—Original Article—

A Clinical Study of Anestrus Buffaloes in Southern Nepal

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Abstract. Anestrus is one of the most important reproductive disorders in dairy buffaloes. The clinical features of anestrus in buffaloes, however, have not been well described. The objectives of this study were to describe the causes of anestrus in buffaloes and their reproductive performance after treatment under field conditions in southern Nepal. Of 135 anestrus buffalo cows, 61.4% had true anestrus with ovarian dysfunction and 33.3% had silent ovulation. In 111 buffalo heifers, 76.6% were in true anestrus and 18.9% had silent ovulation. The duration of anestrus after calving was longer than 6 months in 83% of buffalo cows and 61.5% of the buffalo cows had durations longer than 10 months. The interval between the last breeding and diagnosis of anestrus was more than 5 months in 67.4% of cows and heifers. Treatment of anestrus with prostaglandin F_{2α} in cows and heifers with a corpus luteum resulted in higher pregnancy rates within one (P<0.01) and two months (P<0.05) after treatment as compared with treatment with a vitamin/mineral mixture. Buffalo cows and heifers with inactive ovaries bearing a dominant follicle were also successfully treated with gonadotropin releasing hormone, resulting in higher pregnancy rate within one month after treatment (P<0.05). In conclusion, the predominant cause of anestrus in dairy buffaloes in this region was true anestrus with inactive ovaries, and the duration of anestrus after calving as well as breeding was extremely long. Routine reproductive examination and adequate hormone treatment may improve the reproductive performance of these buffaloes.

Key words: Anestrus, Buffalo, Inactive ovaries, Infertility

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The buffalo population of the world was estimated to be over 170 million in 2003 [1]. More than 95% of the population of buffaloes are in Asia and the Pacific region [2]. There are 4.269 million buffaloes (2.7%) distributed in all the ecological regions (4% in the high hills, 54% in the mid hills and 42% in the Terai region) of Nepal [3]. Buffaloes play a prominent role in overall social development by maintaining a sustainable food producing system and power for agricultural operations in developing countries whose agricultural populations comprise 60–80% of their total populations [4–6]. About a million buffaloes in lactation contribute 70% of the total milk production and 65% of the meat production in Nepal [7]. In spite of the expansion of buffalo farming with remarkable potentialities, productivity remains poor [8], largely due to insufficient improvement in management techniques and inadequate genetic selection for productivity [9].

Reproductive efficiency is the primary factor affecting productivity. The average herd size of buffalo farmers in Chitwan in the southern region of Nepal is approximately 7.5, with a range of between three and 12 including buffalo cows, heifers and calves. Buffaloes are mated naturally. When cows are in estrus, the animals are brought to a male buffalo for mating. Although information on reproductive performance in buffaloes in Nepal or this region is not available, it is generally believed that buffaloes have severe reproductive problems. Among the various causes of infertility, anestrus and repeat breeding are the two major reproduc-

tive disorders [10,11]. However, the available information on the clinical aspect of anestrus in buffaloes is insufficient.

The aim of this study was to show some clinical features of anestrus buffaloes in Chitwan District, Nepal.

Materials and Methods

Animals

This study was conducted on Murrah graded buffaloes that were referred to infertility camps organized by the Veterinary Teaching Hospital, Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan District, southern region of Nepal, in collaboration with some of the dairy cooperatives and village development committee offices in the region during the breeding season in three consecutive years from 2001 to 2003. Three hundred and six buffaloes in which estrus had not been observed for more than two months after calving or after breeding were brought to the camps for diagnosis and treatment.

Breeding history, milk yield, feed and fodders fed were obtained by interviewing the owners before a gynecological examination. Body condition was checked and recorded as good, fair or poor. Among the 306 buffaloes in which estrus had not been observed after calving or breeding, 60 (19.6%) were diagnosed as pregnant by palpation per rectum and were, therefore, excluded from the group of animals for further investigation. The 246 anestrus animals were subjected to different treatments based on the clinical findings, the interval from calving to diagnosis and their age.

Parity of anestrus buffaloes: Of the 246 anestrus buffaloes, 111 (45.1%) were heifers and 135 (54.9%) were cows. The parity of the buffalo cows ranged between 1 and 9, with the majority being

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Table 1. Interval in months from last calving to diagnosis of anestrus in buffaloes

Months after calving	Numbers of animals	Percentages
2–3	7	5.2
4–6	16	11.8
7–9	29	21.5
10–12	27	20.0
13–15	13	9.6
16–	43	31.9
Total	135	100.0

between 1 and 4.

Breeding history: In the buffaloes with postpartum anestrus, the intervals in months between the last calving and diagnosis were greater than 6 months in 83% of the buffaloes; 79 of the 135 (61.5%) buffalo cows were at 10 months after calving or later (Table 1).

Of the 246 cows and heifers, 135 animals had post-breeding anestrus; they did not return to estrus after breeding, although they were not pregnant. The interval from last breeding to diagnosis in 66.0% of the heifers and 63.0% of the cows were longer than 5 months, and approximately 10.0% of the heifers and 12.0% of the cows were already at 11 months after breeding or later (Table 2).

Milk yield and feeding: The buffalo cows were milked twice a day, in the morning and evening. The lactation period ranged between 240 and 270 days. The buffaloes generally suckled their calves only when they were milked. Farmers allow the cows to suckle only for a few minutes to stimulate the udder before they start milking and again for another few minutes after completion of milking. The calves are then separated from the cows. The duration of suckling is four to six months.

The daily milk yield at the day of diagnosis was 1 to 2 kg in 19 buffaloes (28.4%), 3 to 6 kg in 31 buffaloes (46.3%) and 7 to 12 kg (25.4%) in 17 buffaloes. The buffaloes were fed 5 to 10 kg of rice straw, 5 to 20 kg of green fodders and 0.5 to 2 kg of wheat bran depending on their milk yield. Concentrate feed was not consistently fed to low producers (less than 2 kg per day), but for high producers (5 kg or above), 2 to 5 kg of concentrate feed was given.

Body condition: The body conditions of 42% of the heifers and 35% of the cows were poor. All the other cows and heifers had moderate body conditions.

Reproductive examination

All the buffaloes with anestrus that were brought to the infertility camps during the period from 2001 to 2003 were palpated per rectum for morphological examination of the reproductive tract by the authors themselves. In the buffalo cows, a vaginoscopic examination was also conducted.

Diagnosis and treatment

Buffaloes with a corpus luteum (CL): Anestrus buffalo cows with a normal CL about 10 mm in diameter or larger were considered to be in the luteal phase after silent ovulation. Among the

Table 2. Interval in months from last breeding to diagnosis of anestrus in buffalo cows and heifers

Months after breeding	Numbers of animals	Percentages
3–4	44	32.6
5–6	38	28.1
7–8	20	14.8
9–10	6	4.4
11–12	14	10.4
13–	13	9.6
Total	135	100.0

anestrous animals, those that were already 12 months or more after calving were injected intramuscularly with 25 mg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Pronalgon F, dinoprost tromethamine, Pfizer, Tokyo, Japan). All other animals were prescribed administration per os of a vitamin/mineral mixture for three weeks [12]. Heifers with a CL at the age of 2.5 years or more were treated with $PGF_{2\alpha}$, and the others were treated with a vitamin/mineral mixture.

Buffaloes with a dominant follicle (DF) 10 to 12 mm in diameter or larger and without a CL: These buffaloes were considered to probably not be cycling but to have a DF that may respond to GnRH. Anestrus buffalo cows 12 months after calving with DFs greater than 12 mm and without a CL were injected intramuscularly with 50 μ g fertirelin acetate (Schering-Plough Animal Health, Tokyo). All the other cows were treated with a vitamin/mineral mixture. Heifers with a DF that were at least 2.5 years old were injected with GnRH and the others were given a vitamin/mineral mixture.

Buffaloes without a CL and DF: Buffalo cows and heifers with inactive ovaries were treated with oral administration of a vitamin/mineral mixture. In the anestrus buffaloes with indications of cervicitis, one liter of 1% Lugol's solution was infused into the vagina. Anestrus buffaloes in which infection with internal parasites was suspected because of extremely poor nutrition were orally administered 3 g oxclozanide and 3 g tetramisole hydrochloride for deworming.

Buffaloes with large follicles without a CL: These buffaloes were considered to have had anestrus-type follicular cysts if they had a follicular structure 2.0 cm in diameter or larger without co-existence of a CL. These cows and heifers were treated intramuscularly with 50 μ g GnRH analog.

Mating and pregnancy diagnosis after treatment

All the treated anestrus buffaloes were observed for estrus behavior and signs for two months or longer. Breeding was basically performed by natural mating after detection of estrus. The animals were checked for pregnancy 2 to 3 months after the last breeding.

Statistical analysis

Differences in percentages between two groups were analyzed by Fisher's exact test.

Table 3. Reproductive performance in anestrus buffalo heifers and cows after treatment

Clinical findings	Number of buffalo heifers and cows treated						Total N=246
	CL N=66		DF / No CL N=92		No CL / No DF N=76	F Cyst N=12	
Treatment	PGF ₂ α	Vit/M	GnRH	Vit/M	Vit/M	GnRH	
No. of heifers treated	22	44	11	81	76	12	246
Cows in estrus / mated within 1 month	22	28	11	52	49	12	174
Estrus detection rate (%)	100.0	63.6	100.0	64.2	64.5	100.0	70.7
Cows conceived within 1 month	19	22	10	41	39	10	141
Pregnancy rate within 1 month (%)	86.4 ^a	50.0 ^b	90.9 ^x	50.6 ^y	51.3	83.3	57.3
Cows conceived within 2 months	3	13	1	25	24	2	68
Pregnancy rate within 2 months (%)	100.0 ^x	79.5 ^y	100.0	81.5	82.9	100.0	85.0

^{a,b} Values with different superscripts in the same row are significantly different ($P<0.01$). ^{x,y} Values with different superscripts in the same row are significantly different ($P<0.05$).

Results

Clinical findings

Ovaries: On palpation per rectum, CLs 10 mm in diameter or larger, DFs and ovarian cysts were found in 33.3, 33.3 and 5.2% of the anestrus buffalo cows, respectively. Twenty-eight percent of cows had neither a CL nor a DF. In the heifers, 18.9% had a CL, 42.3% had a DF and 4.5% had ovarian cysts, while 34.2% had neither a CL nor a DF.

Uterine horns: The diameters of uterine horns estimated by palpation per rectum ranged between 1.0 and 2.0 cm in the heifers. Of the 135 cows, 116 (85.9%) had uterine horns 1 to 2 cm in diameter, and 19 cows (14.1%) had uterine horns 2.5 to 3.5 cm in diameter.

Uterine contraction was not detected in 66.7% of the heifers and 74.8% of the cows.

Vaginal mucus discharge: Purulent discharge was detected on vaginoscopy in 2.7% of the heifers and 2.9% of the cows. No mucus was seen in 91.2% of the heifers and 73.3% of the cows.

Effects of treatment

Since the effects of the treatments in terms of reproductive performance did not differ between the heifers and cows, the data of the heifers and cows were combined to compare the pregnancy rate after the different methods of treatment (Table 3). Treatment of anestrus cows and buffaloes with a CL using PGF₂α resulted in higher pregnancy rates within one ($P=0.0064$) and two months ($P=0.0239$) after treatment than treatment with a vitamin/mineral mixture.

Buffaloes with inactive ovaries treated with GnRH showed a higher pregnancy rate within one month after treatment ($P=0.0201$) than those treated with a vitamin/mineral mixture (Table 3).

Discussion

Anestrus in cattle and buffaloes is generally caused by ovarian dysfunction (true anestrus), silent ovulation and missing heat. One clinical survey conducted many years ago in cases of reported anestrus showed that 58.4% of cases were true anestrus and 33.3%

of cases were silent ovulation [13]. In the present study, among the 135 buffalo cows that were brought to the infertility camps due to anestrus in the southern region of Nepal, 61.4% had inactive ovaries (true anestrus), while 33.3% had silent ovulation or missing heat, which corresponds well with the previous observations. Ovarian cysts were diagnosed in only 5.2% of the cows in this study, which was also well comparable with the figure reported earlier [13]. In the anestrus heifers, 76.6% were true anestrus, and 18.9% were silent ovulation. It has been generally observed in the field that lactating cows are better fed than heifers. Concentrate feed is, for example, fed to only cows in lactation and is not fed to non-lactating animals, including heifers. Comparison of body condition between cows and heifers may be needed to clarify the causes of the difference in the incidence of true anestrus.

It is commonly accepted that dairy buffaloes have two calves every three years [13], the calving interval being approximately 18 months. The buffalo cows may need to become pregnant again within 8 to 8.5 months after calving to achieve this calving interval. In this survey, 61.5% of the anestrus cows were already at 10 months after calving or later, and 31.9% were at more than 16 months postpartum. 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 of the cows.

Buffaloes with a CL responded well to PGF₂α, with high estrus induction and pregnancy rates, as already reported by a number of studies [14–16]. It has also been reported that GnRH is effective in inducing ovulation in buffaloes [17, 18]. The results of this study correspond to these earlier reports.

The efficacy of PGF₂α and GnRH in anestrus 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 buffa-

loes in this region were true anestrus and silent ovulation, 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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