EFFECT OF HORMONAL PROTOCOLS ON BLOOD SERUM PROFILE IN POSTPARTUM ANOESTRUS BUFFALOES DURING NONBREEDING SEASON

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ABSTRACT

The study was conducted on 40 postpartum anoestrus buffaloes during non-breeding season. The selected animals were divided into 4 equal groups. Group I was treated with standard Ovsynch + CIRD protocol, Group II with CIDR alone, and Group III was administered with Ovsynch protocol alone, all with FTAI on day 10, while Group IV served as untreated control. Blood samples were collected on day 0 (initial), day 7 (day of PG inj.) and on day 10 (FTAI) of each protocol for determination of haemato-biochemical profile. The overall mean values of WBCs, RBCs, haemoglobin, plasma glucose, and serum total protein, total cholesterol, calcium and phosphorus were statistically similar on all days in all groups. The serum levels of Cu, Fe and Zn increased significantly ($p<0.01$) on day 7 and day 10 as compared to day 0 in treatment group, and were also higher in group I than others. The serum progesterone concentration differed significantly on all days, the level increased significantly on day 7 and dropped significantly ($p<0.01$) to basal level on day 10, *i.e.,* at induced estrus/FTAI. It was concluded that except serum progesterone and trace minerals, none of the other haemato-biochemical parameters were influenced by the synchronization protocols used in anoestrus buffaloes during low breeding season.

Key words: Hormonal protocol, Haemato-biochemical profile, Anoestrus buffaloes, Non-breeding season.

INTRODUCTION

The buffalo is considered as sluggish breeder as the reproductive efficiency of buffalo is adversely affected by certain constraints such as late maturity, poor expression of the estrus signs particularly during summer, irregular estrous cycle, silent heat, seasonality in breeding, poor conception rate/early embryonic mortality and prolonged inter-calving interval. Buffalo in tropical and subtropical parts of the world is considered as short day breeder and its reproductive efficiency is greatly influenced by biometerological factors. About 35% of buffaloes experienced anoestrus even during the breeding season from October to December (Anwar *et al.*, 2003) hampering their productivity by increasing the service period. A seasonal breeding of water buffalo has also been reported in many studies (Sheth *et al.*, 1978; Janakiraman *et al.*, 1980; Kaker *et al.*, 1981). In many parts of Asia, malnutrition and climatic stress are the main causes of poor reproductive performance and anoestrus in buffaloes. Efforts, however, have been made to breed the buffaloes throughout the year by using different hormone treatments (Rajamahendran and Thamotharam, 1983). Hence, the present research study was designed to study the effect of hormonal protocols on blood profile in postpartum anoestrus buffaloes during non-breeding season.

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MATERIALS AND METHODS

Total 40 postpartum anoestrus buffaloes were selected from Cattle Breeding Farm, Nagpur Veterinary College, Nagpur and Private Dairy Farms in and around Nagpur region during non-breeding season. All selected buffaloes were critically examined for reproductive status. The animal owners were advised to deworm and give mineral mixture @ 50 gm/day/head along with concentrate for study duration to their animals. The selected animals were divided into 4 equal groups, each of 10 animals. Group I was treated with standard Ovsynch + CIRD protocol. The CIDR was removed on day 7 and GnRH was administered @ 10 µg i/m on day 10, that is day of fixed time AI (FTAI). Group II was inserted with CIDR (Progesterone impregnated intra-vaginal device) alone intra-vaginally for 7 days, and Group III was administered with Ovsynch protocol alone, both with FTAI on day 10. Group IV served as untreated control.

Blood samples were collected from jugular vein of all buffaloes on day 0, 7 and 10 in ETDA vials for haematology, in fluoride vacutainers for plasma glucose estimation, and in serum activator vials for serum biochemistry. The blood samples were centrifuged at 3000 rpm for 15 minutes and the serum/plasma was stored in properly labeled sterilized 2 ml vials at -20°C until analyses. The haematological parameters (WBCs, RBCs, Hb, MCV, MCH and MCHC) were evaluated immediately after collection of the blood samples on

Hemo-analyser (Model ABX Micros ESV60, HORIBA Medical, Japan). The plasma glucose and serum total protein, total cholesterol, calcium and phosphorus concentrations were determined with the help of semi-automated biochemical analyser (Seac STAR-21, Rapid Diagnostics Pvt. Ltd. Model no. S/N 322437) using standard procedures and commercial kits procured from Coral Clinical System, Goa. Serum Cu, Fe, Zn, Co and Mn values were determined in wet digested samples on Atomic Absorption Spectrophotometer (Lab India). The data were analyzed statistically as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

In the present study, no significant changes were observed in the mean values of WBCs and RBCs in treatment and control groups of buffaloes including overall means over periods or days. (Table 1 & 3). Aggarwal *et al.* (2016) noted normal values of WBCs and RBCs for buffaloes in the range of 10253 ± 162 to 10249 ± 31 cell/ ìl, and 7.85 ± 0.16 to 7.23 ± 0.10 million/ìl, respectively.

On day 0 and 7, overall mean Hb values did not differ significantly, but on day 10, it was found significantly ($p<0.01$) increased in Group I, but not in other groups (Table 3). Ali and Shukla (2012) recorded haemoglobin levels as 9.81 ± 0.21 and 12.63 ± 0.49 mg/dl, and 9.87 ± 0.21 and 12.73 ± 0.49 mg/dl on day 0 and day 3 in anoestrus and normal cycling buffaloes, respectively, whereas Haque *et al.* (2013) reported it as 11.94, 13.41, 13.70 and 14.23 g/dl in buffaloes kept at ambient temperatures of 22°, 40°, 42° and 45°C, respectively.

The MCV levels before and after treatment, were significantly higher ($p<0.01$) in Group I and II than III and IV, whereas the post-treatment MCH values observed were significantly higher ($p<0.01$) in treatment group I than Group II, III and lowest in control. There was no significant difference in mean MCHC values between or within group (Table 1 & 3). Aggarwal *et al.* (2016) noted MCV and MCH in buffaloes ranges between 46.83±1.44 to 48.63±1.01 fl and 17.22±0.57 to 16.71±0.41 pg. The present non-significant changes found in values of MCV, MCH and MCHC between days and between groups concurred well with Kumar *et al.* (2015[a]), who also recorded a non-significant difference in values of the said traits on the day of treatment and on the day of estrus with Ovsynch and Ovsynch + CIDR treatment.

The mean plasma glucose level was found to be significantly increased after than before treatment in all three protocols, bt not in control group (Table 1 & 3). Blood glucose level is expected to be on lower side during summer as body system tries to compensate thermoregulatory function on priority and much energy is constantly utilized for the same. Ali and Shukla (2012) recorded 50.94 ± 1.86 and 71.85 ± 2.44 mg/dl, 51.39 ± 2.24 and 72.38 ± 2.65 mg/dl glucose on day 0 and day 3 in anoestrus and normal cycling buffaloes, respectively. Chabukdhara *et al.* (2015) noted glucose level of 49.79±1.64 mg/dl.

Day 0 to day 10, protein and cholesterol level did not increased significantly overall or in any of the groups. It was found under normal range (Table 3). Shrikhande *et al.* (2008) reported higher level of serum total protein during summer season in lactating cows. Ali and Shukla (2012) recorded total cholesterol level as 74.83 ± 2.06 and 130.86 ± 3.80 mg/dl, 75.32 ± 1.92 and 132.49 ± 3.39 mg/dl on day 0 and day 3 in anoestrus and normal cycling buffaloes, respectively. Chabukdhara *et al.* (2015) noted cholesterol value of 93.85±5.0 mg/dl. Whereas, Kumar *et al.* (2015[a]) observed the mean cholesterol level non-significantly higher on the day of estrus as compared to the day of treatment in Ovsynch and Ovsynch + CIDR protocol.

Serum calcium and phosphorus levels also did not vary significantly overall or between days and/or between groups. Similar findings were recorded by Shrikhande *et al.* (2008) in serum calcium during summer season in lactating cow. Ali and Shukla (2012) recorded 3.92 ± 0.14 and 6.02 ± 0.18 mg/dl, 3.94 ± 0.14 and 6.22 ± 0.15 mg/dl on day 0 and day 3 in anoestrus and normal cycling buffaloes, respectively.

The Cu, Fe and Zn levels were significantly ($p<0.01$) higher in group I than Group II, III and IV before and/or after treatment, while Co showed reverse trend (Table 2). There were no significant differences in overall mean Co and Mn levels during the study period in any of the groups (Table 2 & 3). Kumar *et al.* (2015[b]) recorded Zn level as 1.0 ± 0.1 (PPM) in cyclic buffalo during summer season.

Serum progesterone level was found to be lower on day 0 in all four groups. On day 7, it was found to be increased significantly ($p<0.01$) in Group I, II and III. However, in group IV it did not increase significantly on any day. Whereas on day 10, progesterone level was found to be decreased significantly ($p<0.01$) at basal level among Group I, II and III than Group IV. Serum progesterone level differed significantly ($p<0.01$) on day 0 and 10 in all three treatment groups. Endocrinological studies of buffalo during summer season reveal suboptimal functioning of the hypothalamus-pituitary gonadal axis, low FSH and LH release, variable plasma progesterone and elevated plasma prolactin. This low breeding season coincides with the period of highest temperatures, relative humidity and day lengths (Sheth *et al.,* 1978).

This study supports the observations of Bahga and Gangwar (2016) who investigated blood serum progesterone as 0.65 ± 0.77 ng/ml during summer season and observed that overall reproductive performance was impaired due to deficiency of progesterone. Mungad *et al.* (2017) analyzed blood samples of true anoestrus buffaloes during summer and reported progesterone levels on day 0, 7 and 10 during Ovsynch protocol as 0.43±0.06, 2.47±0.45, 0.51±0.09 ng/ml, whereas the same were 0.44±0.09, 2.70±0.33, 0.66±0.21 ng/ml in Triu-B protocol. It was a common observation that progesterone levels in buffaloes were low during non-breeding season, which were responsible for reduced fertility noted in terms of poor fertilization rates, conception rates and cyclicity (Aggrawal *et al.,* 2016). Considering the progesterone level during summer season, standard protocols have been supported with pre-exposure to progesterone and even the PRID + Ovsynch protocol has showed higher success than Ovsynch protocol only due to progesterone availability.

It can be concluded that the during non-breeding season the hormonal protocols used has no significant influence on blood picture during treatment period in postpartum anoestrus buffaloes. Similarly serum concentration of total protein, cholesterol and macro-minerals calcium and phosphorus were also not altered significantly by the protocols. The micro-minerals Cu, Fe & Zn were significantly higher, and Co lower in group I as compared to other groups. The serum progesterone level was significantly increased on day 7 in each protocol and dropped to basal level on day 10 (induced estrus/FTAI).

REFERENCES

Aggarwal Anjali, Sohan Vir Singh, Khan Farheen Badrealam, Renuka and Anil Kumar (2016). *Haematological and hormonal profile of various breeds of cattle and buffalo under varied seasons and environmental conditions*. ICAR National Dairy Research Institute, Karnal. Publication No. **146**: 47.

Ali, R. and S.P. Shukla (2012). Haemato-biochemical changes in postpartum anoestrus buffaloes during low breeding season. *Researcher*, **4** (9): 55-58.

Anwar, M., N. Ullah, A. Mehmood and S.M.H. Andrabi (2003). Postpartum anoestrus of Nili-Ravi buffaloes maintained under rural and peri-urban management around Islamabad. *Pakistan Vet. J.,* **23**: 114-117.

Bahga, C.S. and P.C. Gangwar (2016) Seasonal variations in plasma hormones and reproductive efficiency in early postpartum buffalo. *Theriogenology*, **86**: 1209-1223.

Chabukdhara Prasanta, Shashi Nayyar, Rajesh Jindal and Chandrasekhar Mukhopahyay (2015). Heat stress induced alteration in blood biochemical profile of lactating buffaloes during summer season. *Indian Vet. J.,* **92**: 16-20.

Haque, N., A. Ludri, S.A. Hossain and M. Ashutosh (2013). Impact on hematological parameters in young and adult Murrah buffaloes exposed to acute heat stress. *Buffalo Bulletin*, **32**: 321-326.

Janakiraman, K., M.C. Desai, D.R. Amin, A.R. Sheth, S.B. Mudbidri and K.B. Wadadekar (1980). Serum gonadotrophin levels in buffaloes in relation to phases of estrous cycle and breeding periods. *Indian J. Anim. Sci.,* **50**: 601-606.

Kaker, M.L., M.M. Galhotra and M.N. Razdan (1981). Serum prolactin levels of cycling Murrah buffaloes during summer and winter. *Indian J. Dairy Sci*., **34**: 391-395.

Kumar Ashwani., Utsav Sharma, Rajiv Singh, Sudhir Kumar and Sharad Kumar (2015[a]). Changes in hemato-biochemical profile in postpartum anoestrus Murrah buffaloes subjected to different hormonal protocols. *Indian Vet. J.,* **92**: 50-52.

Kumar Parveen, Anand Kumar Pandey, S.K. Phulia, Sandeep Kumar, R.K. Sharma, Nidhi Bishnoi and Kailash Kumar (2015[b]). Effect of Ovsynch estrus synchronization protocol on certain mineral profile of cyclic Murrah buffaloes in summer and winter season. *J. Anim. Res.,* 5: 909-913.

Mungad, K.S., J.A. Patel, A.J. Dhami, K.K. Hadiya, D.N. Borkhatariya and N.P. Sarvaiya (2017). Use of estrus induction strategy in true anestrus buffalo for reducing non-productive period. *Indian J. Anim. Reprod.,* **38**: 15-19.

Rajamahendran, R. and M. Thamotharam (1983). Effect of progesterone releasing intravaginal device (PRID) on fertility in the postpartum buffalo cow. *Anim. Reprod. Sci.*, **6**: 111-118.

Sheth, A.R., K.B. Wadadekar, S.B. Mudbidri, K. Janairaman and M. Parameshwaran (1978). Seasonal alteration in the serum prolactin and LH levels in the water buffaloes. *Curr. Sci.*, **47**: 75-77.

Shrikhande, G.B., A.M. Rode, M.S. Pradhan and A.K. Satpute (2008). Seasonal effect on the composition of blood in cattle. *Vet. World,* **1**: 341-342.

Snedecor, G.W. and Cochran, W.G. (1990*). Statistical Methods*. 6th ed. Oxford and JBH Publishing, New York, USA.

Table 1: Mean (±SE) haematological parameters of anoestrus buffaloes before and after use of different estrus synchronization protocols

Grp	WBCs (10^3/µl)		RBCs (10^6/µl)		Hb (g/dl)		MCV (ft)		MCH (pg)		MCHC (%)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
G1	8.70±0.37	9.89±0.45	7.13±0.41	8.68±0.41	10.95±0.40	12.26±0.26*	55.00±1.28^{a}	56.46±1.10^{a}	17.85±0.50	19.48±0.48^{a}	32.56±0.53	34.09±0.55
G2	8.34±0.23	9.43±0.32	6.96±0.45	7.59±0.32	10.93±0.62	12.21±0.69	53.42±1.80^{a}	54.31±1.86^{a}	17.43±0.46	18.61±0.66a^{b}	33.27±1.22	34.40±1.14
G3	8.46±0.18	9.47±0.33	6.84±0.28	7.85±0.29	10.33±0.30	11.39±0.41	49.49±0.52^{b}	50.71±0.59^{b}	16.94±0.37	18.06±0.46^{b}	33.59±0.72	34.81±0.86
G3	8.93±0.25	9.81±0.37	7.78±0.26	8.57±0.35	10.04±0.35	10.83±0.33	49.61±0.73^{b}	49.23±0.81^{b}	16.44±0.33	16.52±0.26^{c}	33.59±0.72	34.45±0.73
CD	NS	NS	NS	NS	NS	NS	3.41	4.57	NS	1.41	NS	NS

* Significant at P<0.05 before and after treatment for a trait.
Means bearing uncommon superscripts within column differ significantly between groups.

Table 2: Mean (±SE) blood glucose and serum trace minerals profile of anoestrus buffaloes before and after use of different estrus synchronization protocols

	Cu (ppm)		Fe (ppm)		Zn (ppm)		Co (ppm)		Mn (ppm)		Glucose (mg/dl)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
G1	0.82±0.02^{a}	1.00±0.06^{a}	1.76±0.12	2.04±0.06^{a}	1.73±0.15	2.45±0.24^{a}	0.36±0.01	0.45±0.01^{b}	0.34±0.03	0.42±0.05	53.51±0.98	58.43±0.94^{a*}
G2	0.72±0.03^{bc}	0.84±0.04^{c}	1.55±0.12	1.77±0.15^{ab}	1.56±0.12	1.78±0.10^{b}	0.41±0.02	0.52±0.06^{ab}	0.39±0.02	0.48±0.02	53.96±1.66	59.11±1.28^{a*}
G3	0.70±0.03^{bc}	0.87±0.04^{ab}	1.53±0.10	1.74±0.15^{ab}	1.75±0.17	1.91±0.16^{b}	0.58±0.04	0.57±0.03^{a}	0.41±0.03	0.49±0.02	53.13±0.79	58.11±1.07^{a*}
G3	0.78±0.03^{ab}	0.86±0.03^{ab}	1.52±0.09	1.60±0.08^{b}	1.84±0.03	1.91±0.04^{b}	0.58±0.03	0.57±0.03^{a}	0.43±0.03	0.46±0.02	53.42±1.48	53.80±1.12^{b}
CD	0.09	0.15	NS	0.40	NS	0.52	NS	0.12	NS	NS	NS	3.84

* Significant at P<0.05 before and after treatment for a trait.
Means bearing uncommon superscripts within column differ significantly between groups.

Table 3: Effect of hormonal protocols on hamato-biochemical profile in postpartum anoestrus buffaloes during non-breeding season (Overall)

Parameters	Days of treatment/protocols			CD (5%/1%)
	Day 0	Day 7	Day 10	
WBCs (10^3/µl)	8.26±0.31	8.11± 0.17	8.15±0.29	NS
RBCs (10^6/µl)	7.19±0.22	7.39±0.12	7.72±0.11	NS
Hb (g/dl)	9.76±0.07	10.06±0.14	10.49±0.39	NS
MCV (ft)	42.58±1.43	42.90±1.85	43.15±2.89	NS
MCH (pg)	15.24±0.29	15.44±0.31	15.77±0.60	NS
MCHC (%)	30.29±0.69	31.21±1.12	32.47±1.21	NS
Plasma glucose (mg/dl)	49.05^{b}±0.21	52.33^{a}±0.71	53.64^{a}±1.61	1.32/1.67
Serum protein (g/dl)	6.28±0.06	6.57±0.10	6.69±0.12	NS
Serum cholesterol (mg/dl)	99.06±2.09	101.67±1.71	103.03±2.59	NS
Serum Ca (mg/dl)	8.42±0.04	8.75±0.07	8.95±0.11	NS
Serum P (mg/dl)	5.46±0.13	5.67±0.19	5.80±0.21	NS
Serum Cu (ppm)	0.74^{b}±0.02	0.78^{b}±0.01	0.80^{a}±0.01	0.05
Serum Fe (ppm)	1.41^{b}±0.05	1.50^{ab}±0.01	1.54^{a}±0.02	0.08
Serum Zn (ppm)	1.41^{b}±0.09	1.61^{a}±0.10	1.63^{a}±0.09	0.15
Serum Co (ppm)	0.50±0.02	0.53±0.03	0.54±0.02	NS
Serum Mn (ppm)	0.38±0.01	0.43±0.01	0.44±0.01	NS
Serum progesterone (ng/ml)	0.76 ^{b}±0.07	3.46 ^{a}±0.08	0.27 ^{c}±0.01	0.35/0.82

Means bearing different superscripts (a,b,c) within the row differ significantly (p<0.01) between days.