Application of Ultrasonography to Investigate Postpartum Anestrus in Water Buffaloes

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ABSTRACT

Anestrus is one of the most important production limiting disorders in dairy buffaloes and its underlying causes have been a current topic of studies. The objectives of this study were to explore the causes of anestrus in buffaloes with the application of ultrasonography. Two examinations were performed by transrectal ultrasonography at 12 days apart in buffalo cows that were not seen in oestrus at 60 or more days postpartum. As high as 54.5% buffaloes had silent ovulation and 45.5% suffered from the true anestrus with ovarian dysfunction. The duration of anestrus after calving was 60～90, 91～120, 121～180 and 181～365 days in 27%, 32%, 18% and 23% buffalo cows, respectively. Treatment with prostaglandin of cyclic buffalo cows with a corpus luteum (72.7%) resulted in higher estrous rate as compared with close observation of estrus (23.1%) by the farmer (p=0.021). Acyclic buffalo cows without any corpus luteum on ovaries were successfully treated with gonadotropin releasing hormone (70%), resulting in higher estrous detection rate than those treated with a vitamin-mineral mixture (20%) (p=0.035). In conclusion, poor heat detection due to silent ovulation is the most important cause of apparent anoestrus in buffaloes; however the percentage of the true anestrous is also quite high in postpartum buffaloes.

(Key words : Ultrasonography, Postpartum anestrus, Water buffalo, Ovulation, Pregnancy)

INTRODUCTION

Postpartum ovarian activity and subsequent conception may be affected by several factors such as breed, nutrition plan, milk yield, suckling, uterine involution, season of calving (Baruselli, 2001; Pipaon *et al.*, 2002). The recent application of ultrasonographic techniques in the study of buffalo follicles is elucidating the patterns of follicular growth, development and regression has greatly improved our perceptive of the follicular dynamics in buffaloes (Henao *et al.*, 2000; Presicce *et al.*, 2003; Vassena *et al.*, 2003). A good understanding of the processes involved in the growth and differentiation of vesicular follicles destined for ovulation is also essential in order to optimize buffalo reproduction. Ultrasonography for the assessment of ovarian structures is a reliable and accurate method for identifying and measuring follicles (Rahman *et al.*, 2011), especially important since manual palpation per rectum in buffalo is not completely accurate. Gonadotropin releasing hormone (GnRH) administration in buffaloes induces ovulation in 60～86% of the treated animals (Aboul-Ela *et al.*, 1985). This researcher's also reported that, interval between GnRH administration and ovulation was 33±8.3 hours. Some other elegant study on this issue noted that, the presence of a dominant follicle at the time of GnRH administration is a pre-requisite for a successful induction of ovulation (Baruselli, 2001; De Rensis *et al.*, 2005). Additionally, it is also reported that, administration of prostaglandins $F_{2\alpha}$ ($PGF_{2\alpha}$) as early as Day 5 of the cycle induces CL regression (luteolysis), resulting in a dramatic decrease in

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progesterone concentrations, allowing for ovulation within 2～6 days after the treatment (Brito *et al.*, 2002). Single or double (with 11～14 days interval) $PGF_{2\alpha}$ administration induces oestrus and ovulation in 60～70% of dairy buffaloes during the breeding season (Brito *et al.*, 2002). Prostaglandins were only induces oestrus in buffalo, if a CL is present and therefore can be useful in case of silent ovulation. All of these methods for synchronization of estrus and ovulation are currently practiced in field condition, but still the rate of estrus and ovulation are remained questionable. Some researcher's assume that, this condition might be subjected due to poor heat detection and silent heat. However, to date, the literature has not detailed a set of specific causes of anestrus *in situ* in indigenous buffalo of Bangladesh. Therefore, the current study was designed to explore the cyclical status of apparent anoestrus postpartum buffaloes and to test the effectiveness of oestrus induction in cyclic and non-cyclic buffaloes at 60 or more day postpartum with the application of ultrasonography.

MATERIALS AND METHODS

The present study was conducted in the Field Fertility Clinic Laboratory (FFC), Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh (BAU), Bangladesh.

Animal Selection and Management

In total, 44 indigenous river-type buffaloes with postpartum anoestrus at 60 or more days were selected randomly from Trishal upazila and BAU dairy farms. The body condition score (BCS) of the buffalo cows varied from 2.5 to 4.0 (1.0=very thin to 5.0=very fat). Whereas, the parity of the buffaloes ranged from 1 to 6. The buffaloes were milked twice daily with their calves used for stimulating milk let down. Calves survived on residual milk after the hand milking. Weaning was not controlled in the buffaloes. The buffaloes were fed on rice straw, cut-and-carry grass and milling by-product as concentrate with limited grazing on roadside and community land. At lactation period, 0.5 to 1.5 kg concentrate (crashed rice and mustard oil cake) daily was given to each buffalo. Visual inspection of estrus sign and mating with fertile buffalo bull were practiced regularly.

Data Collection

Data was collected from selected farm families and the dairy farm throughout the study period. Parity, duration of postpartum anestrus, milk yield and body condition score of the buffaloes with 60 or more days postpartum were recorded. The BCS of the buffalo cows (1.0=very thin to 5.0=very fat) was evaluated according to the procedures described elsewhere (Ahmed *et al.*, 1999; Alapati *et al.*, 2010).

Reproductive Examination

Buffalo cows with ≥60 days postpartum were examined by rectal palpation and transrectal ultrasonography (two examinations 12 days apart) to diagnose the underlying disorders. B-mode real-time ultrasonography (Tringa Linear VET®, Esaote Pie Medical, Genova, Italy)

Table 1. Descriptive statistics of the study buffaloes

Variable	Breedable cases / farm	Number of buffaloes	Percentage of farms
Cows in studied farms	1	16	31.4
	2	20	39.2
	3	9	17.6
	4～5	6	11.8
Body condition score of buffaloes	2.5	4	9.1
	3.0	18	40.9
	3.5	17	38.6
	4.0	5	11.4
Parity of study buffaloes	1～2	26	59.1
	3～4	14	31.8
	5～6	4	9.1
Milk production (litre) per buffalo	≤2.0	19	43.2
	2.5～3.0	15	34.1
	≥3.5	10	22.7

was used transrectally to examine the ovaries of buffaloes. After adequate restraining, the scanner was placed at a sensible distance from the cow on the side opposite to the operator's arm engaged in rectal palpation. All fecal materials from the rectum were evacuated prior to introduction of the transducer. The transducer face was lubricated with a suitable coupling medium (Ultrasonic Gel® for Medical use-250 mg) and was usually covered by a lubricated plastic sleeve before insertion. The transducer was then progressed cranially along the rectal floor to overlie the reproductive tract. The ultrasound screen and the human eye were at similar level for accurate interpretation of ultrasound images. The ultrasound examination was carried out to identify the cyclic and non-cyclic buffaloes, based on the presence of corpus luteum (CL). To confirm the cyclic status, a second ultrasound examination was carried out 12 days later on those animals having no CL at the first monitoring (De Rensis *et al.*, 2005). Based on the ovarian structures, the postpartum anestrous buffaloes were classified as follows:

1. True anestrus (both ovaries found small, smooth, firm, with no corpus luteum).
2. Silent heat (a corpus luteum was found on one of the ovaries while the uterus was considered to be symmetric).
3. Uterine infection (detection of purulent material echogenic secretion in the body of uterus).
4. Ovarian cyst (detection of a follicular structure or corpus luteum with ≥ 25 mm in diameter and persisting 10 or more days).

Experimental Design (Fig. 1)

Treatment of the Anoestrus Buffaloes

Buffalo cows with a CL at either of the two examinations were considered as cyclic. They were injected intramuscularly with 25 mg $PGF_{2\alpha}$ or advised to monitor heat carefully to breed on observed oestrus. Buffalo cows with inactive ovaries were treated with oral administration of a vitamin-mineral mixture or intramuscular injection of GnRH and bred at heat.

Statistical Analysis

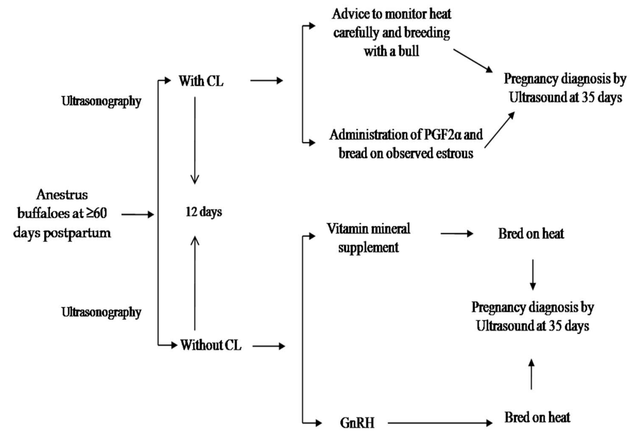

Fig. 1. Schematic diagram showing the experimental designs.

Descriptive statistics was done to determine the prevalence of anestrus in buffaloes. Differences in percentages of treatment outcome between two groups were analyzed by Fisher's exact test. All statistical analyses were performed by using the Statistical Package for the Social Sciences (SPSS, 16.0 versions) software for windows.

RESULTS

Prevalence of Anestrus

The prevalence of anestrus in postpartum buffaloes was 40% (44 out of 110). The prevalence was higher in summer (48.4%) than in winter (29.2%). In the anestrus buffaloes, 54.5% (24 out of 44) was cyclic evidenced by the presence of a corpus luteum (CL) and 45.5% (20 out of 42) buffalo cows were acyclic, which was manifested by small ovaries without a corpus luteum (Table 2).

The duration of anestrus after calving was 60～90, 91～120, 121～180 and 181～365 days in 27%, 32%, 18% and 23% buffaloes respectively (Table 3).

Treatment of Anestrus

Treatment of cyclic buffalo cows having a CL with prostaglandin (72.7%) resulted in higher rate of detection

Table 2. Characteristics of apparently anestrous water buffaloes

Season	Farms studied	Total buffaloes	Anestrus buffaloes (%)	Ovarian cyclicity	
				Cyclic (%)	Acyclic (%)
Hot	31	62	30 (48)	20 (67)	10 (33)
Cold	20	48	14 (29)	4 (29)	10 (71)
Total	51	110	44 (40)	24 (55)	20 (46)

Table 3. Interval in months from last calving to diagnosis of anestrus in buffaloes

Days postpartum	Number of animals	Percentage (%)
60～90	12	27.3
91～120	14	31.8
121～180	8	18.2
181～365	10	22.7

than that with close observation (23.1%) for estrus by the farmer (p=<0.050; Table 4). Acyclic buffalo cows bearing no corpus luteum on ovaries were successfully treated with GnRH (70%) resulting in higher rate of estrous detection than those treated with a vitamin-mineral mixture (20%) ($p<0.05$; Table 4).

The buffaloes having post partum anestrus ≥60 days were allocated primarily into two groups buffaloes with CL on ovaries (non-detected estrus) and without CL (true anestrus). Later on, the buffalo cows with CL on ovary were treated by either $PGF_{2\alpha}$ or assigned to close observation of estrus by farmer to detect the buffaloes at heat. Whereas, buffaloes without detectable CL on ovary (true anestrus), were treated by GnRH or vitamin-mineral premix.

DISCUSSION

Prevalence of Anestrus

Anestrus in buffaloes is generally caused by ovarian dysfunction or silent ovulation. Previous clinical surveys reported cases of anestrus showed that about 60% of cases were true anestrus and 33% of cases were silent ovulation (Sah and Nakao, 2010). In the present study, among the 110 buffalo cows examined 45% had inactive ovaries (true anestrus), while 55% had silent ovulation or missing heat. Ovarian cysts were diagnosed in only 5.2% of the cows (Reddy et al., 1986), which was also not well comparable with this study. In this survey, 32% of the anestrus cows were at 90～120 days postpartum and 42% were at more than 120 days postpartum. In the survey, 61.5% of the anestrus cows were already at 10～15 months after calving and 31.9% were at more than 16 months postpartum (Sah and Nakao, 2010). This is due partly to the limited availability of veterinary service and to the producer's attitude towards cows; as long as the cows are producing a certain amount of milk, they do not pay much attention to re-breeding the cows. Season of calving influences the reproductive performance. Numerous worker reported that buffaloes calving in the rainy and monsoon seasons had shorter anestrous periods than those calved in other seasons (Reddy *et al.*, 1986; Sule *et al.*, 2001). Nevertheless, longer anestrous periods were reported for out of season (156±34 and 91±12 days) than normal breeding season calvers (57±6 and 56±5 days) (Qureshi *et al.*, 1998). The prevalence of anestrus in postpartum buffaloes was higher in summer than in winter in the present study. Majority of the buffaloes during summer had silent ovlution (Chaudhry, 1988), characterized by less intense overt signs of oestrus with shorter duration (Jainudeen and Hafez, 2000). A research in the Pakistani river buffalo showed that 51.5% of estrus was the silent heat (Qureshi and Ahmad, 2008). Whereas, someother found problems of difficult calving was highest during autumn, followed by rainy and summer months (Kaushik and Mathur, 2005). It is well know that in buffalo species, the ability of detect estrus declines during the warmest hours of the day and in animals with heat stress (Vale *et al.*, 1988).

Treatment of Anestrus

Treatment of silent ovulation with prostaglandin in buffalo cows with a corpus luteum (CL) resulted in higher oestrus rate within one month after treatment as compared with treatment with close observation for heat by the farmer. Buffaloes with a CL responded well to $PGF_{2\alpha}$, with high estrus induction and pregnancy rates, as already reported by a number of studies (Chauhan *et*

Tabel 4. Reproductive performance of anestrus buffalo cows after $PGF_{2\alpha}$ treatment

Clinical findings	Number of buffalo cows treated			
	With CL (N=24)		Without CL (N=20)	
Treatment	$PGF_{2\alpha}$	Observation	GnRH	Vitm-min mix
Number treated	11	13	10	10
Cow in estrus / mated within a month	8	3	7	2
Estrus detection rate (%)	73^a	23^b	70^x	20^y
Number of conception	5	2	4	2
First service conception rate (%)	62.5	66.7	57.1	100

^{a,b} Values with different superscripts in the same row differ with each other ($p<0.05$).
^{x,y} Values with different superscripts in the same row are different ($p<0.05$).

al., 1982; Dhaliwal *et al.*, 1988). The efficacy of the prostaglandin treatment in buffaloes to regress a functional CL was reported to be dependent on plasma progesterone concentration, the CL size and the ovarian follicular status before prostaglandin administration (Brito *et al.*, 2002). Nevertheless, in buffaloes the occurrence of the inadequate luteal phase due to short time CLs or characterized by insufficient progesterone production has been reported, which in turn may explain the unsatisfactory response to prostaglandin administration for control of reproductive function (Zicarelli *et al.*, 1988). However, improvements are recorded when prostaglandin is followed by GnRH administration (Neglia *et al.*, 2011). In addition with the possibility of the use of hormonal control of the estrous cycle, seasonal pattern can be overcome and they can be bred through the synchronization of the estrous cycle in year around (Vale and Ribeiro, 2005).

Buffalo cows with inactive ovaries were successfully treated with GnRH resulting in higher oestrus rate within one month after treatment than those treated with vitamin-mineral mixture. The interval after GnRH to ovulation was 36 h in buffaloes (Baruselli *et al.*, 2003). During the last few years, several studies have been attempted to treat the prolonged postpartum anestrum in buffaloes by using hormonal treatments such as GnRH, prostaglandin $F_2\alpha$ (Metwelly, 2001; 2006). It has also been reported that GnRH is effective in inducing ovulation in buffaloes (Pattabiraman *et al.*, 1986; Thakur *et al.*, 1993). The results of this study correspond to these earlier reports. The efficacy of PGF2α and GnRH in anestrous buffaloes shown by the present study and the earlier reports mentioned above suggests that routine reproductive examination of buffaloes that are not observed to be in estrus and adequate treatment may improve reproductive efficiency in buffaloes. The commonly practiced method of treatment of anestrus in buffaloes in this region has been use of a vitamin/mineral mixture, which was shown to be less effective than administration of hormones.

In conclusion, the predominant causes of anestrus in dairy buffaloes studied were silent ovulation; however true anestrus was also common and the duration of anestrus after calving and breeding was extremely long. Routine reproductive examination and adequate hormone treatment may improve the reproductive performance of these buffaloes.

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