

Inclusion of CIDR in ovsynch protocol to improve fertility in postpartum subestrus buffaloes*

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ABSTRACT

Twenty four postpartum subestrus buffaloes not showing observed estrus within 60 days after calving were randomly and equally divided into ovsynch-subestrus and ovsynch plus CIDR-subestrus groups. Ovsynch-subestrus group buffaloes were subjected to ovsynch protocol which consisted of 100 µg i.m. injection of GnRH on the day of start of synchronization (d 0), 25 mg i.m. injection of PGF₂α seven days later (d 7), another 100 µg i.m. injection of GnRH 48 h after PGF₂α (d 9) and timed insemination 16 to 18 h after second GnRH injection (d 10). Ovsynch plus CIDR-subestrus buffaloes were subjected to ovsynch plus CIDR protocol which included intra vaginal placement of CIDR device for a period of 7 days starting from first GnRH injection (d 0) to PGF₂α injection (d 7) for synchronization of ovulation. Blood samples were collected from all experimental animals on d -10 and d 0, d 7, and d 9 days after insemination for the estimation of progesterone. During the treatment protocol none of the buffaloes exhibited visible premature estrus signs. However progesterone estimation showed that 25 per cent had exhibited premature estrus. The estrus detection rates were 41.66 and 58.33 per cent in ovsynch-subestrus and ovsynch plus CIDR-subestrus respectively. Most buffaloes showed intermediate signs of estrus. The ovulatory response and first service conception rates were 83.33 and 33.33 and 100 and 41.66 per cent in ovsynch-subestrus and ovsynch plus CIDR-subestrus groups respectively.

Key words : Ovsynch, CIDR, subestrus buffaloes, fertility

The major limitation of GnRH and PGF₂α based synchronization protocols was the inability of GnRH to turnover dominant follicles late in the estrous cycle leading to premature estrus in 8 to 10 per cent of treated cows (Geary *et al.*, 2000). Alternatively the inclusion of an exogenous such as CIDR during the interval between GnRH and PGF₂α injection prevented premature estrus and increased estrus response and conception rates (Steckler *et al.*, 2002). Although much work has been done using ovsynch and ovsynch plus CIDR in synchronization of ovulation in cattle, information on their use in buffaloes especially postpartum lactating subestrus Murrah buffaloes is limited. Hence the present

study was taken up (i) To study the effect of ovsynch protocol in induction of ovulation in postpartum subestrus buffaloes. (ii) To study the effect of CIDR-B on estrus synchronization in ovsynch treated postpartum subestrus buffaloes. (iii) To compare fertility rate among ovsynch and ovsynch plus CIDR-B treated postpartum subestrus buffaloes.

MATERIALS AND METHODS

Twenty four apparently healthy lactating buffaloes maintained at Central Cattle Breeding Farm, Alamathi, with a history of absence of observed estrus signs within 60 days were selected and subjected to rectal examination twice at an interval of ten days. All the selected animals had corpus luteum during rectal examination. Similarly serum progesterone levels were estimated twice (-d 10 and d 0) to confirm cyclic activity of the animals. The selected postpartum subestrus buffaloes were randomly and equally divided into ovsynch-subestrus and ovsynch plus CIDR-subestrus groups.

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The ovsynch subestrus group buffaloes were injected to administration of 100 µg i.m. injection of GnRH (Gonadorelin Acetate, Fertiline®, Vetoquinol, Canada) on the day of start of synchronization (d 0), 25 mg i.m. injection of PGF₂α (Dinoprost. tromethamin, Lytalyse®, Pharmacia, Belgium) seven days later (d 7), another 100 µg i.m. injection of GnRH 48 h after PGF₂α (d 9) and timed insemination 16 to 18 h after second GnRH injection (d 10). The ovsynch plus CIDR group buffaloes were treated with same ovsynch protocol and in addition intra vaginal placement of CIDR-B device (EAZI-BREED CIDRTM, Inter Ag, Hamilton, New Zealand) for a period of 7 days starting from 1st GnRH injection (d 0) to PGF₂α injection (d 7). The experimental buffaloes were observed frequently for estrus signs during the period of treatment and after PGF₂α injection. The percentage of estrus detection rate was estimated as the number of buffaloes detected in estrus during the 66 h after PGF₂α administration. Onset of estrus was calculated in hours from the time of PGF₂α administration or / and CIDR removal to the time of first appearance of estrus signs. The intensity of estrus was studied using behavioral changes, physiological changes and gynaecological observations and it was scored as described by Rao and Rao (1981) with slight modifications.

Blood samples were collected from all experimental animals on - d 10, d 0, d 7, and d 9 and the sera samples were subjected for the estimation of progesterone. All the animals had more than 1 ng/ml progesterone either at -d10 or d 0 indicating that they were in cyclicity without observable estrus signs. The serum progesterone level was estimated using solid-phase Radio Immuno Assay technique with the help of progesterone kits (Coat-A-Count, Diagnostic Products Corporation, USA). Ovulatory response was assessed by rectal examination at 10 days after induced ovulation which was later confirmed by serum progesterone levels. First service conception rate was calculated as percentage of animals that conceived to fixed time insemination at induced estrus in each group. Pregnancy was confirmed by palpation per rectum at 60 days post insemination.

RESULT AND DISCUSSION

In the present study, none of the animals exhibited visible premature estrus signs between d 0 and d 7 of the treatment period. However, Gabor *et al.* (2002) reported that the incidence of premature estrus to be 11

to 14 per cent while using ovsynch protocols. Although in the present study no visible premature estrus signs were observed, progesterone levels on day 7 and day 9 indicated that 25.00 per cent of the ovsynch subestrus group animals had less than 1 ng/ml indicating that they were exhibited premature estrus however none of the CIDR treated exhibited premature estrus. The inclusion of CIDR in the ovsynch protocol in the present study may have potentially prevented the occurrence of premature estrus in ovsynch plus CIDR buffaloes. Estrus detection rate, onset of estrus, duration of estrus and intensity of estrus were presented in table-I. An increased percentage of estrus detection rate of 16.67 per cent (Table 1) for ovsynch plus CIDR-subestrus over the ovsynch-subestrus buffaloes might be due to the inclusion of CIDR in ovsynch protocol in the present study.

There was no significant difference with regards to onset of estrus among the two treatment groups. Stevenson *et al.* (1999) reported the interval to onset of estrus after PGF₂α was 54±13 and 55±4.4 h in ovsynch 33 (2nd GnRH 33 h after PGF₂α) and ovsynch 48 (2nd GnRH 48 h after PGF₂α) treated Holstein cows. The intensity of estrus was almost the same in ovsynch-subestrus and ovsynch plus CIDR- subestrus groups. Neglia *et al.* (2003) reported that mucus and estrus behaviour were only observed in rare occasions. However, 88 per cent of Italian Mediterranean buffaloes treated with ovsynch protocol had a tonic uterus on the day of AI.

The ovulatory response and first service conception rate were presented in Table 1. The ovulatory response for ovsynch-subestrus buffaloes (83.33%) in the present study was similar to those reported by Steckler *et al.* (2002) in cows. Higher ovulatory responses have been reported by Berber *et al.* (2002) (93.3 per cent) in buffaloes, while a lower ovulatory response (52.9 per cent) have been reported by Santos *et al.* (2002) in Holstein lactating cows. The ovulatory response of 100 per cent observed for ovsynch plus CIDR-subestrus buffaloes in the present study was higher than those reported by Steckler *et al.* (2002) (85 per cent) for cyclic postpartum cows. Between ovsynch and ovsynch plus CIDR groups a higher ovulatory response was observed for the ovsynch plus-CIDR group. The inclusion of progesterone in an ovsynch protocol increased LH pulse frequency (Garcia-Winder *et al.*, 1986) thereby providing a favourable condition for the follicles to grow. As follicles grew, the increased production of estrogens

Table 1. Estrus pattern, ovulatory response and first service conception rate in Ovsynch and ovsynch plus CIDR treated subestrus buffaloes

Reproductive parameters	Estrus detection rates (per cent)	Onset of estrus		Intensity of estrus at the time of AI No. of animals (per cent)			Ovulatory response	First service conception rate
		Mean±SE (h)	Range (h)	Intense	Intermediate	Weak		
Ovsynch per cent (no./no.)	41.66 (5/12)	48.80±7.74	24-61	2 (16.67)	8 (66.66)	2 (16.67)	83.33 (10/12)	33.33 (4/12)
Ovsynch plus CIDR per cent (no./no.)	58.33 (7/12)	36.71±2.65	24-48	2 (16.67)	8 (66.66)	2 (16.67)	100 (12/12)	41.66 (5/12)

(Garcia-Winder *et al.*, 1987) most likely increased the positive feed back of estradiol - 17 β on LH release from the pituitary once GnRH was injected on d 9. As a result of the increased concentrations of estradiol - 17 β in cows treated previously with progesterone, more LH was released after GnRH (Thompson *et al.*, 1999) which may have resulted in the increased ovulatory response. Higher conception rates following treatment with ovsynch have been reported by Berber *et al.* (2002) (61.7 per cent) in postpartum buffaloes and High conception rates of 49 and 72 per cent have been reported by Pursley *et al.* (2001) and Steckler *et al.* (2002) in ovsynch plus CIDR treated postpartum dairy cows as against the 41.66 per cent conception rate obtained in the present study for postpartum buffaloes.

The results of the present study have shown that the addition of progesterone in ovsynch plus CIDR protocol potentially could prevent the premature occurrence of estrus prior to or following PGF₂ α . Ovsynch plus CIDR-subestrus group responded better with increased estrus response, higher ovulatory response and conception rates when compared to Ovsynch-subestrus group. Thus, to conclude, inclusion of a CIDR device with ovsynch protocol may be an ideal strategy for dealing with lactating postpartum subestrus buffaloes.

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