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Research Article

Detection of early embryonic mortality through ultrasound scanning in water buffaloes*

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ABSTRACT

The objective of present experiment was to detect embryonic mortality by ultrasound scanning in water buffaloes during early stages of pregnancy. The uterus of freshly bred buffaloes (n=22) not returning to estrus 23 days after service were scanned by ultrasound scanner on days 23, 25, 28 and 30 post-service. The blood samples were collected on day 23 post-breeding for estimation of plasma progesterone. The reproductive tract of these animals was palpated per-rectum for confirmation of pregnancy between 60 and 70 days post-service. The early embryonic mortality was recorded between days 23 and 25 in one animal, between days 25 and 28 in one animal and between days 30 and 60 in one animal. Thus, total 3 cases of early embryonic mortality were recorded out of 17 early pregnancies yielding overall incidence of early embryonic mortality to be 17.64%. It may be concluded that early embryonic mortality may be detected through ultrasound scanning in water buffaloes.

Keywords: Water buffaloes, Ultrasound, Plasma progesterone, Early pregnancy, Early embryonic mortality.

NTRODUCTION

The estimation of plasma progesterone concentration (Rao *et al.*, 1983; Nanda *et al.*, 1984) and ultrasonography (Pawshe *et al.*, 1994; Glatzel *et al.*, 2000; Bhosrekar and Hangare, 2000) has been successfully used for detection of early pregnancy in buffaloes. However, the diagnostic accuracy and reliability of these techniques are affected owing to early embryonic mortality. The detection of early embryonic mortality has been extensively studied in cattle (Chaffaux *et al.*, 1986; Pieterse *et al.*, 1990; Totey *et al.*, 1991); however, such study is lacking in water buffaloes. In perspective, the objective of present work was to detect embryonic mortality through ultrasound scanning in water buffaloes during early stages of pregnancy.

MATERIALS AND METHODS

A total of 22 freshly bred buffaloes were selected at Livestock Research Station, Sardar Krushinagar-Dantiwada Agricultural University, Sardar Krushinagar, Gujarat for present study. The estrous females were naturally served with fertile buffalo bull. Those that did not return to estrus 23 days after service were selected for serial examinations through ultrasound scanner on days 23, 25, 28 and 30 post-service. Their reproductive tracts were palpated per-rectum for confirmation of pregnancy between 60 and 70 days post-service.

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The uterus of animals were scanned using a real-time B-mode ultrasound scanner (Sigma-110 Master-Vetson, Kontron Medical, SAS, France) equipped with a 6.5 MHz convex linear array transducer designed for intra-rectal placement. After evacuating the faecal matter the transducer was placed in rectum and positioned in close proximity to the dorsal surface of the each uterine horn. After initial orientation, each uterine horn was scanned on its dorsal and lateral surface for signs of pregnancy. Positive diagnosis of pregnancy was based on the presence of a non-echogenic round or elongated area of varying size in the lumen of an echogenic uterine horn representing the fluid filled allantoic cavity, the embryonic vesicle (Pieterse *et al.*, 1990). The presence of embryo within the embryonic vesicle was confirmed by observing an echogenic (white) area with rhythmic pulsation representing heartbeats (Ginther, 1995). Blood samples were collected on day 23 post-breeding for estimation of plasma progesterone. Progesterone concentration was estimated in plasma samples using a radioimmunoassay (RIA) kit (Immunotech-SA, Marsielle Cedex, France) employing standard technique (Kubasic *et al.*, 1984). The sensitivity of the assay was 0.1 ng/ml. Intra-assay coefficient of variation was 5.4%, while inter-assay variation was 9.1%. Presence of progesterone $\geq$ 1.0 ng/ml in blood plasma on Day 23 post-service was considered positive for diagnosis of pregnancy (Nanda *et al.*, 1984).

RESULTS AND DISCUSSION

The scanning of uterus by real-time ultrasound waves allowed the visualization of embryonic vesicle and embryo in freshly bred buffaloes. On day 23, 17 animals were found pregnant, whereas 16 animals were detected pregnant on day 25. Later, 15 animals were detected pregnant on days 28 and 30; however only 14 animals were confirmed pregnant by palpation per rectum (Table1). The mean plasma progesterone concentration in all 22 freshly bred animals on day 23 post-breeding is presented in Table 2.

In the present study, the plasma progesterone concentration ranged from 1.10 to 6.00 ng/ml, with an average of 2.94 ± 0.53 ng/ml in pregnant animals on day 23, which approximates with earlier reports in buffaloes (Jain and Pandey, 1991; Glatzel *et al.*, 2000). However, some authors reported higher levels of plasma progesterone at early stages of pregnancy in buffaloes (Sarvaiya *et al.*, 1991; Chohan *et al.*, 1992).

The two animals detected pregnant based on plasma progesterone level were subsequently found non-pregnant by palpation per rectum. Present finding approximates with observation of Rao *et al.* (1983) who reported that 3 out of 11 animals with plasma progesterone level > 1.0 ng/ml were found non-pregnant by palpation per rectum. However, both these animals were found pregnant on Day 23 and later one animal was found pregnant on Day 25 by ultrasound scanning suggesting early embryonic mortality between Days 23 and 25 in one animal. The other animal, which was detected pregnant on Days 23 and 25, was later diagnosed as non-pregnant on Days 28 and 30 by ultrasound scanning indicating loss of embryonic vesicle in this animal between Days 25 and 28. Thus, two animals recorded early embryonic mortality between Days 23 and 30. On the other hand, two animals had plasma progesterone level < 1.0 ng/ml and both were found pregnant by ultrasound scanning on all the four(4) examinations between Days 23 and 30 post-service. However, one animal with plasma progesterone level of 0.85 ng/ml was diagnosed non-pregnant and the other animal with plasma progesterone level of 0.90 ng/ml was found pregnant by palpation per-rectum. In non-pregnant animal with plasma progesterone level of 0.85 ng/ml, probably luteolysis had just begun prior to ultrasound scanning, thus allowing embryonic vesicle to be observed through ultrasound scanning. There could have been gradual decline in plasma progesterone concentration resulting in loss of embryo between Days 30 and 60 prior to palpation per rectum. This speculation was consistent with the report of Chaffaux *et al.* (1986) who suggested that deficiency of progesterone could be the cause of embryonic mortality resulting in delayed death of embryo thereby allowing it to reach the size necessary to be detected by ecograph. However, it disagreed with the observation of Bazer and First (1983), who reported that pregnancy wastage was a result of conceptus deficiencies rather than of maternal origin. Thus, 3 cases of early embryonic mortality were recoded in present study out of 17 early pregnancies giving overall incidence of early embryonic mortality as 17.64% between Days 23 and 60. The present observation corroborates with early reports in cattle (Chaffaux *et al.*, 1986).

Surprisingly, one animal with plasma progesterone level of 0.90 ng/ml on Day 23 post-service was detected pregnant through ultrasound scanning on all the four(4) examinations between Days 23 and 30 post-service and subsequently found pregnant by palpation per rectum. Since the plasma progesterone levels were not recorded at later stages, it seems possible that embryo might have produced luteotropic substance(s) to sustain corpus luteum. Therefore, corpus luteum might have started functioning with full potential and resumed secreting progesterone in an amount that was sufficient to maintain pregnancy till its detection by palpation per rectum. The present observation further suggests that plasma progesterone concentration should also be estimated at later stages of early pregnancy for confirmation.

We conclude that serial ultrasound examination is useful in detecting the early embryonic mortality in water buffaloes and thereby it is helpful in identifying the non-pregnant animals during early stages of suspected pregnancy.

Table 1. Diagnostic results of early pregnancy diagnosis by ultrasound scanning and plasma progesterone assay in Mehsana buffaloes

Diagnostic results by palpation per rectum	Days of ultrasound scanning				Plasma progesterone assay
	23	25	28	30	
Diagnosis pregnant correct (n)	14	14	14	14	12
Diagnosis pregnant incorrect (n)	4	2	1	1	2
Diagnosis non-pregnant correct (n)	4	6	7	7	7
Diagnosis non-pregnant incorrect (n)	0	0	0	0	1

Table 2. Mean and range of plasma progesterone concentration in different status of pregnancy diagnosis on day 23 in Mehsana buffaloes

Diagnostic results by palpation per rectum	Progesterone concentration (ng/ml)	
	Mean ± SE	Range
Diagnosis pregnant correct (n=12)	2.94 ± 0.52	1.10-6.00
Diagnosis pregnant incorrect (n=2)	2.33 ± 0.53	1.80-2.85
Diagnosis non-pregnant correct (n=7)	0.25 ± 0.07	0.15-0.40
Diagnosis non-pregnant incorrect (n=1)	0.90	0.90

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