EFFECT OF OVSYNCH PROTOCOL ON FERTILITY IN POSTPARTUM SUBESTRUS AND ANESTRUS BUFFALOES*

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ABSTRACT

Postpartum buffaloes (n = 23) not resuming or not showing observed estrus within 60 days after calving were classified as cyclical subestrus or anestrus based on palpable ovarian structures were divided into ovsynch-subestrus (n = 12) and ovsynch – anestrus (n = 11) groups. Ovsynch 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 PGF_2a seven days later (d 7), another 100 µg i.m. injection of GnRH 48 h after PGF_2a (d 9) and timed insemination 16 to 18 h after second GnRH injection (d 10) for synchronization of ovulation. Blood samples were collected from all experimental animals on d -10 and d 0 for the estimation of progesterone. The estrus detection rates were 41.66 and 27.27 per cent in ovsynch-subestrus and ovsynch-anestrus respectively. Most buffaloes showed intermediate signs of estrus in ovsynch-subestrus group but buffaloes of ovsynch-anestrus group showed predominantly weak estrus. The ovulatory response and first service conception rates were 83.33 and 33.33 and 54.54 and 0 per cent in ovsynch-subestrus and ovsynch-anestrus groups, respectively.

Key words: Ovsynch, Postpartum, buffaloes, Subestrus, Anestrus, Fertility.

INTRODUCTION

In cows, the use of GnRH 7 days prior to synchronization of estrus with PGF2a can alter the follicular development. Treatment with GnRH produces preovulatory follicles which are more homogeneous, more estrogen-active and more dominant and there is a greater size difference between the preovulatory and subordinate follicles prior to estrus (Wolfenson *et al* .,1994). The combined use of these two drugs (GnRH administered seven days prior to PGF_2a) in estrus synchronization programmes not only improved estrus detection rates and synchrony of estrus, but also induced fertile estrous cycles in both cyclic and anestrus bovine females (Wolfenson *et al* .,1994). Addition of a second GnRH injection 48 h after PGF_2a injection to induce the preovulatory LH surge and subsequent ovulation and cows were then inseminated once at fixed time, 16 to 18 h after second GnRH (Pursley *et al.*, 1998) is called ovsynch. Although much work has been done using ovsynch in synchronization of ovulation in cattle, information on their use in buffaloes especially in postpartum lactating subestrus and anestrus 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 and anestrus buffaloes. (ii) To compare the fertility rate among ovsynch treated postpartum subestrus and postpartum anestrus buffaloes.

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MATERIALS AND METHODS

Twenty three apparently healthy lactating buffaloes maintained at Central Cattle Breeding Farm, Alamathi, with a history of absence or presence of estrus signs within 60 days post partum and had palpable or absence of palpable ovarian structures during rectal examination were selected and divided into ovsynch-subestrus and ovsynch – anestrus groups. Serum progesterone levels were estimated twice (d 0 and -d 10) to confirm presence or absence of cyclic activity of the selected buffaloes.

The ovsynch subestrus (n=12) and anestrus (n=11) group buffaloes were injected with of 100 µg i.m. injection of GnRH (Gonadorelin Acetate, Fertilineâ, Vetoquinol, Canada) on the day of start of synchronization (d 0), 25 mg i.m. injection of PGF_2a (Dinoprost tromethamin, Lytalyseâ, Pharmacia, Belgium) seven days later (d 7), another 100 µg i.m. injection of GnRH 48 h after PGF_2a (d 9) and timed insemination 16 to 18 h after second GnRH injection (d 10). The experimental buffaloes were observed frequently for estrus signs during the period of treatment and after PGF_2a injection. Blood samples were collected from all experimental animals on d 0 and -d 10 and the sera samples were subjected for the estimation of progesterone. The percentage of estrus detection rate was estimated as the number of buffaloes detected in estrus during the 66 h after PGF_2a administration. Onset of estrus was calculated in hours from the time of PGF_2a administration 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. 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.

RESULTS AND DISCUSSION

Estrus detection rate, onset of estrus, duration of estrus and intensity of estrus, ovulatory response and first service conception rate are presented in table-I. The percentage of buffaloes exhibiting visible estrus discharge during the 66 h period following PGF_2a administration was 41.66 and 27.27 per cent in ovsynch- subestrus and ovsynch-anestrus groups respectively. Stevenson *et al.* (1996) opined that the low estrus detection rate could be due to the fact that heifers and cows when treated with GnRH- PGF_2a-GnRH rarely showed overt symptoms of estrus after PGF_2a or before or after the second GnRH injection. Signs of nervousness, mucus discharge and occasional mounting activity were observed by them in only 3 of 85 cows (3.5 per cent). Synchronization of ovulation with GnRH caused LH surge of normal amplitude (12 ng/ml) but of shorter duration (5 h) than expected for spontaneous surges (Lucy *et al.*, 1986). Further more, the concentration of estradiol 17-b decreased from 11 to 3 pg/ml within 12 h of GnRH injection (Kobayashi *et al.*, 1995) due to decreased expression of mRNA to P450 aromatase in the granulosa cells (Voss and Fortune, 1993) and thus very few cows showed heat even though they were ovulating.

The estrus response was higher in Ovsynch - subestrus than Ovsynch - anestrus buffaloes. The low estrus detection rate of 27.27 per cent in Ovsynch - anestrus buffaloes in the present study might be due to the fact that the effect of GnRH on inactive ovaries depended on the stage when the follicular wave was arrested. Cows with static ovaries bearing follicles smaller than 8.5 mm might not respond to GnRH or gonadotropic hormones having LH-like activity (Wiltbank *et al.*, 2002). Responsiveness of dominant follicle to LH was lowered in cows with severe shortage of energy (Butler, 2000). Even if follicles responded to LH with ovulation, estrus signs were not expressed, since cows with inactive ovaries were not exposed to progesterone (Nakao, 2002).

In the present study the overall mean time taken for the onset of estrus was 48.8 ± 7.74 and 52.67 ± 4.18 h in ovsynch-subestrus and ovsynch-anestrus groups, respectively. There was no significant difference with regards to onset of estrus among different treatment groups. Stevenson *et al.* (1999) reported the interval to onset of estrus after PGF_2a was 54 ± 13 and 55 ± 4.4 h in ovsynch 33 (2^{nd} GnRH 33 h after PGF_2a) and ovsynch 48 (2^{nd} GnRH 48 h after PGF_2a) treated Holstein cows.

Most buffaloes in the present study showed intermediate signs of estrus except those of ovsynch anestrus group in which most buffaloes showed weak estrus. 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. Berber *et al.* (2002) reported that the presence of mucus at the time of AI did not influence conception rate in ovsynch treated half bred Murrah x Mediterranean buffaloes. The high percentage of animals exhibiting weak estrus in the ovsynch-anestrus group might be due to the fact that more than 54.84 (6/11) per cent of buffaloes of this group did not respond to ovsynch as evinced by their progesterone concentrations.

Geary *et al.* (1998) reported that the ovsynch protocol was capable of inducing fertile ovulation and increased pregnancy rates in cyclic and anestrus beef cows. The ovulatory response for ovsynch-subestrus buffaloes (83.33 per cent) in the present study was similar to those reported by Mialot *et al.* (2003) in cows. Higher ovulatory responses have been reported by Berber *et al.* (2002) (93.3 per cent) in buffaloes. The ovulatory response for ovsynch-anestrus buffaloes (54.54 per cent) in the present study was lower than the response of 76 and 92 per cent reported by Geary *et al.* (2001) and Thompson *et al.* (1999) respectively but, higher than the response of 45.5 per cent reported by Mialot *et al.* (2003) following synchronization of ovulation in anestrus cows.

The lower ovulatory response of 54.54 per cent in ovsynch-anestrus buffaloes might be due to the fact that more animals would have been in anestrus or in luteal phase at the time of AI (or) with late induced ovulation without luteal phase d 10 after AI (Mialot *et al.*, 2003).The first service conception rate was 33.33 and 0

per cent in ovsynch- subestrus and ovsynch-anestrus groups respectively. The results of the present study have shown that ovsynch - subestrus group responded better with increased estrus response, higher ovulatory response and conception rates when compared to ovsynch-anestrus group. Thus, to conclude ovsynch protocol may be an ideal strategy for dealing with lactating postpartum subestrus than anestrus buffaloes.

Table 1

Estrus pattern, ovulatory response and first service conception rate in Ovsynch treated subestrus and anestrus buffaloes

Reproductive parameters	Estrus detection rates Per cent (no./no.)	Onset of estrus		Intensity of estrus at the time of AI No. of animals (per cent)			Ovulatory response Per cent (no./no.)	First service conception rate Per cent (no./no.)
		Mean ± SE (h)	Range (h)	Intense	Intermediate	Weak		
Ovsynch-Subestrus	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-Anestrus	27.27 (3/11)	52.67± 4.18	48-61	1 (9.09)	2 (18.18)	8 (72.72)	54.54 (6/11)	0 (0/11)

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