# RESEARCH RTICLE

# Effect of PGF<sub>2</sub> and PGF<sub>2</sub> along with vitamin and phosphorus combination on fertility parameters of postpartum subestrous surti buffaloes as well as Ca: P ratio

■ A.S. REDE, C.T. KHASATIYA<sup>1</sup>, D.K. SONI<sup>1</sup>, M.D. PATEL<sup>3</sup>, S.P. KATKAR<sup>1</sup> AND S.S. CHAUDHARY<sup>2</sup>

#### Members of the Research Forum

#### Associate Author:

<sup>1</sup>Department of Veterinary Gynaecology and Obstetrics, Navsari Agricultural University, Navsari Campus, NAVSARI (GUJARAT) INDIA

<sup>2</sup>Department of Physiology and Biochemistry, Navsari Agricultural University, Navsari Campus, NAVSARI (GUJARAT) INDIA

<sup>3</sup>Livestock Research Station, (NAU) NAVSARI (GUJARAT) INDIA

# $\label{eq:author} \mbox{Author for correspondence}: \\ \mbox{A.S. REDE}$

Department of Veterinary Gynaecology and Obstetrics, Navsari Agricultural University, Navsari Campus, NAVSARI (GUJARAT) INDIA **Abstract :** Postpartum suboestrous surti buffaloes of an organized farm confirmed by twice per-rectal palpation 11 days apart from 45 days post-partum were treated with 2 ml (500 µg) of inj. cloprostenol sodium I/M route in first group (n=6) and 2 ml (500 µg) of inj. cloprostenol sodium I/M route along with 5 ml inj. Vit. AD<sub>3</sub>E preparation and 15 ml inj. Toldimphos sodium preparation I/M route in second group (n=6) on 55 days postpartum after confirmation of ovarian cyclicity. Six animals of same status were kept as control to see the oestrus induction response and conception rate including evaluation of serum mineral profile, just before (0 day) treatment and 24 hr, 48 hr and 72 hr after treatment. The service period and oestrus induction interval in days was found significantly lower in PGF<sub>2</sub> $\alpha$  treated (T<sub>1</sub> and T<sub>3</sub>) groups as compared to T<sub>2</sub> and control (T<sub>4</sub>) group clearly showed the luteolytic effect of PGF<sub>2</sub> $\alpha$  on ovaries and earlier resumption of ovarian activities as compared to treatment (T<sub>2</sub>) and control (T<sub>4</sub>) groups. The serum Ca: P ratio of suboestrous surti buffaloes in different treatment and control groups at different time intervals was found to be ranging from 1.44:1 to 1.63:1. The ratio of serum Ca: P should be between 1.5:1 and 2.5:1 for efficient reproduction in dairy bovines.

**Key words:** Sub-oestrus buffaloes, Hormone therapy, Vitamin and phosphorus, Postpartum period, Ca: P ratio

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### INTRODUCTION

Silent oestrus is one of the major impediments in understanding reproductive parameters and assisted reproduction

in buffaloes (Mondal *et al.*, 2010). The period of post-partum sub-oestrus is usually longer in buffalo than in cattle under comparative managemental conditions (Azawi *et al.*, 2012). Sub-oestrus is a condition in which genital organs are undergoing normal cyclical changes but behavioural signs of oestrus are not manifested. Prostaglandins are commonly used in the therapy of suboestrus condition and reproductive management in cattle and buffaloes, have a wide application in female animal reproduction. 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 (Rahaman *et al.*, 2012). Keeping all this in view, the study was undertaken on suboestrous buffaloes to see the efficacy of  $PGF_2\alpha$  and  $PGF_2\alpha$  along with vitamin and phosphorus combination in relation to oestrus response and fertility.

## RESEARCH METHODOLOGY

A study was carried out on 24 postpartum suboestrous Surti buffaloes of Livestock Research Station, Navsari Agricultural University, Navsari (Gujarat). All these buffaloes had normal calving and subsequent normal genital health as assessed gynaeco-clinically. During the course of this study, 24 buffaloes beyond 45 days postpartum were identified through twice per-rectal palpation 10 days apart as suboestrous. They were randomly divided into four equal groups consisting 6 surti buffaloes in each group viz., T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. The buffaloes in T<sub>1</sub> group were treated with 2 ml of Inj. Cloprostenol sodium (Inj. Cyclix) (500 μg, PGF<sub>2</sub>α analogue, I/M route); the buffaloes in T<sub>2</sub> group were treated with [inj. Vit. AD<sub>2</sub>E preparation (5 ml, I/M route) + inj. Toldimphos sodium preparation (15 ml, I/M route)]; the buffaloes in T<sub>3</sub> group were treated with 2 ml of Inj. Cloprostenol sodium (Inj. Cyclix) (500 μg, PGF<sub>2</sub>α analogue, I/M route) + [inj. Vit. AD<sub>3</sub>E preparation (5 ml, I/M route) + inj. Toldimphos sodium preparation (15 ml, I/M route) M route)] and the buffaloes in group T<sub>4</sub> were kept as sub-oestrous control group. All these buffaloes were followed for oestrus induction response, reproductive performance for upto 120 days post-partum. Approximately, 10 ml blood samples in serum clotting vaccutainers were collected from all those selected animals on 0 hr (prior to treatment), 24 hr, 48 and 72 hr post-treatment aseptically by jugular vein puncture. The vaccutainers containing blood samples were kept in slanting position at room temperature for 1-2 hours. Finally, serum was separated by centrifugation at 3000 rpm for 15 minutes and stored in properly labelled sterilized 4.5 ml plastic storage vials at -20°C in deep freezer until analysis. Estimation of calcium and phosphorus levels was determined in blood serum samples by using standard assay kits and an auto analyzer (Merck's Micro-lab 300 analyzer, Vital Scientific, DIEREN-Netherlands).

## RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads:

#### **Fertility parameters:**

In PGF<sub>2</sub> $\alpha$  (T<sub>1</sub>) group, 100 per cent (6/6) treated buffaloes responded with normal oestrus signs at a mean interval of 3.50±0.34 days (ranging from 3 to 5 days) post-treatment and all of them conceived. The number of services per conception in this group was 1.33 with 100 per cent conception rate.

In Vit+ P ( $T_2$ ) group, 66.67 per cent (4/6) treated buffaloes responded with normal oestrus signs at a mean interval of 16.75 $\pm$ 2.56 days (ranging from 10 to 22 days) post-treatment and all of responded buffaloes were conceived. The number of services per conception in this group was 1.50 with 66.67 per cent conception rate.

In PGF<sub>2</sub> $\alpha$ +Vit+ P(T<sub>3</sub>) group100 per cent (6/6) treated buffaloes responded with normal oestrus signs at a mean interval of 3.83±0.31 (ranging from 3 to 5 days) days post-treatment and all of them conceived. The number of services per conception was an average of 1.33 in this group.

In suboestrus control ( $T_4$ ) group 50.00 per cent (3/6) treated buffaloes responded with normal oestrus signs at a mean interval of 20.67 $\pm$ 2.03 days (ranging from 17 to 24 days) post-treatment and all of responded buffaloes conceived with an average of 1.33 services per conception.

The mean intervals (days) from calving to conception for treatment groups- I ( $T_1$ ), II ( $T_2$ ), III ( $T_3$ ) and control group- IV ( $T_4$ ) were observed to be  $66.83\pm4.07$  days,  $91.50\pm3.48$  days,  $67.33\pm3.85$  days and  $96.00\pm8.50$  days, respectively. The overall service period of all the groups and treatment to oestrus induction interval was  $80.42\pm4.98$  and  $11.19\pm1.31$  days, respectively, with an overall conception rate 79.17 per cent (19/24).

The service period and treatment to oestrus induction interval of the suboestrus surti buffaloes for  $T_1$  (66.83±4.07; 03.50±0.34 days) and  $T_3$  (67.33±3.85; 03.83±0.31days) treatment groups as well for  $T_2$  (91.50±3.48; 16.75±2.56 days) and control  $T_4$  (96.00±8.50; 20.67±2.03 days) group did not differ significantly. However, the service period and treatment to oestrus induction interval of the suboestrus surti buffaloes in  $T_1$  (66.83±4.07; 03.50±0.34 days) and  $T_3$  (67.33±3.85; 03.83±0.31 days) treatment groups differed significantly from treatment  $T_2$  (91.50±3.48; 16.75±2.56 days) and control  $T_4$  (96.00±8.50; 20.67±2.03 days) groups. It was observed that service period in the PGF<sub>2</sub> $\alpha$  treated ( $T_1$  and  $T_3$ ) groups had been minimized upto 30 days earlier than that of treatment ( $T_2$ ) and control ( $T_4$ ) group (Table 1).

The service period and oestrus induction interval in days was found significantly lower in  $PGF_2\alpha$  treated ( $T_1$  and  $T_3$ ) groups as compared to  $T_2$  and  $T_4$  control group which clearly showed the luteolytic effect of  $PGF_2\alpha$  on ovaries and earlier resumption of ovarian activities as compared to treatment ( $T_2$ ) and control ( $T_4$ ) groups. Moreover, there was no significant difference found in oestrus induction interval (days) between phosphorus and vitamins treated group ( $T_2$ ) and control group ( $T_4$ ); however, apparently lower oestrus induction interval in days (16.75±2.56) was found in treatment ( $T_2$ ) group as compared to control group (20.67±2.03) which might be attributed to the influence of exogenous inorganic phosphorus and vitamins given to the animals in treatment ( $T_2$ ) group. Earlier workers (Kumar *et al.*, 2010) also have used vitamins and inorganic phosphorus as a therapy in the suboestrous cattle with

Treatment/ Group (n=6)	Service period (days)	Number of services per conception	Treatment to oestrus induction interval (days)	Conception rate (%)	No. of animals responded to the treatment	No. of pregnant animals (n=6)
$T_1$	66.83±4.07 <sup>a</sup>	1.33 <sup>a</sup>	$03.50\pm0.34^a$	100.00	6(100.00%)	6
$T_2$	91.50±3.48 <sup>b</sup>	1.50 <sup>a</sup>	16.75±2.56 <sup>b</sup>	66.67	4(66.67%)	4
$T_3$	67.33±3.85 <sup>a</sup>	1.33 <sup>a</sup>	$03.83 \pm 0.31^a$	100.00	6(100.00%)	6
$T_4$	$96.00\pm8.50^{b}$	1.33 <sup>a</sup>	$20.67\pm2.03^{b}$	50.00	3(50.00%)	3
Overall	$80.42\pm4.98$	1.37	11.19±1.31	79.17	19(79.17%)	19/24

Means bearing different superscripts within a column (group) differ significantly ( $p \le 0.05$ ) Group-I =  $T_1$  (PGF<sub>2</sub> ) Group-II =  $T_2$  (Vit+ P) Group-III =  $T_3$  (PGF<sub>2</sub> +Vit+ P) Group-IV =  $T_4$  (Control)

Table 2 : Comparison of serum Ca: P ratio of suboestrus buffaloes in different treatment and control groups at different tim intervals (Mean±SE)								
Time intervals/	Parameters	Groups (n=6)						
days	rarameters	$PGF_2$ $(T_1)$	Vit+ P (T <sub>2</sub> )	PGF <sub>2</sub> +Vit+ P (T <sub>3</sub> )	$Control(T_4)$			
0 hour	Calcium (mg/dl)	10.56±0.29	9.05±0.22	9.57±0.29	9.24±0.22			
	Phosphorus (mg/dl)	$6.74\pm0.22$	6.21±0.17	5.92±0.27	$5.87 \pm 0.22$			
	Ratio	1.57:1	1.46:1	1.62:1	1.57:1			
24 hour	Calcium (mg/dl)	10.54±0.29	9.06±0.22	9.52±0.29	9.22±0.22			
	Phosphorus (mg/dl)	6.72±0.22	6.23±0.17	5.94±0.27	$5.88 \pm 0.22$			
	Ratio	1.57:1	1.45:1	1.60:1	1.57:1			
48 hour	Calcium (mg/dl)	10.57±0.29	9.08±0.22	9.53±0.29	9.21±0.22			
	Phosphorus (mg/dl)	6.72±0.22	6.30±0.17	5.90±0.27	5.90±0.22			
	Ratio	1.57:1	1.44:1	1.61:1	1.56:1			
72 hour	Calcium (mg/dl)	10.59±0.29	9.13±0.22	9.57±0.29	9.23±0.22			
	Phosphorus (mg/dl)	6.72±0.22	6.32±0.17	$5.89\pm0.27$	5.85±0.22			
	Ratio	1.58:1	1.44:1	1.63:1	1.58:1			

encouraging results.

Additional exogenous injection of inorganic phosphorus along with vitamins might have helped to resolve the problem of suboestrus in surti buffaloes upto some extent and it is true in treatment group  $T_2$ , had higher conception rate (66.67 %) as compared to 50.00 per cent in  $T_4$  control group. However, the number of services per conception did not differ significantly among all the experimental suboestrus treatment and control groups. Moreover, cent per cent conception rate in  $PGF_2\alpha$  ( $T_1$ ) and  $PGF_2\alpha+Vit+P$  ( $T_3$ ) groups as compared to 66.67 per cent and 50.00 per cent conception rate in simple Vit+P ( $T_2$ ) and suboestrus control ( $T_4$ ) groups, respectively, might be under the influence of various treatments in above period (45 to 120 days) with an overall 79.17 per cent (19/24) conception rate. That is the reason why phosphorus and vitamin therapy could have frequently been used as an initial package of practices in the treatment of suboestrus in the field conditions to augment fertility in those animals.

The service period, treatment to oestrus induction interval, percentage of animals responded to the treatment and conception rate in  $T_1$  and  $T_3$  groups are in agreement with the findings of Khasatiya *et al.* (2006), who reported that 100 per cent animals exhibited pronounced oestrus within 3.40±0.40 days as against 91.33±76.86 days in control group from the date of treatment schedule, with 100 and 66.66 per cent conception rate in suboestrus  $PGF_2\alpha$  treated and control surti buffaloes. Rao and Rao (1988) observed cent per cent animals responded with  $PGF_2\alpha$  analogue by IVSM route as compared to 88.89 per cent by I/M route. While, Deshpande (2007) reported cent per cent oestrus induction with an average interval (in I/M and I/U groups) 3.66±0.21 days post-treatment with lower conception rate in both groups as 83.33 and 66.66 per cent, respectively.

Moreover, oestrus induction interval in days in  $T_1$  (3.50±0.34) and  $T_3$  (3.83±0.31) groups were corroborated more or less by the findings of earlier workers (Rao and Rao, 1988 and Sahasrabudhe and Pandit, 1999).

On the other hand, 100.00 per cent animals responded with I/U route as compared to 85.71 per cent by IVSM route with 40.00 per cent conception rate in both the routes reported by Gautam *et al.* (1990). On the contrary, no significant changes in the conception rate between prostaglandin treated and control animals observed by (Espana *et al.*, 1992). Whereas, significantly higher conception rate obtained by Sinha *et al.* (2002) in  $PGF_2\alpha$  (Dinoprost) treated than control cows (70.00 vs. 33.33%) which supports present findings (100.00 vs. 50.00%) in surti buffaloes.

As compared to present findings, lower oestrus response 72.20 per cent, 75.00 per cent, 77.50 per cent, 80.00 per cent, 85.70 per cent, 87.50 per cent and 88.89 per cent and 90.00 per cent have been observed by (Rahaman *et al.*, 2012; Kharche and Srivastava, 2001; Pant and Singh, 1991; Sharma, 2002; Totewad *et al.*, 2009; Honparkhe *et al.*, 2008; Nzar, 2004; Cairoli *et al.*, 2006; Rao and Rao, 1988 and Sahasrabudhe and Pandit, 1999) with varying conception rate ranging from 40.00 to 80.00 per cent. The reason for the difference found by various workers in terms of oestrus induction interval, oestrus response and conception rate might be attributed to different PGF $_2\alpha$  analogue (dinoprost and cloprostenol), its dose, route and regimen used by various workers. In the study, 50.00 per cent suboestrus surti buffaloes in control group T $_4$  exhibited oestrus after 20.67±2.03 days of placebo treatment and conceived which is very higher as compared to the findings of Deshpande (2007) who reported that 16.66 per cent (1/6) suboestrus crossbred cows in control group exhibited oestrus after 8.00±0.00 days of placebo treatment and conceived which could be attributed to the place, species and breed difference. Thus, PGF $_2\alpha$  can successfully be employed to tackle the problem of sub-oestrus in the field conditions so as to improve reproductive efficiency and ultimately the production in buffaloes.

#### Serum calcium: Phosphorus ratio:

The serum Ca: P ratio of suboestrous surti buffaloes in different treatment and control groups at different time intervals was found to be ranging from 1.44:1 to 1.63:1. The ratio of serum Ca: P should be between 1.5:1 and 2.5:1 for efficient reproduction in dairy bovines reported by Carnahan (1974). Whereas, positive correlation between Ca: P ratio and fertility was achieved best at 2:1 in cattle stated by Marinov (1978). As compared to present findings, Shrivastav *et al.* (1981) reported higher Ca: P ratio in Murrah buffaloes as 1.74:1 and Quayam *et al.* (1985) estimated very higher Ca: P ratio in primiparous non-suckled buffaloes as 3.25:1 at the time of parturition and continued to be maintained till 60 days postpartum. The findings and fertility are in close apposition and highly correlated with Ca: P ratio and was found to be higher in T<sub>1</sub> and T<sub>3</sub> groups having cent per cent conception rate with treatment as

compared to  $T_2$  group and 50.00 per cent conception without any interference was found in  $T_4$  (control) group might be true and attributed to the normal Ca: P ratio in this group (Table 2).

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