CHANGES OF FECAL AND PLASMA PROGESTERONE LEVELS IN SWAMP BUFFALO COWS (*BUBALUS BUBALIS*) DURING THE ESTROUS CYCLE AND PREGNANCY

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ABSTRACT

The fecal and plasma progesterone profiles during the estrous cycle and pregnancy in swamp buffalo (*Bubalus bubalis*) were investigated in this study. The stability of progesterone presented in blood obtained from pregnant cows was also examined. Five pregnant and 5 cycling cows were used. A 5-10 gm fecal sample along with 5 ml of blood was collected from each animal. Samples were taken from pregnant cows once weekly until parturition and from non-pregnant cows 3 times weekly for a period of 2 months. The plasma was separated from the blood and stored, along with the fecal sample at -20 C. The progesterone values were then measured using an enzyme immunoassay.

For the stability test, the progesterone concentrations in blood samples obtained from pregnant cows can be maintained in the room temperature (22-25 C) at least 24 hours. It is suggested that unlike dairy cow's blood samples, the buffalo ones are quite stable and their progesterone data are low and reliable if the assay method is sensitive enough.

For the reproductive monitoring, the results showed that the 5 cycling cows during the follicular phase, the plasma progesterone levels were found to fluctuate between 0.4-0.8 ng/ml and fecal progesterone between 50-100 ng/g; during the luteal phase, the plasma progesterone levels fluctuated between 1.5-3.0 ng/ml and the fecal progesterone fluctuated between 150-400 ng/g. And the pregnant test, 3 out of 5 cows had persistantly high levels of plasma and fecal progesterone indicating pregnancy, while the remaining 2 cows were found to be open. These data indicate that the fecal progesterone values from swamp buffaloes are nearly 100 folds greater than the plasma progesterone levels. This in turn indicates that the measurement of fecal progesterone can potentially be used for monitoring the reproductive status in swamp buffaloes.

Key words: buffalo, enzyme immunoassay, fece, progesterone, reproduction.

INTRODUCTION

Progesterone concentrations in blood are commonly used for monitoring the reproductive status in domestic animals. Unfortunately, the blood levels of progesterone in swamp buffaloes are quit low and the difference between those in the follicular phase and the luteal phase is small, leading easily for miss diagnosis (Avenell *et al.*, 1985; Chauhan *et al.*, 1985). One of reasons for such miss diagnosis may be due to the unproper storage of blood samples like dairy cows in which the progesterone presented in blood decreases dramaticly when stored at room temperature (Wisemen *et al.*,1982/1983; Choi *et al.*, 1989). In addition, the recent reports have demonstrated that the steroid contents in feces are much higher than those in blood (approximately 100-300 folds) and are applicable to evaluate the aminal reproduction (Lin *et al.*, 1991).

The present study was, therefore, designed with the following objectives: (a) to examine the stability of progesterone presented in heparizing blood of prengant buffaloes; (b) to investigate the fecal sample as a potential source of progesterone determination for monitoring the reproductive status in swamp buffaloes.

MATERIALS AND METHODS

Animals: Ten swamp buffaloes, 3-4 years old, were used in this study. Five of them were mated in May 1991 for pregnancy study and the other 5 were kept for the estrous study. They were well nourished in good health. A 5-10 g fecal sample along with 5 ml of blood was collected from each cow. Samples were taken from cyclic cows 3 times weekly for a period of 2 months (May-July 1991) and from pregnant ones once weekly until parturition. Fecal samples were collected from rectum directly and blood samples were obtained from jugular venipuncture and placed in heparinized tubes. The blood samples for stability test of progesterone were obtained from 3 pregnant cows. Following incubation at room temperature (22-25 C) and 4 C, the plasma was separated and stored at -20 C until assayed. The progesterone in plasma and fece were measured using an enzyme immunoassay described briefly as follows.

Extraction of progesterone from feces: Fecal samples, 0.5 gm, are added with 0.5 ml of distilled water and 4 ml of methanol in test tubes (16x125 mm), and vortexed for 30 minutes. To remove non-polar lipid, 3 ml of petroleum ether are added and vortexed for other 15 seconds. The tubes are frozen at -20 C for subsequent analysis. The methanol phase is used directly for the progesterone assay after appropriate dilution with assay buffer. The recovery of added 3H-progesterone was 88±5%.

Enzyme immunoassay (EIA) of progesterone: The procedure of the EIA for progesterone in plasma and feces is described briefly as follows (Lin *et al.*, 1991:

Prakesh *et al.*, 1992). The plasma samples and fecal methanol fractions are diluted appropriately with 0.1 M phosphate buffer, pH 7.0 (20-50 x for the plasma and 200-500 x for the fecal methanol fraction). All of the diluted samples are vortexed and then aliquots of 300 µl were added to antibody coated star tubes (Nunc 470319, Denmark) via duplicate pipetting. This is immediately followed by the addition of 500 µl progesterone-horse-radish peroxidase conjugate diluted in assay buffer to initiate a competition reaction for the coated antibody. The tubes are then incubated for 15 minutes at room temperature. Following incubation, separation of free from bound progesterone is achieved by decanting the tubes and washing three times with assay buffer. The amount of conjugate is determined by adding 1 ml OPD (o-phylenediamine dihydrochloride) substrate solution containing 0.003% H_2O_2 to each tube, allowing to incubate for further 15 minutes, and then terminating the reaction with 1 drop of 8N H_2SO_4. The optic density is measured at 492 nm and the amount of progesterone is obtained by comparing O.D. of the progesterone standards (1-1000 pg/ml).

The monoclonal antibody (code no. G7) was made from Balb/c mice against progesterone-11a-hemisuccinyl:bovine serum albumin (Steraloids, no. Q 3252). Out of 15 different steroids tested the cross-reactivity with progesterone was 100% and with all other steroids including 17a-OHprogesterone, 20a-OHprogestin, pregnanediol, pregnenolone, cortisol, corticosterone, testosterone, estradiol, estrone etc. was less than 0.1%. The intra- and inter-assay coefficiences of variation of this EIA system were 8% and 15%, respectively. The sensitivity was 1 pg/tube.

Statistical analysis: Averages were calculated as mean ± standard deviation and the significance of differences between means were evaluated using Student's t-test.

RESULTS AND DISCUSSION

Progesterone stability in blood: Quantitation of circulating levels of progesterone in animals is important for both research and clinical use. Only the valid data can reflect the real physiological condition of animals. Progesterone is very unstable in bovine blood, especially in dairy cows. The decline apparently begins immediately after collection and at 12-48 hour incubation in room temperature the progesterone averaged only 5% of the original data without incubation (Wilseman *et al.*, 1982/1983). Therefore, the collection of bovine blood samples for progesterone assays is very critical. In our study, concentrations of progesterone measured from 3 pregnant buffaloes indicated that heparinized blood incubated at 4 C and room temperature keeps consistent values over 24 hours and no significant differences are found (Fig. 1). It is suggested that the low progesterone levels is the character of the buffalo itself and they exist in blood stably.

Estrous cycle: There were only 10 cycles seen by observation and 13 cycles found by progesterone monitoring from the 5 animals during May-July 1991. The duration of estrous cycle of swamp buffaloes is 20.5±2.6 days, based on the fecal progesterone profile. In the follicular phase, the plasma progesterone levels were found to fluctuate between 0.4-0.8 ng/ml and fecal progesterone between 50-100 ng/g; whereas in the lutreal phase, those data increased to 1.5-3 ng/g and 150-400 ng/g, respectively. The pattern of plasma progesterone levels during estrous cycle in swamp buffaloes is similar to that in dairy cows, althought the progesterone levels during the luteal phase are about only a half lower than the dairy cows. These low values of progesterone during the luteal phase and pregnancy (ref. Figs 2 & 3), results in a marked reduction of the accuracy of diagnosis for monitoring the reproductive status of buffaloes (Avenell *et al.*, 1985; Chauhau *et al.*, 1985; Prakash *et al.*, 1992). However, it is clear from the fecal data that make the diagnosis more meaningful.

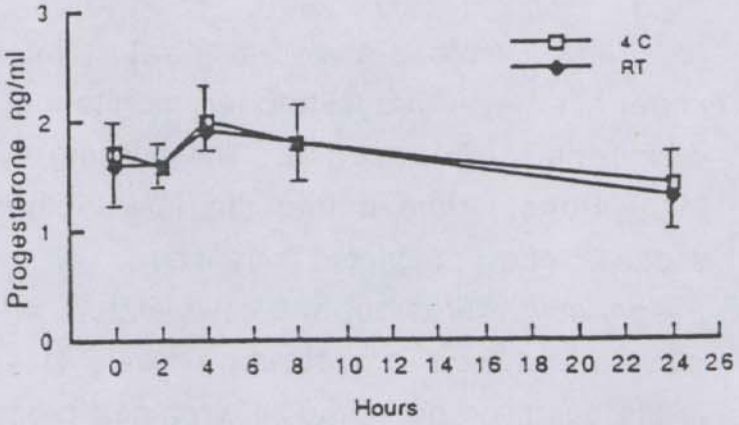

Figure 1. Progesterone concentrations of plasma samples obtained from 3 pregnant buffalo's bloods which were incubated at 4 C and room temperature for up to 24 hours before centrifugation.

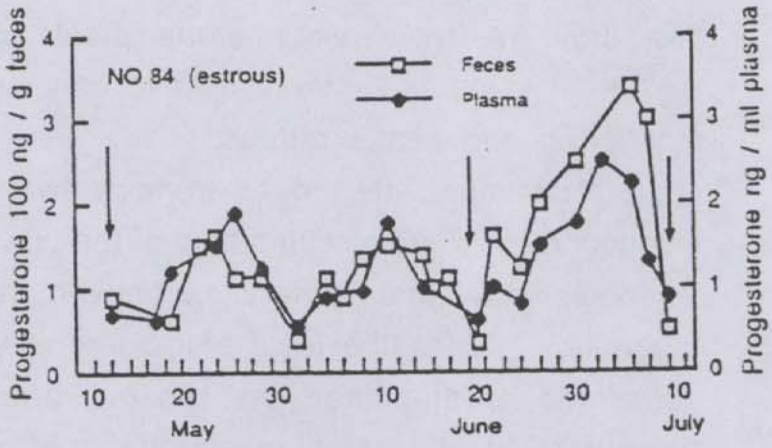

Figure 2. Correlation of progesterone profiles between plasma and fece in a cow (#84) which exhibited 4 ovarian cycles, but showed 3 estrous behaviors (arrows) only during the 2 month study.

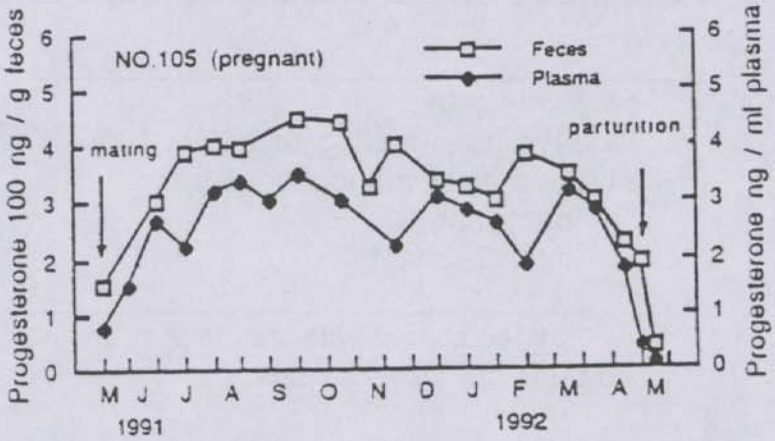

Figure 3. Correlation of progesterone profiles between plasma and fece samples in a cow (#105) which was mated on 15 May, 1991 and calved on 14 April, 1992. Her gestation period was 335 days.

An example is shown in Fig 2. The cow (#84) experienced 4 estrous cycles under the fecal progesteroner monitoring but showed 3 behavioral cycles only during the 2-month period. The missing cycle and the low values of plasma and fecal progesterone during the luteal plhase in May-June 1991 implies a weak seasonal effect presented in Taiwan.

Pregnancy: Three out of 5 cows mated in May 1991, and persisently high levels of plasma and fecal progesterone levels (1-4 ng/ml and 100-500 ng/g, respectively) in the gestation period. The averaged gestation period from these 3 pregnant cows (i.e. 335, 340 and 341 days, respectively) was 338.7±3.2 days which is about 9 days longer than the data (330 days) of Jainudeen and Hafez (1987) and 19 days longer than the record (320±10 days, N=30) of the Hualien Breeding Station. Such differences may be due to the small number used and the different methods made in this study. A typic progesterone profile during the gestation period is shown in Fig. 3. The other 2 cows failed to conceive were confirmed by the progesterone monitoring and estrus returns.

In summary, the progesterone in swamp buffalo is stable and can be used for monitoring the reproductive status of the cows, if the assay method is good enough to distinguish the small difference betwen the follicular phase and luteal phase or pregnancy. Since the fecal progesterone values from swamp bufalloes are nearly 100 folds greater than the plasma progesterone levels, suggesting that the measurement of fecal progesterone may produce more reliable results (Table 1). To regard this opinion, a further study with a large scale survey is needed.

Table 1: Characteristics of the progesterone levels (mean ± S.D.) in different reproductive status of swamp buffalo.

	plasma, ng/ml	fece, ng/g
on estrus (10)	0.4 ± 0.1	84 ± 33
14 days after mating or estrus (10)	1.4 ± 0.7 *	390 ± 78 *
21 days after mating or estrus		
pergnant (3)	1.3 ± 0.5 *	370 ± 21 *
non-pregnant (7)	0.7 ± 0.2	95 ± 31

* $P < 0.01$, compared with the data of on estrus. The number in parenthese indicates the animals used.

ACKNOWLEDGEMENTS

We thank financial supports from the cooperative science program between the National Science Council, China (80-0409-B002-88) and the Austrian Science Foundation, Austria.

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