentration range (0.00 to 12.60 ng/ml) found in skim milk of most of the domesticated livestock species. From time to time, two freeze-dried skim milk samples were included with samples as external quality control. These quality controls were analyzed three times within an assay i.e. in the beginning, middle and at the end.

RESULTS AND DISCUSSION

Signs of oestrus

In the present study, bellowing alone was noted to be the only sign of oestrus in 23% buffaloes with average milk progesterone concentration of 0.41 ng/ml. Mucus discharge was observed in 27% animals having milk progesterone level of 0.17 ng/ml. Both bellowing and mucus discharge were noted in 46% animals with milk progesterone value of 0.49 ng/ml. Bellowing with mucus discharge and restlessness was noted in only 4% buffaloes showing milk progesterone level of 0.11 ng/ml. It means that mucus discharge with other signs occurred in 77% oestrus buffaloes, while bellowing with other signs was noted in 73% animals. Marked swelling of valva was observed in 39% buffaloes, while moderate to slight swelling was seen in 60% animals, only 1% buffaloes showed no vulvar swelling during oestrus.

Singh *et al*. (1984) and Chohan (1988) reported bellowing as a sign of oestrus in 58.7 and 52.27% buffaloes, respectively. Danell (1987) closely monitored oestrus symptoms in Surti buffaloes and noted bellowing in 27.6% and mucus discharge in 100% animals. Vulvar swelling was noted in 100% buffaloes. Minor discrepancies among results of various studies can be attributed to differences in breed and managemental practices. Since buffalo is a seasonal breeder (Noakes *et al*., 2001), environment also affects oestrus signs in this species.

Milk progesterone concentrations

In the present study, milk samples were collected from 100 buffaloes diagnosed to be in oestrus on the basis of visual signs and rectal examination of genitalia. Among these, 94% animals showed the milk progesterone concentrations less than 1 ng/ml (range 0.01 to 0.90 ng/ml). In 6% buffaloes, progesterone concentrations at the time of insemination were more than 1.00 ng/ml (range 3.31 to 7.44 ng/ml). The progesterone level of more than 1 ng/ml has been considered to occur during luteal phase (Rajamahendran *et al*., 1993). Thus, among animals assumed to be in oestrus on the basis of visual signs and rectal examination, 94% were in true oestrus, while 6% buffaloes were inseminated during luteal phase of the cycle. According to Rajamahendran *et al.* (1993), 32 out of 667 cows (4.80%) were inseminated during luteal phase. Since detection of oestrus on the basis of visual signs and rectal examination is based on the experience of the inseminator, its accuracy can vary from person to person. Nevertheless, rectal examination of the genitalia, which showed 94% accuracy in this study, remains the convenient and reasonably reliable method of oestrus detection in individually kept large animals.

Among 94 buffaloes confirmed to be in oestrus on the basis of milk progesterone levels, 45.75% buffaloes showed mean milk progesterone concentration of 0.04 ± 0.004 ng/ml on day of oestrus (Table 1). In 42.55% animals, milk progesterone concentration averaged 0.25 ± 0.016 ng/ml, while in the remaining 11.70% buffaloes it was 0.66 ± 0.03 ng/ml (Table 1). For 400 Nili-Ravi buffaloes, Shah *et al*. (1990) recorded progesterone concentration of 0.34 ± 0.12 ng/ml in whole milk at oestrus. According to Pope *et al*. (1976), at the time of oestrus, progesterone concentration was 0.86 ng/ml for whole milk and 0.14 ng/ml for skim milk. Qureshi *et al*. (2000) observed that milk progesterone level in buffaloes fell to as low as 0.10 ng/ml on day of oestrus, with mean value of 0.30 ± 0.98 ng/ml. This indicates that milk progesterone level at oestrus remains below 1.00 ng/ml.

Among 94 buffaloes examined during mid-cycle (10-12 day of oestrus cycle), milk progesterone concentration was 2.14 ± 0.08, 3.95 ± 0.46 and 6.04 ± 0.21 ng/ml in 36.17, 43.62 and 20.21% animals, respectively. Overall mean progesterone concentration during mid-cycle was 3.72 ± 0.16 ng/ml, the range was 1.12 to 8.31 ng/ml (Table 1). For 400 Nili-Ravi

Table 1: Mean milk progesterone concentrations (ng/ml) in Nili-Ravi buffaloes on the day of insemination and 10-12 and 21-22 days post insemination

No of samples (%)	Mean	Range
Day of insemination*		
43(45.75)	0.04 ± 0.004	0.01 – 0.10
40(42.55)	0.25 ± 0.016	0.11 – 0.48
11(11.70)	0.66 ± 0.030	0.52 – 0.90
Mean	0.215 ± 0.224	-
10-12 days post AI		
19 (20.21)	6.04 ± 0.21	5.09 – 8.31
41 (43.62)	3.95 ± 0.46	3.13 – 4.80
34 (36.17)	2.14 ± 0.08	1.12 – 2.94
Mean	3.72 ± 0.16	-
21-22 days post AI		
14 (16.67)	6.85 ± 0.32	5.07 – 8.57
27 (32.14)	4.08 ± 0.12	3.14 – 5.00
9 (10.71)	2.77 ± 0.05	2.64 – 2.92
34 (40.48)	0.14 ± 0.03	0.01 – 0.82
Mean	2.69 ± 0.29	-

- Data for 6 buffaloes with progesterone levels >1.00 ng/ml have been excluded.

buffaloes, Shah *et al*. (1990) reported progesterone concentration of 13.22 ± 6.74 ng/ml in whole milk during mid cycle. In fat free milk, progesterone concentration on day 11 of the cycle was 3.6 ng/ml (Vale *et al*., 1990).

A total of 84 buffaloes were examined on day 21-22 post insemination. Among these, milk progesterone concentration was 6.85 ± 0.32, 4.08 ± 0.12, 2.77 ± 0.05 and 0.14 ± 0.03 ng/ml in 16.67, 32.14, 10.71 and 40.48% animals, respectively. The overall mean milk progesterone level on day 21-22 post insemination in these animals was 2.69 ± 0.29 ng/ml, the range was 0.01 to 8.57 ng/ml. Thus, out of 84 buffaloes examined 21-22 days post insemination, in 50(59.52%) buffaloes milk progesterone level was above 2.5 ng/ml and these animals were assumed to be pregnant. In 34(40.48%) buffaloes the milk progesterone level was less than 1 ng/ml and these were considered as non pregnant.

Among 50 buffaloes that were assumed to be pregnant on the basis of milk progesterone concentrations (above 2 ng/ml) on day 21-22 post insemination, only 41 could be examined for pregnancy through rectal examination 60 days after insemination. Among these, 36(87.81%) were confirmed to be pregnant. Whether embryonic death have occurred in the remaining 5(12.19%) buffaloes, is not known. An accuracy rate of 82-88% have been reported in cows when pregnancy was diagnosed through milk progesterone levels 24 days after insemination (Heap *et al*., 1976).

In buffaloes that were confirmed pregnant 60 days after insemination, milk progesterone levels on day 0 (insemination day) were 0.13 ± 0.04 ng/ml versus 0.37 ± 0.17 ng/ml in those found non pregnant 60 days after insemination. The respective values for the two groups were 3.72 ± 0.16 versus 2.40 ± 0.34 ng/ml on day 10-12 and 4.36 ± 0.38 versus 1.53 ± 1.28 ng/ml on day 21-22 after insemination.

It was concluded that milk progesterone assay may be used to evaluate the accuracy of clinically diagnosed oestrus, as well as for early pregnancy diagnosis in Nili-Ravi buffaloes.

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