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RESEARCH ARTICLE

Fertility parameters and Ca: P ratio of postpartum surti buffaloes having inactive ovaries

D.K. SONI, C.T. KHASATIYA[1], A.S. REDE[1], M.D. PATEL[2] AND K.K. TYAGI[2]

Members of the Research Forum

Associate Author :
[1]Department of Veterinary Gynaecology and Obstetrics, Vanbandhu College of Veterinary Science and Animal Husbandry, Navsari Agricultural University, NAVSARI (GUJRAT) INDIA

[2] Livestock Research Station (NAU) NAVSARI (GUJRAT) INDIA

AUTHOR FOR CORRESPONDENCE :
D.K. SONI
Department of Veterinary Gynaecology and Obstetrics, Vanbandhu College of Veterinary Science and Animal Husbundry, Navsari Agricultural University, NAVSARI (GUJRAT) INDIA

Abstract : Total 18 post-partum acyclic buffaloes were randomly divided into three equal groups consisting 6 surti buffaloes in each group *viz.*, T_1, T_2 and control (T_3) group. The buffaloes in T_1 group were treated with 5ml of inj. Buserelin acetate (20 µg, GnRH analogue, I/M route); the buffaloes in T_2 group were treated with 5ml of inj. Buserelin acetate (20 µg, GnRH 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 T_3 group were kept as anoestrus control. Number of animals responded to the treatment were 6, 6 and 4 in T_1, T_2 and control (T_3) groups, respectively. The service period and treatment to oestrus induction interval and conception rate of the inactive ovarian condition of surti buffaloes in T_1 (71.17±4.42; 12.67±1.11days and 100%) and T_2 (70.83±3.80; 12.33±1.11 days and 100%) treatment groups differed significantly ($p<0.05$) from T_3 control (94.50±5.43; 30.75±3.95 days and 66.66 %) groups. It was observed that service period in the GnRH treated (T_1 and T_2) groups has been minimized up to 23 to 24 days *i.e.* one cycle earlier in treatment groups than that of control (T_3) group. However, slightly lower service period and oestrus induction interval in days was found in T_2 group as compared to T_1 group, which might be attributed to the influence of exogenous inorganic phosphorus and vitamins along with GnRH given to the animals in T_2 group. The serum Ca: P ratio of acyclic surti buffaloes under study in different treatment and control groups at different time intervals was found to be ranging from 1.61:1 to 1.72:1. The ratio of serum Ca: P should be between 1.5:1 and 2.5:1 for efficient reproduction in dairy bovines.

Key words : Acyclic surti buffaloes, Hormone therapy, Vitamin, Phosphorus preparation, Postpartum period, Ca: P ratio

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INTRODUCTION

Anoestrus due to ovarian inactivity is considered to be the most important, frustrating and challenging problem associated with buffalo reproduction in India. It is the main cause of lowered fertility in buffaloes and responsible for

tremendous economic losses to the farmers by decreasing milk yield and the number of calves produced in a cow's lifetime. True anoestrus is the condition in which both the ovaries are small, smooth, inactive with the absence of either Graffian follicle or corpus luteum and characterized by cessation of sexual cycle as well as psychic manifestation of oestrus. Postpartum anoestrus is affected by several factors such as nutrition plane, milk yield, body condition score (BCS) at calving, suckling, parity, calving season and other factors as documented by (Shah *et al*., 1986, Barile, 2005 and El-Wishy, 2007).

Various hormonal treatments like PMSG, GnRH, Estrogen and Progesterone either alone or in combination have been tried with variable success (Nayak *et al*., 2009, Rao and Rao, 1984 and Singh *et al.*, 2004). Keeping all this in view, the study was undertaken on acyclic buffaloes to see the efficacy of GnRH and GnRH along with vitamin and phosphorus combination in relation to oestrus response and fertility.

RESEARCH METHODOLOGY

A study was carried out on 18 postpartum acyclic Surti buffaloes of University farm, Navsari (Gujarat). All these buffaloes had normal calving and subsequent normal genital health as assessed gynaeco-clinically. During the course of this study, 18 buffaloes beyond 45 days postpartum were identified through twice per-rectal palpation at 11 days apart as acyclic or inactive ovarian condition. They were randomly divided into three equal groups consisting 6 Surti buffaloes in each group *viz.*, T_1, T_2 and control (T_3) group. The buffaloes in T_1 group were treated with 5ml of inj. Buserelin acetate (20 µg, GnRH analogue, I/M route); the buffaloes in T_2 group were treated with 5ml of inj. Buserelin acetate (20 µg, GnRH 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_3 were kept as anoestrus control group. Approximately 10 ml blood samples in serum clotting vaccutainers were collected from all those selected animals on 0 day (prior to treatment), 7th, 14th and 21st days 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). The data were analyzed using standard statistical procedures (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been given in Tables 1,2 and 3.

Fertility parameters :

The service period and oestrus induction interval in days was found significantly lower (p>0.05) in GnRH treated T_1 (71.17±4.42; 12.67±1.11 days) and T_2 (70.83±3.80; 12.33±1.11 days) groups as compared to control group

Table 1 : Effect of different treatments on reproductive performance of postpartum acyclic/anestrous 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	71.17±4.42^a	1.33^a	12.67±1.11^a	100.00	6(100.00%)	6
T_2	70.83±3.80^a	1.33^a	12.33±1.11^a	100.00	6(100.00%)	6
T_3 (Control)	94.50±5.43^b	1.50^a	30.75±3.95^b	66.66	4(66.66%)	4
Over all	76.88±3.66	1.38	17.06±2.29	88.89	16(88.89%)	16/18

Means bearing different superscripts within a column (group) differ significantly (P>0.05)
Group-I = T_1 (GnRH analogue)
Group-II = T_2 (GnRH analogue + Vit.+ P)
Group-III = T_3 (Acyclic Control)

(94.50±5.43; 30.75±3.95 days) clear cut showed the effect of GnRH treatment on ovary and earlier resumption of ovarian activities as compared to control group. It was observed from the above findings that service period in the GnRH treated (T_1 and T_2) groups has been minimized up to 23 to 24 days *i.e.* one cycle earlier in treatment groups than that of control group. Additional exogenous injection of inorganic phosphorus along with vitamins might have helped to resolve the problem of anoestrus (acyclic ovarian condition) in surti buffaloes and it is true in treatment group (T_2). However, the number of services per conception did not differ significantly among all the experienced anoestrous treatment and control groups.

Moreover, cent per cent conception rate in T_1 (GnRH alone) and T_2 (GnRH + Vit.+ P) groups as compared to 66.66 per cent conception rate in acyclic control T_3 group, respectively might be under the influence of various treatments in period (45 to 120 days) with overall 88.89 per cent (16/18) conception rate.

The service period, treatment to oestrus induction interval, percentage of animals responded to the treatment and conception rate in T_1 and T_2 groups are in agreement with the findings of Sirmour *et al.* (2006), who reported the oestrus induction interval (16.60 ± 2.7) days with 100 per cent conception rate in Tonophasphane and Receptal treated group and Dhoble and Gupta (1986) used 21 µg GnRH and reported 9 anoestrus cows responded earlier (4-10 days) than present finding, whereas, 3 cows responded (14-15 days) which is corroborated with our study while very long oestrus induction response (94 days) in control group noted by them as against 30.75±3.95 days in present findings. In addition to this, earlier oestrus induction intervals 7.75 days and 4 to 7 days with less response to treatment 57.10 per cent and 66.67 per cent with 75 per cent conception rate as compared to the study reported by (El-Shamaa *et al.*, 1996 and Markandeya and Patil, 2003). On the contrary, little bit longer oestrus induction intervals 16.06 ±0.65 days with less response (80%) and low conception rate 62.50 per cent reported by Singh (2003).

Table 2 : Weekly serum profile of calcium and phosphorus in postpartum acyclic (GnRH treated and control) surti buffaloes (Mean±SE), (n=6)

Time intervals/ days	Calcium (mg/dl)			Phosphorus (mg/dl)		
	T_1	T_2	T_3 (Control)	T_1	T_2	T_3 (Control)
0 day	$9.98\pm0.21_a^w$	$10.13\pm0.17_a^w$	$9.91\pm0.05_a^w$	$5.97\pm0.20_a^w$	$5.97\pm0.14_a^w$	$5.90\pm0.18_a^w$
7th day	$9.94\pm0.21_a^w$	$10.06\pm0.17_a^w$	$10.00\pm0.05_a^w$	$5.92\pm0.20_a^w$	$5.98\pm0.14_a^w$	$5.88\pm0.18_a^w$
14th day	$10.05\pm0.21_a^w$	$10.28\pm0.17_a^w$	$10.11\pm0.05_a^w$	$5.95\pm0.20_a^w$	$6.08\pm0.14_a^w$	$5.87\pm0.18_a^w$
21st day	$10.08\pm0.21_a^w$	$10.30\pm0.17_a^w$	$10.07\pm0.05_a^w$	$6.01\pm0.20_a^w$	$6.20\pm0.14_a^w$	$5.95\pm0.18_a^w$
Over all	$10.01\pm0.21_a$	$10.19\pm0.17_a$	$10.04\pm0.05_a$	$5.96\pm0.20_a$	$6.01\pm0.14_a$	$5.90\pm0.18_a$

Means bearing common superscripts within a column (group) and means bearing common subscripts within a row (between the groups) do not differ significantly (p >0.05).

Table 3 : Comparison of serum Ca: P ratio of acyclic buffaloes in different treatment and control groups at different time intervals (Mean±SE)

Time intervals/ days	Parameters	Groups (n=6)		
		T_1	T_2	Control
Day 0	Calcium (mg/dl)	9.98±0.21	10.13±0.17	9.97±0.05
	Phosphorus(mg/dl)	5.97±0.20	5.97±0.14	5.90±0.18
	Ratio	1.67:1	1.70:1	1.68:1
Day 7th	Calcium (mg/dl)	9.94±0.21	10.06±0.17	10.00±0.05
	Phosphorus mg/dl)	5.92±0.20	5.98±0.14	5.88±0.18
	Ratio	1.61:1	1.68:1	1.70:1
Day 14th	Calcium (mg/dl)	10.05±0.21	10.28±0.17	10.11±0.05
	Phosphorus (mg/dl)	5.95±0.20	6.08±0.14	5.87±0.18
	Ratio	1.68:1	1.69:1	1.72:1
Day 21st	Calcium (mg/dl)	10.08±0.21	10.30±0.17	10.07±0.05
	Phosphorus (mg/dl)	6.01±0.20	6.2±0.14	5.95±0.18
	Ratio	1.68:1	1.66:1	1.69:1

Macro-minerals (Calcium and Phosphorus) profile :

The overall serum calcium and phosphorus values in T_1, T_2 and T_3 (control) groups were 10.01±0.21 mg/dl, 10.19±0.17 mg/dl and 10.04±0.05 mg/dl; 5.96±0.20 mg/dl, 6.01±0.14 mg/dl and 5.90±0.18 mg/dl, respectively. The mean serum calcium and phosphorus of acyclic surti buffaloes did not differ significantly ($p > 0.05$) at 0 day, 7th day, 14th day and 21st day within and between all the treatment and control groups at any time interval including overall mean values in all the groups.

In case of mean serum calcium concentration, the observations made are in agreement with the findings of Khasatiya *et al*. (2005), who reported that overall pooled calcium level in GnRH treatment group did not vary significantly from control group (10.32±0.28 vs. 10.25±0.19 mg%) in surti buffaloes. Similarly, Shah (1999) did not observe significant variation in weekly mean plasma calcium levels in GnRH treated and their control groups of buffaloes postpartum, the overall mean plasma calcium concentration in GnRH treated buffaloes was lower than their control (9.49 ± 0.17 vs. 10.07 ± 0.17 mg). Moreover, Patel (2008) reported the mean values of plasma calcium in post-partum buffaloes in GnRH treatment group did not vary significantly from control group (9.77±0.05 vs. 9.17±0.04 mg %).

The trend and values of serum phosphorus closely corroborated with the observations of Shah (1999), who reported that the mean inorganic phosphorus levels ranged between 5.40 ± 0.35 to 6.19 ± 0.30 mg per cent for GnRH treated and 5.27 ± 0.88 to 6.71 ± 0.27 mg per cent for untreated group without any significant variation during different weeks postpartum and it was found to be lower in treated group than the control group with the overall mean of 5.64 ± 0.11 vs 6.29 ± 0.09 mg per cent. On the contrary, Khasatiya *et al*. (2005) reported that the overall pooled mean of blood plasma phosphorus in GnRH treatment group was significantly higher than the control group (5.07±0.21 vs. 4.37±0.23 mg %) in surti buffaloes.

Serum calcium: Phosphorus ratio :

The serum Ca: P ratio of acyclic surti buffaloes under study in different treatment and control groups at different time intervals was found to be ranging from 1.61:1 to 1.72:1. 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 these findings, Shrivastava *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 time of parturition and continued to be maintained till 60 days postpartum. These findings and fertility were in close apposition and highly correlated with Ca: P ratio and was found to be higher in T_1 and T_2 groups having cent per cent conception rate with treatment. Whereas, in T_3 control group conception rate was found to be 66.66 per cent that was more than 50 per cent usually found in the organized farm, without any interference might be true and attributed to the normal Ca: P ratio in that groups.

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