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RESEARCH ARTICLE

Reproductive performance and progesterone profile in post-partum suboestrous surti buffaloes

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Abstract : Post-partum 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_3E 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 progesterone, 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 $PGF_2\alpha$ treated (T_1 and T_3) groups as compared to T_2 and control group which clear cut showed the luteolytic effect of $PGF_2\alpha$ on ovaries and earlier resumption of ovarian activities as compared to treatment (T_2) and control groups. Statistical analysis of the data generated in respect of the treatment on the progesterone concentration of the blood serum did not show any significant difference among the four groups of suboestrus surti buffaloes at 0 hr (prior to the treatment) and at 24 hr (post-treatment). Moreover, the mean serum progesterone values at 48 and 72 hr post-treatment between T_1 and T_3 ($PGF_2\alpha$ treated) groups as well as between treatment T_2 (Vit+P) and T_4 group (Control) did not differ significantly. However, the mean serum progesterone levels of T_1 and T_3 ($PGF_2\alpha$ treated) groups differed significantly from T_2 and T_4 groups at 48 and 72 hr post-treatment.

Key words : Suboestrous surti buffaloes, $PGF_2\alpha$, Progesterone, Postpartum period

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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).

Progesterone profile in clinically observed suboestrus buffaloes can suffice to detect ovarian status and resumption of cyclic activity postpartumand useful in the detection of oestrus and suboestrus condition.

RESEARCH METHODOLOGY

The present study was conducted on twenty four suboestrous surti buffaloes from 45 to 120 days post-partum. They were randomly divided into four groups (T_1, T_2, T_3 and T_4) comprising of six animals in each group. All these buffaloes had normal calving and subsequent normal genital health as assessed Gynaeco-clinically. Oestrus occurrence was detected daily in them with the help of teaser bull parading in morning and evening hours. The animals which were not exhibiting overt signs of oestrus during routine heat detection programme were segregated and subjected to rectal palpation. The animals with palpable structures either corpus luteum (CL) or follicle, on either of the ovaries were selected for another palpation after eleven days apart to ascertain their cyclic nature and considered as silent heat/subestrous buffaloes. The buffaloes in T_1 group were treated with 2 ml of Inj. Cloprostenol sodium (Inj. Cyclix) (500 µg, $PGF_2\alpha$ analogue, I/M route); the buffaloes in T_2 group were treated with 2 ml of Inj. Cloprostenol sodium (Inj. Cyclix) (500 µg, $PGF_2\alpha$ analogue, I/M route) + [inj. Vit. AD_3E preparation (5 ml, I/M route) + inj. Toldimphos sodium preparation (15 ml, I/M route)] and the buffaloes in group (T_4) were kept as sub-oestrous control group. All these buffaloes were then 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. Serum progesterone concentration was measured by standard Enzyme Linked Immuno Sorbent Assay (ELISA) technique using assay kits and procedure described by Labor Diagnostika Nord GmbH and Co. KG, Nordhorn.

RESULTS AND DISCUSSION

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

Reproductive performance :

In $PGF_2\alpha$ (T_1) 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 were 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±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 $PGF_2\alpha$+Vit+ P (T_3) 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 were 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±2.03 days (ranging from 17 to 24 days) post-treatment and all of responded buffaloes were conceived with an average of 1.33 services per conception (Table 1).

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±4.07 days, 91.50±3.48 days, 67.33±3.85 days and 96.00±8.50 days, respectively. The overall service period of all the groups and thereby, treatment to oestrus induction interval was 80.42±4.98 and 11.19±1.31 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 in between 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 as in between 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 $PGF_2\alpha$ 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.

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 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 (Bearden and Fuquay, 1997; Kahlon and Singh, 2004; Kumar *et al*., 2010; Agrawal and Pandit, 1991; Tapas, 1996 and Giri and Yadav, 2001) also have used vitamins and inorganic phosphorus as a therapy in the suboestrous cows and buffaloes with encouraging results.

Additional exogenous injection of inorganic phosphorus along with vitamins might have helped to resolve the problem of suboestrus in treatment group T_2 of surti buffaloes with 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 P $GF_2\alpha$ (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.

The findings about 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.

Table 1 : Effect of different treatments on reproductive performance of postpartum suboestrous surti buffaloes (Mean±SE)

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^a	1.33^a	03.50±0.34^a	100.00	6(100.00%)	6
T_2	91.50±3.48^b	1.50^a	16.75±2.56^b	66.67	4(66.67%)	4
T_3	67.33±3.85^a	1.33^a	03.83±0.31^a	100.00	6(100.00%)	6
T_4	96.00±8.50^b	1.33^a	20.67±2.03^b	50.00	3(50.00%)	3
Overall	80.42±4.98	1.37	11.19±1.31	79.17	19(79.17%)	19/24

Means bearing different superscripts within a column (group) differ significantly ($p \leq 0.05$)
Group-I = T_1 ($PGF_{2\alpha}$) Group-II = T_2 (Vit+ P) Group-III = T_3 ($PGF_{2\alpha}$+Vit+ P) Group-IV = T_4 (Control)

Serum progesterone profile :

The mean serum progesterone concentration (ng/ml) at different time intervals/days in sub-estrus treated and control groups of animals are presented in Table 2. The mean serum progesterone concentration in all the suboestrus surti buffaloes under study prior to the treatment was widely ranging from 1.45 to 5.52 ng/ml which was above 1 ng/ml during the sampling period.

In T_1 group, the mean serum progesterone levels of suboestrous surti buffaloes at 0 hr, 24 hr, 48 hr and 72 hr were 4.13±0.15, 3.43±0.15, 1.51±0.15 and 0.41±0.15 ng/ml, with widely ranging from 2.88 to 5.36 ng/ml, 2.27 to 4.74 ng/ml, 0.97 to 2.25 ng/ml and 0.17 to 0.72 ng/ml, respectively.

In T_2 group, the mean serum progesterone levels of suboestrous surti buffaloes at 0 hr, 24 hr, 48 hr and 72 hr were 3.59±0.26, 3.73±0.26, 3.83±0.26 and 3.95±0.26 ng/ml, respectively. The serum progesterone concentration of the suboestrus surti buffaloes in the referred group (T_2) at 0 hr, 24 hr, 48 hr and 72 hr was widely ranging from 1.98 to 5.52 ng/ml, 2.22 to 5.45 ng/ml, 2.34 to 5.22 ng/ml and 2.53 to 5.15 ng/ml, respectively. The mean serum progesterone values of the referred group (T_2) differed non-significantly at every time interval during the sampling period.

In T_3 group, the mean serum progesterone levels of suboestrous surti buffaloes at 0 hr, 24 hr, 48 hr and 72 hr were 3.82±0.14, 2.95±0.14, 1.26±0.14 and 0.35±0.14 ng/ml, respectively. The serum progesterone concentration of the suboestrus surti buffaloes in the referred group (T_3) at 0 hr, 24 hr, 48 hr and 72 hr was widely ranging from 2.65 to 5.11 ng/ml, 2.23 to 3.85 ng/ml, 0.58 to 2.11 ng/ml and 0.17 to 0.64 ng/ml, respectively. A sharp decline in the mean progesterone concentration at 24 hr, 48 hr and 72 hr post-treatment was observed in the T_1 and T_3 groups which differed significantly. The trend of decline in mean serum progesterone concentration at 24 hr, 48 hr and 72 hr post-treatment may be due to the effect of $PGF_2\alpha$ as luteolytic.

In T_4 group, the mean serum progesterone levels of suboestrous surti buffaloes at 0 hr, 24 hr, 48 hr and 72 hr were 3.29±0.23, 3.38±0.23, 3.48±0.23 and 3.62±0.23 ng/ml, respectively. The serum progesterone concentration of the suboestrus surti buffaloes in the referred group (T_4) at 0 hr, 24 hr, 48 hr and 72 hr was widely ranging from 1.45 to 5.20 ng/ml, 1.69 to 5.05 ng/ml, 1.86 to 4.88 ng/ml and 2.03 to 4.84 ng/ml, respectively. There was no significant difference observed in the mean serum progesterone concentration at any of the time intervals post-treatment.

In T_2 groups steady increase during sampling procedure after palpation as well as in group T_4 fluctuating nature in the mean concentration of progesterone refers to constant presence of active corpus luteum in those groups.

Statistical analysis of the data generated in respect of the treatment on the progesterone concentration of the blood serum did not show any significant difference among the four groups of suboestrus surti buffaloes at 0 hr (prior to the treatment) and at 24 hr (post-treatment). Moreover, the mean serum progesterone values at 48 and 72 hr post-treatment between T_1 and T_3 ($PGF_2\alpha$ treated) groups as well as between treatment T_2 (Vit+P) and T_4 group (Control) did not differ significantly. However, the mean serum progesterone levels of T_1 and T_3 ($PGF_2\alpha$ treated) groups differed significantly from T_2 and T_4 groups at 48 and 72 hr post-treatment.

These findings are in close agreement with those of Jain (1994), who reported progesterone concentration of most of the crossbred cows under suboestrus status was above 1 ng/ml prior to $PGF_2\alpha$ treatment with mean concentration value of 3.05±0.63 ng/ml (ranging from 0.31 to 5.86 ng/ml); though, Mondal and Prakash (2003) reported that progesterone levels were lower (1.39±0.13 ng/ml) in cows that exhibited silent oestrus compared to overt oestrus (1.94±0.22 ng/ml). The mean concentration of progesterone was found to be higher in suboestrus as

Table 2 : Serum progesterone concentration (ng/ml) pattern at different time intervals/ days in sub -estrus treated and control groups of animals (Mean±SE)

Time intervals/ days	Groups (n=6)			
	$PGF_2\alpha$ (T_1)	Vit+ P (T_2)	$PGF_2\alpha$+Vit+ P(T_3)	Control (T_4)
0 hr/0 Day	$4.13\pm0.15_a^w$	$3.59\pm0.26_a^w$	$3.82\pm0.14_a^w$	$3.29\pm0.23_a^w$
24 hr/1st Day	$3.43\pm0.15_a^w$	$3.73\pm0.26_a^w$	$2.95\pm0.14_a^x$	$3.38\pm0.23_a^w$
48 hr/2nd Day	$1.51\pm0.15_a^x$	$3.83\pm0.26_b^w$	$1.26\pm0.14_a^y$	$3.48\pm0.23_b^w$
72 hr/3rd Day	$0.41\pm0.15_a^y$	$3.95\pm0.26_b^w$	$0.35\pm0.14_a^z$	$3.62\pm0.23_b^w$

Means bearing different superscripts within a column (group) differ significantly ($p<0.05$). Means bearing different subscripts within a row (between the groups) differ significantly ($p<0.05$).

compared to anoestrus as 0.46±0.00 vs. 0.54±0.01 ng/ml and 1.30 ± 0.04 vs. 0.91 ± 0.04 ng/ml, respectively in buffalo heifers (Sharma *et al.* 1999) and in surti buffaloes (Khasatiya *et al*., 2006).

The declining trend of progesterone concentration of the animals in T_1 and T_3 groups following $PGF_2\alpha$ treatment are supported by the findings of Khasatiya *et al*. (2006) in surti buffaloes and Deshpande (2007) in crossbred cows, they had also found same trend in the respective species.

The findings about mean serum progesterone levels of suboestrous surti buffaloes in T_2 and T_4 groups reveal that the ovaries were cyclic with palpable structure when examined per-rectally and was further confirmed by serum progesterone estimation which was strongly corroborated by the findings of (Chohan *et al.*, 1992) who reported peripheral progesterone concentrations were minimal on the day of oestrus (0.1 ng/ml), rise to peak concentrations of 1.6 to 3.6 ng/ml on days 13 to 15 of the cycle or even on day 17 before declining to basal levels at the onset of next oestrus. Same phenomenon may be applied in our study during rectal palpation of ovarian structure and random sampling of the animals, they might be between 5 to17 days of luteal phase of oestrous cycle that revealed by further estimation of progesterone concentration to be high in T_1 (4.13±0.15 ng/ml), T_2 (3.59±0.26 ng/ml), T_3 (3.82±0.14 ng/ml) and T_4 (3.29±0.23 ng/ml) groups prior to treatment in those suboestrous animals. Further, the findings are in close agreement with (Ahmed *et al*., 1977 and Raizada *et al*., 1977), they reported serum progesterone value in normal cyclic murrah buffaloes and heifers 5.20 ng/ml between 13th to 15th day of cycle, while lowest value was 0.1 to 0.5 ng/ml on the day of oestrus.

From this study, our conclusive statement revealed that per rectally we could palpate the CL in large animals but couldn't say regarding its viability in term of its function and nature and for that ultrasonography and estimation of progesterone have been best tools for further accurate diagnostic confirmation supported by Honparkhe *et al*. (2008) and the need for correlation of repeated per-rectal palpation, ultrasonography and progesterone measurement for critical differential diagnosis of suboestrus from true anoestrous condition.

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