NOTE *Theriogenology*

Effects of Treatment for Anestrus in Water Buffaloes with $PGF_{2\alpha}$ and GnRH in Comparison with Vitamin-Mineral Supplement, and Some Factors Influencing Treatment Effects

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ABSTRACT. The effect of treatment for anestrus in buffaloes with a $PGF_{2\alpha}$ or GnRH injection and vitamin-mineral (Vit-M) supplementation for 1 to 2 months and some factors influencing the treatment effect were studied. In anestrus buffaloes with CL, an injection of $PGF_{2\alpha}$ tended to show higher estrus detection and pregnancy rates within 17 days after treatment than Vit-M supplementation ($P<0.10$). In those with inactive ovaries, effect of GnRH and Vit-M did not differ. Body condition score of the animals before treatment affected pregnancy rate within 17 days after treatment ($P<0.05$). Pregnancy rate within 4 months after treatment was adversely influenced by low serum concentrations of calcium ($P<0.01$) and gastrointestinal parasitic infection before treatment ($P<0.05$).

KEY WORDS: anestrus buffalo, gastrointestinal parasite, hormonal treatment, nutritional parameters, vitamin-mineral supplementation.

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Buffalo rearing is very important for rural development in many countries including Nepal. In Nepal, water buffaloes (*Bubalus bubalis*) contribute to 71% of total milk and 52.8% of total meat production [19]. Productivity of buffaloes which depends largely on age of first calving and calving intervals is still low. There is a plenty of room for increasing productivity of buffaloes through improvement of reproductive performance.

Anestrus is the most important cause of poor reproductive performance in buffaloes [6, 10, 13, 24]. It is also a big reproductive problem in modern dairy cow production worldwide [18, 21]. Dairy herds are regularly visited by veterinarians at two to four week intervals, and cows with anestrus are treated with hormones for synchronization of estrus and/or ovulation without delay. Regular reproductive examination of buffaloes at an appropriate interval is practically difficult in Nepal due to constraints of expense and availability of veterinary service in rural areas. For many marginal buffalo farms, infertility camps which are organized once a year or at a longer interval mainly by district livestock offices, veterinary schools, dairy cooperatives and some other organizations are the occasions to have their buffaloes examined and treated by veterinarians. Anestrus buffaloes are conventionally treated with vitamin-mineral mixture (Vit-M) supplements with the variable effects [24]. Several methods of estrus and ovulation induction using hormones have been recently developed in buffaloes for treating anestrus and improving reproductive efficiency [2, 7, 20, 27]. More veterinarians have started to use hormones, such as $PGF_{2\alpha}$ and GnRH, for the treatment of anestrus in buffaloes in Nepal. The effectiveness of these treatments under the field conditions, however, is yet to be described, since only limited information on the effectiveness of different methods of treatment for anestrus in buffaloes has been available. This is due to the fact that no follow up examination of animals has been conducted after treatment at the infertility camps.

Thus, it is imperative to describe the most useful method of treatment based on accurate diagnosis for anestrus, since the presence of a dominant follicle (DF) and/or corpus luteum (CL) in the ovary indicates the success of hormonal treatment [7]. Management and nutritional factors that may influence the response of anestrus buffaloes after treatment have not been well described either. Aims of this study were to show the effectiveness of $PGF_{2\alpha}$ or GnRH in comparison with the conventional Vit-M treatment on anestrus in buffaloes and analyze some nutritional and managemental factors influencing the effectiveness of treatment.

A total of 104 Murrah graded water buffaloes, 32 heifers and 72 cows, from small holders in Chitwan district of the Southern Nepal that had not shown any signs of estrus for at least 2 months after reaching the age for breeding or after calving or the last breeding were enrolled for clinical reproductive examination at the infertility camps organized in November, 2009. The buffaloes were examined by transrectal palpation and/or ultrasonography (Honda, HV-1500, Honda Electronics, Tokyo, Japan) to diagnose the causes

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of anestrus. The information about the calving history, interval from calving to the examination, parity, feeding and management was obtained from the owners. Of 104 animals with anestrus, 28 buffaloes had a normal size of CL, larger than 10 mm in diameter, and 43 others had neither Graafian follicles nor CL. The remaining 33 buffaloes including 22 buffaloes which were already pregnant, seven buffaloes with cervicitis and/or endometritis, two buffaloes with ovarian cysts and two buffaloes with Graafian follicles showing signs of approaching to estrus were excluded from the study.

The 28 anestrus buffaloes with CL were randomly divided into two groups; the first group was injected IM with 25 mg $PGF_{2\alpha}$ (Pronalgon F, dinoprost tromethamine, Pfizer, Tokyo, Japan), and the second group was administered orally with 30 g Vit-M supplement daily for 1 to 2 months. The supplement contained vitamins A (700,000 IU), D_3 (70,000 IU) and E (250 mg), nicotinamide (1,000 mg), iodine (325 mg), iron (1,500 mg), magnesium (6,000 mg), manganese (1,500 mg), potassium (100 mg), sodium (5.9 mg), zinc (9,600 mg), P (12.75 mg), selenium (10 mg), sulphur (0.72%) and Ca (25.5%) per kg (Agrimin forte®, Virbac Animal Health India Pvt. Ltd., Mumbai, India). Other 43 anestrus buffaloes that had inactive ovaries were also divided at random into two groups and were either injected IM with 50 μg GnRH analogue (Fertirelin acetate, Takeda-Schering Plough Animal Health, Tokyo, Japan) or given 30 g Vit-M daily for 1 to 2 months. Later, 23 of the 28 buffaloes with CL and 31 of the 43 with inactive ovaries were followed-up by farm visits to investigate their reproductive performance after treatment. The first follow-up visit was conducted within 17 days after the initiation of treatment to obtain the records for estrus or mating. The second visit was conducted around 4 months after treatment mainly to check animals for pregnancy. Since Vit-M was administered for 1 to 2 months, buffaloes in Vit-M treated group were still under the treatment when they were checked for estrus or mating after treatment.

At the infertility camps, body condition score (BCS) of the buffaloes (range: 1–5) [10] was recorded. The buffaloes were classified into three different BCS groups, <2.5, 2.5 to 3.5 and >3.5. Blood samples were collected via the jugular vein from 17 randomly selected buffaloes. Serum concentrations of calcium (Ca) and inorganic phosphorus (iP) were determined spectrophotometrically by applying an orthocresolphthalein complexone and molybden blue, respectively, using Wako Chemicals Reagent kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum total protein (TP) was measured by using a refractometer. The reference values for Nepalese buffaloes [15] were used to show the normal range of the values obtained.

Feces in the rectum were collected from 24 randomly selected buffaloes to examine for gastrointestinal parasites; helminth eggs and coccidian oocysts. The effects of BCS, parity, calving-treatment interval, blood nutritional parameters and gastrointestinal parasitic infection on reproductive performance after treatment were analyzed. Difference in the reproductive performance among different groups was analyzed by Likelihood-ratio Chi Square test, and difference in the mean values between two groups was compared by the Student's *t*-test, (JMP 5.0.1, SAS Institute Inc., Cary, NC, U.S.A.). The probability values of $P<0.05$ were considered as significant.

Anestrus buffaloes with CL treated with $PGF_{2\alpha}$ tended to show higher estrus detection or mating rate (70.0 vs 30.8%) and pregnancy rate (60.0 vs 23.1%) within 17 days after treatment than those administered with Vit-M supplement ($P<0.10$). Pregnancy rate within 4 months after treatment was not different between the groups (Table 1). There was no difference in the effect of treatment between GnRH and Vit-M treated groups of buffaloes with inactive ovaries (Table 1).

BCS on the day of treatment affected pregnancy rate within 17 days after treatment with $PGF_{2\alpha}$ in anestrus buffaloes with CL ($P<0.05$) (Table 2). No effect of parity and calving to treatment interval on the pregnancy rate was shown.

Of the 17 anestrus buffaloes measured for blood nutritional parameters, 5 (29.4%) showed subnormal blood level of Ca, 4 (23.5%) of iP and 8 (47%) of TP (Table 3). Similarly, 11 of the 24 (45.8%) anestrus buffaloes examined for gastrointestinal parasites showed positive on fecal test for coccidia (6 buffaloes) or helminth only (2 buffaloes) or a mixed infection (3 buffaloes) of coccidian and helminthes (Table 4). Moreover, pregnancy rate within 4 months after treatment was affected by the blood levels of those nutritional parameters (Table 3) as well as gastrointestinal parasitic infection (Table 4). Buffaloes with serum calcium of lower than the normal range showed lower pregnancy rate within 4 months after treatment ($P<0.01$), and in those with serum TP of lower than the normal range, the pregnancy rate tended to be lower ($P<0.10$). The gastrointestinal parasitic infection reduced pregnancy rate within 4 months after treatment ($P<0.05$) (Table 4).

When compared the blood concentrations of Ca, iP and TP between the anestrus buffaloes that became pregnant and those which failed to become pregnant within 4 months after treatment, blood Ca and TP in buffaloes not becoming pregnant were shown to be lower ($P<0.01$) (Table 5).

The results obtained in the present field study showed that an intramuscular administration of $PGF_{2\alpha}$ for the treatment of anestrus buffaloes with CL showed a tendency to be more effective than feeding Vit-M as a supplement for 1 to 2 months. A number of studies have reported that buffaloes with a CL respond well to $PGF_{2\alpha}$ treatment [5, 9, 11, 22, 24]. It has also been reported that a mid-cycle CL in cyclic buffaloes is sensitive to $PGF_{2\alpha}$ [12], and efficacy of $PGF_{2\alpha}$ in buffalo cow is dependent upon CL size before treatment [3]. The effect of treatment of anestrus buffaloes with $PGF_{2\alpha}$ obtained in this study was closer to the results reported in previous studies. On the other hand, there have been a few, if any, reports on the comparison in effect of treatment for anestrus with CL between $PGF_{2\alpha}$-treated and Vit-M-treated buffaloes. One report from Nepal [24] showed that treatment of anestrus buffaloes with a CL using $PGF_{2\alpha}$ resulted in higher pregnancy rates within one month after treatment than treatment with a Vit-M supplement. Since the benefit of treatment with $PGF_{2\alpha}$ is apparent, a long-standing practice using Vit-M supplement should be replaced by an injection

Table 1. Comparison in the effects of $PGF_{2α}$ and vitamin-mineral (Vit-M) supplement in anestrus buffaloes with corpus luteum (CL) and the effects of GnRH and Vit-M supplement in anestrus buffaloes with inactive ovaries

	Anestrus with CL		Anestrus with inactive ovaries	
Treatment	$PGF_{2α}$	Vit-M	GnRH	Vit-M
No. of buffaloes treated	10	13	7	24
No. of buffaloes bred within 17 days (%)	7 (70.0)	4 (30.8)	2 (28.6)	4 (16.7)
No. of buffaloes conceiving within 17 days (%)	6 (60.0)	3 (23.1)	2 (28.6)	4 (16.7)
No. of buffaloes conceiving within 4 months (%)	7 (70)	8 (61.5)	6 (85.7)	18 (75.0)

Table 2. Influence of body condition score (BCS), parity and calving to treatment interval on pregnancy rate within 17 days after the initiation of treatment with $PGF_{2α}$, GnRH or vitamin-mineral (Vit-M) supplement in anestrus buffaloes

	Pregnancy rate within 17 days after treatment (No. of buffaloes conceiving)			
	Anestrus buffaloes with CL		Anestrus buffaloes with inactive ovaries	
Treatment	$PGF_{2α}$	Vit-M	GnRH	Vit-M
No. of buffaloes treated	10	13	7	24
BCS				
<2.5	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
2.5–3.5	40.0 (4)*	23.1 (3)	28.6 (2)	16.7 (4)
>3.5	20.0 (2)*	0.0 (0)	0.0 (0)	0.0 (0)
Parity				
Heifer	10.0 (1)	0.0 (0)	28.6 (2)	4.2 (1)
1-3 lactation	20.0 (2)	7.7 (1)	0.0 (0)	12.5 (3)
≥4 lactation	30.0 (3)	15.4 (2)	0.0 (0)	0.0 (0)
Calving to treatment interval[a)]				
2–6 months	12.5 (1)	9.1 (1)	0.0 (0)	6.3 (1)
7–12 months	37.5 (3)	9.1 (1)	0.0 (0)	12.5 (2)
>12 months	12.5 (1)	9.1 (1)	0.0 (0)	0.0 (0)

*Significantly higher than the group with BCS <2.5 (P<0.05). a) Only the lactating buffaloes are included.

Table 3. Influence of some blood nutritional parameters on pregnancy rate of anestrus buffaloes within 4 months after treatment

	No. of buffaloes treated	No. of buffaloes conceiving within 4 months	Pregnancy rate within 4 months
Calcium (mg/d*l*)			
<8.5	5	0	0.0*
8.5–11.0	12	10	83.3
Inorganic phosphorus (mg/d*l*)			
<4.5	4	1	25.0
4.5–7.5	13	9	69.2
Total protein (g/d*l*)			
<7.0	8	3	37.5
7.0–9.0	9	7	77.8

* Significantly higher than cows with normal value (P<0.01).

Table 4. Influence of gastrointestinal parasites on pregnancy rate of anestrus buffaloes within 4 months after treatment

Parasitic infection	No. of buffaloes Treated	No. of buffaloes conceiving within 4 months	Pregnancy rate within 4 months
Negative	13	10	76.9
Cocccidia	6	2	33.3*
Helminth with/without coccidia	5[a)]	1	20.0*

*Significantly lower than negative group (P<0.05). a) Infection of helminth with coccidia in 3 buffaloes and helminth only in 2 buffaloes.

Table 5. Comparison in blood concentrations of calcium (Ca), inorganic phosphorus (iP) and total protein (TP) between the anestrus buffaloes that showed or failed to show pregnancy within 4 months after treatment

	Pregnancy within 4 months	
	Positive (n=10)	Negative (n=7)
Ca (mg/d*l*)	9.5 ± 0.3	7.4 ± 0.4*
iP (mg/d*l*)	6.0 ± 0.4	5.2 ± 0.5
TP (g/d*l*)	7.4 ± 0.2	6.2 ± 0.3*

The values are expressed as means ± SEM. **P*<0.01 vs positive group.

of $PGF_{2\alpha}$ after examining the ovaries for the CL. Within 4 months after treatment, pregnancy rate did not differ significantly between the two groups. This may indicate that Vit-M supplementation is effective for the treatment of anestrus in buffaloes, but needs a longer period to show its effectiveness in comparison with $PGF_{2\alpha}$ injection. Pregnancy rate within 4 months after treatment may be affected by a number of factors other than the treatment. Thus, it might be difficult to know whether Vit-M treatment has comparative effects with $PGF_{2\alpha}$ within 4 months after treatment.

Treatment of anestrus with inactive ovaries using GnRH did not show any beneficial effects over Vit-M supplementation. Administration of GnRH to anestrus buffaloes with inactive ovaries has been shown to produce a variable response [1, 22]. In cattle, those with DFs 10 mm in diameter or larger can respond to LH with ovulation [25]. The presence of a DF at the time of GnRH treatment is also a pre-determining factor for ovulation induction in buffaloes [2, 7]. The success of GnRH treatment, therefore, depends largely on the timing of GnRH injection during a follicular wave. There will be no response of buffaloes with inactive ovaries after GnRH, if the animals do not have healthy DF with the adequate size. In their earlier study on the treatment of inactive ovaries in buffaloes, Sah and Nakao [24] gave GnRH to anestrus buffaloes having DF 10 to 12 mm in diameter or larger, which were 6 months postpartum or later, while those that were within 6 months after calving were treated with Vit-M supplement. They found that the GnRH-treated buffaloes showed a significantly higher pregnancy rate within one month after treatment than the Vit-M-treated animals. In the present study, GnRH was injected to anestrus buffaloes regardless of the presence and size of DF. This might have attributed to the poor response of the cases after GnRH treatment. To increase conception rate in non-cycling anestrus buffaloes after treatment, progesterone supplementation for one week in combination with some other hormone treatments, such as Ovsynch [8], was shown to be effective. This has yet to be applied to buffaloes in Nepal.

Nutrition is one of the most important factors influencing reproductive performance in cattle [4]. Several factors including nutrition affect the response of buffaloes with anestrus to treatment [6]. It has been reported that BCS affects the response of anestrus dairy cows after treatment [23] and the success of timed AI after ovulation synchronization in beef cattle [26]. Results of the current study also showed that BCS affected pregnancy rate of anestrus buffaloes with CL after treatment with $PGF_{2\alpha}$. Producers should be informed that buffaloes with low BCS may not respond to the treatment and be encouraged to improve nutritional state of the animals.

It is worthy to know that 29.4, 23.5 and 47% of the anestrus buffaloes had subnormal blood levels of Ca, iP and TP, respectively. Deficiency of blood Ca and TP was associated with a lower pregnancy rate or a tendency for a lower pregnancy rate, respectively, within 4 months after treatment, and buffaloes not becoming pregnant during this period showed lower blood Ca and TP. It is known that Ca is involved in steroidogenesis and ovulation [14], and its deficiency might have caused hormonal incompetence or ovulation failure resulting in pregnancy failure. Protein deficiency may adversely affect reproductive function via a decrease in IGF-1 release in response to exogenous growth hormone [17]. It was reported that true anestrus buffaloes showed lower levels of serum protein as compared to normal cyclic buffaloes [16]. We suggest that Ca and protein deficiency needs to be paid more attention as major causes of subfertility in buffaloes.

Gastrointestinal parasitic infection has been a common problem to cause poor production and sub-fertility in buffaloes. Nearly a half of buffaloes referred to the infertility camps were positive for coccidian and/or helminth infection. The infected buffaloes showed a lower pregnancy rate than those without infection. Implementation of deworming program in buffaloes may improve the reproductive performance.

In conclusion, a single injection of $PGF_{2\alpha}$ is more effective for the treatment of anestrus with CL in buffaloes than treatment with Vit-M supplement. Low BCS affected negatively on pregnancy rate, and deficiency of Ca and protein and gastrointestinal parasitic infection reduced the pregnancy rate after the initiation of treatment. Improvement of nutrition and control of parasitic infection could enhance the treatment effects on anestrus in buffaloes.

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