Oestrus synchronisation and oestrus detection in Swamp Buffaloes (*Bubalus bubalis*)

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ABSTRACT

A trial was undertaken to evaluate the effectiveness of the CIDR-B in buffalo. Twenty four, 20 month old buffalo heifers had CIDR's inserted (day 0) for 12 days. Each heifer was injected intramuscularly with 10 mg of oestradiol benzoate at CIDR insertion and 150 IU of PMSG at CIDR removal. Two buffalo bulls fitted with chin ball harnesses were introduced into each of 2 groups of 12 heifers. Continuous observations of mating activity commenced 24 hours after CIDR withdrawal. The heifers were bled for progesterone analysis on days -20, -9, 0, 2, 7, 12 and 26. 9 CIDR's (38%) lost from the heifers were replaced immediately after the losses were noticed. The mean interval from CIDR removal to onset of mating activity was 50.3 ± 3.4 (mean ± SE) hours and the duration of mating activity was 19.3 ± 5.1 hours. Progesterone concentrations while CIDR's were inserted were high. Based on progesterone analysis 21 of the 24 heifers had ovulated by day 14 after CIDR removal, but only 15 were observed mating with the bulls. CIDR-B can be used for synchronisation of oestrus in buffalo, though there can be a high loss rate. The plastic tail of the CIDR must be cut off to prevent other buffalo removing it. Chin ball harnesses were successful for detecting oestrus, but tail paint was not.

Keywords Buffaloes, CIDR-B, oestrus synchronisation, oestrus detection.

INTRODUCTION

The controlled internal drug release ("CIDR-B", Carter Holt Harvey, Hamilton, New Zealand) intravaginal device is one technique for inducing and synchronising oestrus in cattle in New Zealand but there are no reports of its use in swamp buffaloes. Progesterone releasing intravaginal devices (PRIDs Saini *et al.*, 1988) and prostaglandins ($PGF_2\alpha$ Fletcher 1988) have been successfully used for oestrus synchronisation in buffaloes.

Oestrus detection in water buffaloes is reported to be more difficult than in cattle, as the buffaloes exhibit no obvious behavioural patterns exclusive to oestrus. For example, although both oestrus and non-oestrus buffaloes will mount and stand to be mounted (McCool *et al.*, 1989), homosexual behaviour is infrequent (Kanai and Shimizu 1983) and cervical mucus does not hang as strands from the vulva, but accumulates on the vaginal floor (Toelihere 1988).

This paper reports on an experiment to evaluate the use of the CIDR-B device for oestrus synchronisation, outline the progesterone profile in the blood of buffaloes being treated with the CIDR-B and observe behavioural aspects of buffalo mating.

MATERIALS AND METHODS

On October 4 1990, twenty four 20 month old buffalo heifers were weighed and paint applied over the rump and proximal tail. A buffalo steer treated intramuscularly with 500 mg of testosterone and fitted with a chin ball harness was joined with the heifers. Twenty days later (day 0) the heifers were injected intramuscularly with 10 mg of oestradiol benzoate in 2 ml of peanut oil to remove any active luteal tissue and a CIDR-B was inserted into the vagina of each heifer. At day 12 the CIDR was removed and each heifer injected intramuscularly with 150 IU of PMSG (Folligon, Pharmaco NZ Ltd) to increase ovarian follicle stimulation. The heifers were then randomly allocated to 2 groups based on liveweight and 1 mature buffalo bull (650 kg) and one 20 month old bull (425 kg) joined with each group in 2 adjacent paddocks. From day 13 to 16, continuous observations were undertaken from a caravan parked between the paddocks. Each animal was identified by large numbers painted onto their sides with fluorescent paint and a spotlight and infrared telescope were used to observe mating activity at night. All bull-heifer mountings and heifer-heifer mountings (standing or non-standing) were recorded. The bulls were removed after 7 days (day 19) and reintroduced on days 30-38 to mate returns to service.

The reproductive tract of each heifer was palpated per rectum, once a week over the 20 days before the CIDR's were inserted. Ovarian activity was assessed by the ovarian tone, size and texture. Small, hard, smooth ovaries were classified as inactive, while large, soft ovaries with any degree of protuberance (corpus luteum or follicle) were classified as active. Blood samples were taken by jugular venepuncture from the heifers into heparin treated vacutainers at days -20, -9, 0, 2, 7, 12 and 26. The plasma was removed by centrifuging at 1900 rpm for 10 minutes, then stored at -20°C until assayed for progesterone concentration by radioimmunoassay (Coat-a-Count Assay, Diagnostic Products Corporation, Los Angeles). Means, standard errors and analysis of variance were calculated using the Statistical Analysis System (SAS) computer programme. Analysis of variance was performed using the general linear model procedure.

RESULTS

CIDR Retention

In the first 7 days after insertion, 9 heifers (38%) lost their CIDR's with one animal losing its CIDR twice. The CIDR's were replaced immediately the loss was noticed. On day 7 herdmates were observed chewing on the protruding plastic tails of the CIDR's and once these were cut off the loss of CIDR's ceased. The CIDR-B seemed to be too large for the buffaloes and the animals strained continuously against them when first inserted. Of the nine heifers who lost CIDR's, 6 were subsequently observed mating. The progesterone concentrations suggest 5 of the 6 heifers who lost CIDR's and were observed mating ovulated

during the 14 days after CIDR removal, compared to 1 of the 3 heifers not observed to mate.

Synchronisation and mating activity

Fifteen out of 24 (62%) of the heifers were observed being mated by the bull at least once over the 4 days of mating after CIDR-B removal. Eleven heifers stood for the bull and were repeatedly mated (7.9 ± 3.5 times (mean ± SE)) while 4 heifers stood for only 1 or 2 matings (1.5 ± 0.6 times). The remaining 9 heifers never stood for mating although 4 were mounted by the bull. The mated heifers were significantly heavier ($P<0.01$) than the non-mated heifers (355 ± 9 kg versus 304 ± 11 kg). The mean interval from CIDR removal to standing for the bull was 50.3 ± 3.4 hours, but there was a large range from 31 to 70 hours. In all except three heifers, standing mounts were preceded by non-standing mounts. Standing mounts to the bull for the mated heifers occurred over a period of 19.3 ± 5.1 hours. There was no significant difference in the duration of non-standing mounts to the bull between mated and non-mated heifers (37 ± 8 versus 53 ± 19 hours).

The tailpaint was licked and rubbed off within a few days of application and this was unrelated to mating activity. Prior to CIDR withdrawal, chin ball marks appeared on the rump, withers and midline of the back of the heifers as discrete daubs of paint. A successful mating by the bull resulted in a crescent shaped slash of paint being left in front of the shoulders of the heifer upon dismount of the bull. Ejaculation in the bulls was not distinctive, with a few slow pelvic thrusts followed by the bull sliding off to the side of the heifer. Before being mounted by the bull only 3 heifers were mounted by other heifers and 7 mounted other heifers. The frequency of heifer heifer mounting was low (6.3 ± 0.9) and most of the mountings appeared to be play. The heifers also engaged in head to head pushing with the bulls and generally interfered with the bull heifer mating.

Progesterone Levels

The mean blood progesterone levels were low on days -20 and -9, but had increased to 3.92 ± 0.41 ng/ml by day 0, just prior to CIDR's being inserted (Table 2). At day 0 all of the heifers had progesterone concentrations greater than 1 ng/ml (Table 1), a level considered to be indicative of cyclic ovarian activity (McCool 1989). On day 2 after CIDR insertion, the mean progesterone concentration was 4.57 ± 0.32 ng/ml but this declined to 2.64 ± 0.17 ng/ml by day 12 (Table 2). Fourteen of the fifteen heifers observed to mate and 7 of the 9 heifers not observed to mate had progesterone concentrations greater than 1 ng/ml on day 26 suggesting 21 of the 24 heifers (88%) had ovulated.

Ovarian Activity

The ovaries varied in size from about 0.5 x 0.5 x 0.5 cm to 3 x 2 x 1 cm. Only one heifer was classified as having inactive ovaries at each of three palpations prior to insertion of the CIDR. Three heifers considered to have inactive ovaries on day 0 were considered to have active ovaries on day -9. The four heifers with inactive ovaries on day 0 all had progesterone concentrations greater than 1 ng/ml on day 0 and 26, but 3 were not observed to be mated and one was only mated once (Table 1).

DISCUSSION

Oestrus in buffalo heifers was successfully synchronised by CIDR-B. 62% of the heifers treated were observed to be mated. As a comparison oestrus is induced in 60 % of cattle heifers using CIDR's (MacMillan *et al.*, 1988).

The CIDR-B appeared to be too large for the buffaloes, but the high loss rate was overcome by cutting off the plastic tails. It may be more appropriate to use a smaller type of CIDR. However, CIDR-D (porcine) and CIDR-G (caprine) were subsequently evaluated with the tails removed but the loss rate was 50 % and 100 % respectively (Hill unpublished data). The mean progesterone concentration while the CIDR was inserted was similar to cattle (Macmillan *et al.*, 1990). However, in the current experiment progesterone levels indicated luteal tissue was present in all the heifers at CIDR insertion. This may have been due to a teaser effect of the testosterone treated steer stimulating ovulation. In the first two blood samples taken at -20 and -9 days the mean progesterone concentration was very low suggesting all the heifers had inactive ovaries without luteal tissue. At the time of CIDR insertion the mean progesterone concentration had risen to 3.92 ng/ml, and luteolysis would not have occurred until 7 days after oestrogen injection.

By two weeks after CIDR removal, 21 heifers had ovulated, but only 15 were observed being mated, suggesting either a silent oestrus undetected by the bull had occurred, or a prolonged interval from CIDR withdrawal to oestrus in some heifers. There may have been some unrecorded mating activity because of difficulties in continuing observations throughout the night. Also, difficulty was experienced in identifying when the bulls

TABLE 1 Plasma progesterone concentration (mean ± SE) of 24 buffalo heifers before, during and after treatment with oestrogen, CIDR-B and PMSG to synchronise oestrus. Day 0 = day CIDR-B inserted.

Day	-20	-9	0	2	7	12	26
Treatment	Teasers		CIDR-B				Mating
Progesterone concerntration (ng/ml)	0.37 ±0.03	0.08 ±0.02	3.92 ±0.41	4.57 ±0.32	3.10 ±0.17	2.64 ±0.17	3.66 ±0.66

TABLE 2 Plasma progesterone concentration (P_4 mean ± SE) of 24 buffalo heifers related to observed mating activity after withdrawal of a CIDR-B device

	n	LWT kg	Day 0 P_4	Day 12 P_4	Day 26 P_4	Mean no of times mated
Mated	15	355 ± 9	3.70 ± 2.06	2.54 ± 0.89	3.85 ± 3.13	6.20 ± 1.08
Not mated	9	304 ± 11	4.27 ± 2.01	2.80 ± 0.70	3.35 ± 3.56	0

ejaculated as they did not have the characteristic behaviour of bovine bulls, consisting of a well defined thrust and both hind feet leaving the ground.

Rectal palpation was not an accurate method of predicting ovarian activity. This may be because the corpus luteum is smaller than cattle and deeply imbedded within the stroma of the ovary (Dobson and Kamonpatana 1986).

Heifer-heifer activity was not as important in buffaloes for identifying oestrus females for the bulls. In most instances the bull was the first to institute mating activity. This may have been different if older buffalo cows had been used.

The chin ball harness was effective in identifying mated heifers but tail paint was not. The close social nature of the buffalo and pronounced mutual grooming and rubbing of chins on the rump of the other heifers removed all the tail paint within a few days.

In most tropical countries the onset of puberty in buffaloes is not until 30 to 36 months when the animals weigh between 318 and 350 kg (McCool *et al.*, 1988. Rao and Nagarcenkar 1979). Buffalo heifers given good nutrition can reach liveweights necessary for puberty and be induced into oestrus using CIDR's, oestrogen and PMSG.

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