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Serum Macro-Minerals Profile and Conception Rate following Heatsynch and Heatsynch + PRID Protocols in Subestrus Surti Buffaloes

Swati Saxena, C.T. Khasatiya*, H.R. Savani, S.B. Patel and S.S. Chaudhary

Department of Veterinary Gynaecology & Obstetrics

College of Veterinary Science & Animal Husbandry, NAU, Navsari, Gujarat, India

Corresponding Author: drctkhasatiya@yahoo.in

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Abstract

A study was carried out to interpret the first and second service conception rate along with serum macro-minerals profile for Heatsynch (T1) and Heatsynch + PRID (T2) protocols with fixed timed AI in subestrus Surti buffaloes (n=6 each), keeping one group as untreated control (T3). In Heatsynch protocol, buffaloes were administered i.m. with Inj. Busereline acetate 10 µg on day 0, Inj. Cloprostenol sodium 500 µg on day 7 and Inj. oestradiol benzoate (EB) 0.5 mg on day 8 followed by FTAs twice on day 10, while in Heatsynch + PRID protocol the buffaloes were inserted with PRID (0.96 g of progesterone) intravaginally for 7 days together with above Heatsynch protocol. Blood samples were collected aseptically in vacutainers from all these animals on day 0 (prior to treatment), day 4 (during treatment), day 8 (after PGF₂α inj.), day of induced estrus/FTA and on day 28 (post-treatment, day 18 post-AI), and serum samples were stored at -20°C until analyzed. The buffaloes that became pregnant at 1st service (FTA) in the treatment group T1 & T2 were 50.00 (3/6) and 66.66 (4/6) % and at 2nd cycle 100.00 (3/3) and 50.00 (1/2) %, respectively. The corresponding conception rates for control group T3 were 33.33 (2/6) and 50.00 (2/4) %. The overall pooled CRs of 2 cycles achieved in groups T1, T2 and T3 were 100.00 (6/6), 83.33 (5/6) and 66.66 (4/6) %, respectively. The overall mean serum calcium, phosphorus and magnesium values did not differ significantly (p>0.05) among T1, T2 and T3 groups at any day or within group between different sampling intervals. Heatsynch alone was better and economic over other groups in managing subestrus buffaloes.

Key words: Subestrus buffalo, Heatsynch, PRID, Serum macro-minerals, Conception rate.

Introduction

Buffaloes are premier dairy animals of India, and are popularly known as “Black Diamond”. They play an important role in farmer’s daily life as well as India’s milk production, as they contributes 60 % of the total milk produced in the country (Mondal *et al.*, 2010). Subestrus or silent estrus is clinically characterized by failure of overt symptoms of estrus, though the animal is surprisingly normal. Under these conditions, follicular development and ovulation occurs normally in animals without the manifestation of overt signs of estrus. Hence, such animals can be managed by estrus synchronization protocols like Osynch, Heatsynch with fixed time AI (Kandeil *et al.*, 2012; Mohan

et al., 2009, 2014; Nakrani *et al.*, 2014). Nutritional factors needed for successful reproduction are the same as those needed for maintenance, growth and lactation. Lack of minerals especially calcium and phosphorus and trace elements upset the proper functioning of the genital organs. Magnesium deficiency reduces reproductive efficiency due to loss of appetite (Satish Kumar, 2003). This study was aimed to evaluate the efficacy of Heatsynch and Heatsynch + PRID protocols with fix timed AI in subestrus Surti buffaloes for improving conception rate together with serum macro-minerals profile.

Materials and Methods

The study was conducted on 18 subestrus/silent estrus Surti buffaloes from 45 to 120 days postpartum at Livestock Research Station, NAU, Navsari in South Gujarat. The climate of the region is sub-humid tropical with heavy rainfall. All these buffaloes had normal calving and subsequent normal genital health as assessed gynaeco-clinically. Estrus occurrence was detected daily with the help of teaser bull parading during morning and evening hours. The animals which were not exhibiting overt signs of estrus during routine heat detection program were segregated and subjected to per-rectal palpations. The animals with palpable structures either corpus luteum or follicle, on either of the ovaries were re-examined after 11 days to ascertain their cyclic nature and considered as subestrus (silent heat) buffaloes.

Treatment protocols

In the Heatsynch protocol (T1), six subestrus buffaloes were administered *i/m* with Inj. busereline acetate, a GnRH analogue, 10 µg (Inj. Pregulate, 2.5 ml) on day 0, Inj. cloprostenol sodium, a PGF₂α analogue, 500 µg (Inj. Pregova, 2 ml) on day 7 and Inj. oestradiol benzoate (EB) 0.5 mg (Inj. Pregheat, 1 ml) on day 8 followed by FTAI twice on day 10. Under Heatsynch + PRID protocol (T2), another six subestrus buffaloes were inserted intra-vaginally with PRID (0.96 g of progesterone; Triu-B) for 7 days together with above Heatsynch protocol. No hormonal treatment was given to third group of subestrus animals that served as control (T3). The signs of estrus and per-rectal palpation findings were recorded for each animal initially and at induced/spontaneous estrus and AI.

Blood sampling & Mineral estimation

Blood samples (5-6 ml) were collected aseptically from all animals on day 0 (prior to treatment), day 4 (during treatment), day 8 (after cloprostenol inj.), and day of estrus / FTAI and on day 28 (18th day post-AI) by jugular vein puncture. The serum clotting vacutainers containing blood samples were kept in slanting position at room temperature for 1-2 hours, centrifuged at 3000 rpm for 15 minutes and serum stored at -20°C until analysis.

Estimation of serum macro-minerals (Ca, P and Mg) was done by using assay kits and procedure described by Diatek Healthcare Pvt. Ltd., Kolkata, India. The absorbance and concentrations of macro-minerals were measured using Merck Microlab 300 Biochemistry analyzer at 546 nm and results were expressed as mg/dl.

Statistical analysis

Statistical analysis was carried out using SPSS software version 20.0. Descriptive analysis was carried out and Mean±SEM were calculated for all the designated groups. The test of significance among and within the group for minerals profile was made by analysis of variance (ANOVA) and DMRT at 1 and 5 % level of significance.

Results and Discussion

Conception rates

The buffaloes that became pregnant at 1st and 2nd service in T1 group were 50.00 (3/6) and 100

(3/3) % and in T2 group 66.66 (4/6) and 50.00 (1/2) %, respectively. The corresponding values for control group T3 were 33.33 (2/6) and 50.00 (2/4) %, respectively. The present first service conception rate in T1 group was nearer to 43.75 % reported by Mohan *et al.* (2014) in buffaloes. However, very low to low first service conception rates at induced estrus with Heatsynch protocol were observed by others as 16.66 % in anestrus Sahiwal cows (Caesar *et al.*, 2013); 18.00 and 26.00 % during winter and summer season in cyclic Murrah buffaloes (Mohan *et al.*, 2009); 20.00 to 37.50 % in cyclic buffaloes heifers (Kandiel *et al.*, 2012) and 25.00 % in postpartum anestrus Surti buffaloes (Buhecha *et al.*, 2016). On the other hand, higher 80.00 % first service conception rate was reported by Cevik *et al.* (2010) in lactating dairy cows following Heatsynch protocol. No pertinent literature on buffaloes was available to compare the present conception rates obtained with Heatsynch + PRID protocol (T2) in Surti buffaloes. However, the first service CR (66.66 %) obtained in this group in the present study was higher than 41 to 57 % reported in dairy cattle by some workers (Yusuf *et al.*, 2010; Khodabandehloo *et al.*, 2013).

Minerals profile

The overall mean serum calcium concentrations in T1, T2 and T3 groups were 9.45±0.16 mg/dl (range 7.81 to 12.77), 9.76±0.24 (range 7.02 to 13.23) and 9.54±0.11 (range 8.23 to 10.54) mg/dl, respectively. The overall mean serum calcium levels of subestrus buffaloes, irrespective of groups, at day 0, 4, 8, day of estrus and day 18 post-AI were 9.79±0.22, 9.90±0.31, 9.55±0.25, 9.22±0.16 and 9.45±0.16 mg/dl, respectively (Table 1). The mean serum calcium concentrations did not differ significantly ($p>0.05$) within or between groups for different days.

Table 1: Serum calcium concentration (mg/dl) at different days of treatment and control groups of subestrus buffaloes (Mean ± SEM)

Time intervals / Days	Groups / Treatments (n = 6)			Overall	F-value	P-value NS
	T1	T2	T3			
0 day (before treatment)	9.64±0.32	9.85±0.51	9.89±0.32	9.79±0.22	0.12	0.89
4 th day (during treatment)	9.79±0.60	10.38±0.69	9.55±0.28	9.90±0.31	0.60	0.56
8 th day (after PGF ₂ α inj.)	9.40±0.26	9.88±0.68	9.36±0.24	9.55±0.25	0.43	0.66
Day of estrus/AI	9.29±0.06	8.99±0.45	9.38±0.20	9.22±0.16	0.50	0.62
28 th day (post-treatment)	9.15±0.37	9.69±0.18	9.52±0.27	9.45±0.16	0.94	0.41
Overall	9.45±0.16	9.76±0.24	9.54±0.11	9.58±0.10	0.80	0.45
F-value	0.49	0.87	0.63	1.44	--	--
P-value NS	0.74	0.45	0.65	0.23	--	--

T1= Heatsynch protocol, T2 = Heatsynch + PRID protocol, T3= Untreated Control, NS= Non-significant.

The mean serum calcium concentration and its trend observed between days of treatment closely corroborated with Khasatiya *et al.* (2016) in postpartum subestrus Surti buffaloes and with Nakrani *et al.* (2014) in CIDR implanted Surti buffaloes. While it was higher than 8.32 ±0.36 and 8.63±0.25 mg/dl reported by Jayachandran *et al.* (2013) in regular cyclic and anestrus buffaloes, and lower than 10.55±0.43 and 11.77±0.37 mg/dl reported by Shahzad *et al.* (2016) in normal cyclic and non-cyclic Nilli-Ravi buffaloes, respectively. The differences reported in calcium levels by different

workers could be due to variation in breed, species, nutritional, reproductive and health status of animals, apart from seasonal and analytical differences. Patel (2008) reported the mean plasma calcium values in postpartum buffaloes under GnRH treatment and control groups as 9.77 ± 0.05 and 9.17 ± 0.04 mg/dl, respectively.

The overall mean serum inorganic phosphorus values in T1, T2 and T3 groups were 5.56 ± 0.09 (range 4.51 to 6.45), 5.65 ± 0.12 (range 4.54 to 6.88) and 5.58 ± 0.09 (range 4.45 to 6.54) mg/dl, respectively. While the overall mean serum phosphorus levels of subestrus Surti buffaloes at day 0, 4, 8, day of estrus and day 28 post-treatment were 5.47 ± 0.14 , 5.67 ± 0.13 , 5.50 ± 0.12 , 5.74 ± 0.12 and 5.60 ± 0.14 mg/dl, respectively (Table 2). The mean serum phosphorus concentration of subestrus buffaloes also neither varied significantly ($p > 0.05$) between groups nor within group for different days including the overall mean values.

Table 2: Serum inorganic phosphorus concentrations (mg/dl) at different days of treatment and control groups of subestrus buffaloes (Mean \pm SEM)

Time intervals / Days	Groups / Treatments (n = 6)			Overall	F-value	P-value NS
	T1	T2	T3			
0 day	5.29 ± 0.26	5.47 ± 0.26	5.67 ± 0.22	5.47 ± 0.14	0.59	0.56
4 th day	5.70 ± 0.22	5.61 ± 0.30	5.70 ± 0.21	5.67 ± 0.13	0.04	0.96
8 th day	5.44 ± 0.18	5.60 ± 0.31	5.44 ± 0.16	5.50 ± 0.12	0.17	0.84
Day of estrus/AI	5.64 ± 0.19	5.84 ± 0.31	5.74 ± 0.15	5.74 ± 0.12	0.18	0.84
28 th day	5.73 ± 0.20	5.72 ± 0.28	5.36 ± 0.25	5.60 ± 0.14	0.74	0.49
Overall	5.56 ± 0.09	5.65 ± 0.12	5.58 ± 0.09	5.60 ± 0.06	0.19	0.82
F-value	0.80	0.22	0.74	0.74	--	--
P-value NS	0.53	0.92	0.58	0.57	--	--

T1= Heatsynch protocol, T2 = Heatsynch + PRID protocol, T3= Untreated Control, NS= Non-significant.

The overall mean serum phosphorus concentration corroborated well with 5.58 ± 0.21 mg/dl reported by Khasatiya *et al.* (2005) in postpartum subestrus Surti buffaloes. As compared to present finding, lower mean serum inorganic phosphorus concentration of 4.64 ± 0.33 mg/dl in cyclic Nilli-Ravi buffaloes (Shahzad *et al.*, 2016), 5.12 ± 0.11 mg/dl in CIDR implanted buffaloes (Nakrani *et al.*, 2014) and 5.45 ± 0.04 mg/dl in postpartum Surti buffaloes (Patel, 2008) has been reported. Moreover, higher mean serum inorganic phosphorus concentration as 6.15 ± 0.17 mg/dl in cyclic buffaloes was reported by Jayachandran *et al.* (2013) and 6.92 ± 0.29 mg/dl in Nili-ravi buffalo-heifers by Paul *et al.* (2000).

The overall mean serum magnesium concentrations in T1, T2 and T3 groups were 2.36 ± 0.04 (range 2.14 to 2.80), 2.36 ± 0.03 (range 2.15 to 2.81) and 2.35 ± 0.02 (range 2.12 to 2.55) mg/dl, respectively. While the overall mean serum magnesium levels of subestrus Surti buffaloes at day 0, 4, 8, day of estrus and day 28 post-treatment were 2.36 ± 0.05 , 2.36 ± 0.05 , 2.35 ± 0.04 , 2.36 ± 0.06 and 2.36 ± 0.04 mg/dl, respectively (Table 3). The mean serum magnesium concentration of subestrus Surti buffaloes did not differ significantly ($p > 0.05$) within and between groups including overall means for different time intervals.

The present overall mean serum magnesium concentration of 2.36 ± 0.02 mg/dl closely concurred

with 2.31±0.15 mg/dl reported by Jindal *et al.* (1988) in normal cyclic Murrah buffaloes during proestrus. However, as compared to present findings higher mean serum magnesium concentrations 3.70±0.15 mg/dl in postpartum subestrus Surti buffaloes (Khasatiya *et al.*, 2005), 4.57±0.186 mg/dl in lactating Nili-Ravi buffaloes (Paul *et al.*, 2000) and 4.76±0.22 and 4.98±0.38 mg/dl in cyclic and non-cyclic buffaloes (Shahzad *et al.*, 2016) have been reported. In general, Heatsynch protocol (T1) alone was better and economical over Heatsynch+PRID (T2) and control for improving conception rates in subestrus buffaloes without altering the macro-minerals profile.

Table 3: Serum magnesium levels (mg/dl) at different days of treatment and control groups of subestrus buffaloes (Mean ± SEM)

Time intervals / Days	Groups / Treatments (n = 6)			Overall	F-value	P-value NS
	T1	T2	T3			
0 day	2.36±0.11	2.37±0.07	2.35±0.09	2.36±0.05	0.011	0.99
4 th day	2.36±0.11	2.37±0.08	2.36±0.05	2.36±0.05	0.004	0.99
8 th day	2.35±0.09	2.35±0.08	2.34±0.04	2.35±0.04	0.008	0.99
Day of estrus/AI	2.35±0.11	2.36±0.10	2.37±0.10	2.36±0.06	0.003	0.99
28 th day	2.35±0.11	2.37±0.05	2.35±0.06	2.36±0.04	0.015	0.98
Overall	2.36±0.04	2.36±0.03	2.35±0.02	2.36±0.02	0.019	0.98
F-value	0.004	0.009	0.019	0.018	--	--
P-value NS	1.00	1.00	0.99	0.99	--	--

T1= Heatsynch protocol, T2 = Heatsynch + PRID protocol, T3= Untreated Control, NS= Non-significant.

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Conflict of Interest: Authors declare no conflict of interest for this research work.

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