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HEMATO-BIOCHEMICAL AND BEHAVIORAL CHANGES IN REPEAT BREEDING BUFFALOES TREATED WITH OVSYNCH PLUS CIDR ALONG WITH ANTIOXIDANT

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ABSTRACT

The study was conducted on 24 non infectious repeat breeding buffaloes to study hematology and behavioral changes when treated with Ovsynch plus CIDR along with antioxidant. Selected buffaloes were equally divided into four treatment groups. Group 1 Ovsynch protocol, Group 2 treated with Ovsynch along with antioxidant, Group 3 Ovsynch plus CIDR and Group 4 treated with Ovsynch plus CIDR and antioxidant. Mean Haemoglobin (g/dl), Packed Cell Volume (%), Total Protein (g/dl), Albumin (g/dl) and Globulin (g/dl) values in Group 1, 2, 3 and 4 have no significant difference on different days of treatment. Signs of estrus were more pronounced in buffaloes treated with hormonal protocols along with antioxidant. Estrus was observed as intense, intermediate and weak in Group 1 as (16.66, 66.66 and 16.66%), in Group 2 as (33.33, 50.00 and 16.66%), in Group 3 as (16.66, 83.33 and 00.00%) and in Group 4 as (50.00, 33.33 and 16.66%), respectively.

Keywords: *Bubalus bubalis*, buffaloes, CIDR, Ovsynch, haemato-biochemical, behavioural symptoms

INTRODUCTION

Buffaloes play a prominent role in rural livestock production, predominantly in Asia, and factors affecting productivity are of prime concern and matter of importance to agricultural economics in this region of the world. Inherent late maturity, poor estrus expression, distinct seasonal reproductive patterns, and failure of regular

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breeding and calving at an appropriate time are some of the major factors affecting productivity buffaloes (Singh *et al*., 2000).

Haemato-biochemical profiling helps in diagnosis of reproductive disorders in different livestock species and is important for maintaining functional integrity of the reproductive system (Niazi *et al*., 2003). Alterations in various biochemical constituents may result in reproductive failures (Prabha *et al*., 2000). Haemato-biochemical constituents vary during different reproductive states signifying their important role in reproductive performance of animals (Dutta *et al*., 1988). The present study is based on a white side test, behavioral symptom's, intensity of estrus and haemato-biochemical profile in non-infectious repeat breeding buffaloes.

MATERIALS AND METHODS

White Side Test (Popov, 1969) was performed for the screening the buffaloes and those positive to WST were excluded from the study. Buffaloes negative to WST were examined (Gynaeco-clinical examination) and 24 buffaloes were subjected to four treatment groups. Ovsynch protocol, Ovsynch protocol + antioxidant, Ovsynch protocol + CIDR, Ovsynch protocol + CIDR+ antioxidant. Intensity of estrus and behavioral symptoms were observed. Intensity of estrus was studied on the basis of behavioural changes and gynaeco-clinical observations, and it was scored as intense (14 and above), intermediate (8 to 13) and weak (7 and below) suggested by Rao and Rao (1981) with slight modification. Estrus was classified depending upon the score obtained by individual animal in estrus. Treatment started on day 7 of the cycle. Blood samples were collected aseptically from jugular vein on day 0, 9 and 27 of treatment. Haemato-biochemical estimation (Hb, PCV, Albumin, Globulin and Total protein) was done using standard procedures. Haemoglobin was estimated by using Sahli's haemometer method, (Sastry, 1983) and expressed in g/dl. Packed cell volume was estimated by using Microhaematocrit method (Jain, 1986) and expressed in percentage. Serum Total protein and Serum albumin were estimated to use standard kits using Semi Auto Biochemistry Analyzer (EVOLUTION 3000). Serum globulin concentration was estimated by subtracting albumin concentration from total protein concentration (Doumas and Watson, 1971). The data generated on onset of individual groups were subjected to One Way ANOVA using Statistical Package for Social Sciences software (SPSS 21.0, IBM).

RESULTS AND DISCUSSIONS

White side test

Seventy-two buffaloes having repeat breeding history were screened and selection of the non-infectious repeat breeding buffaloes was made using White side test (WST) 48 (66.66%) were positive and 24 (33.33%) buffaloes were negative.

Behavioural symptoms of estrus

The data generated for all four treatment groups is depicted in (Table 1).

Excitement

Excitement was categorized as intense, intermediate and absent on the basis of the scores in Group 1 and 4, 2 (33.33%), 1 (16.66%) and 3 (50.00%). In Group 2, 1(16.66%), 3(50.00%) and 2 (33.33%). In Group 3, 0 (00.00), 2 (33.33%) and

4 (66.66%) respectively. All the treated buffaloes showed less or higher degree or no excitement during the observation period at estrus suggesting that excitement alone can't be the basis for estrus detection in buffaloes. Variable degrees of excitement was also observed by Singh *et al.* (1983); Mohan *et al*. (2010); Alyas *et al*. (2015); Rathore *et al*. (2017); Wani *et al*. (2018); Mujawar *et al*. (2019) who all reported various percentages of intense, intermediate or moderate or weak or absent type of excitement in buffaloes treated for estrus induction using different treatment protocols.

Bellowing

The following was categorized as frequent, intermediate and absent. In Group 1, 2 (33.33%), 3 (50.00%) and 1 (16.66%); Group 2, 4 (66.66%), 1 (16.66%), and 1 (16.66%); Group 3, 2 (33.33%), 4 (66.66%) and 0 (00.00); Group 4, 3 (50.00%), 3 (50.00%) and 0 (00.00) buffaloes were bellowing as frequent, intermediate and absent, respectively. (Table 3) In Group 3 and 4, all the buffaloes showed remarkable bellowing (frequent to intermediate). Vale (1983); Danell *et al.* (1984); Chohan *et al.* (1992); Amonge *et al.* (1998); Mohan *et al.* (2010) reported frequent bellowing in 59.30, 24.1, 52.27, 56.60 and 20%, respectively. Srivastava and Kharche (1985) reported very frequent, frequent and normal bellowing in 20.58, 32.35 and 47.05% buffaloes. Similarly, Wani *et al*. (2018) reported frequent bellowing as characteristic sign of estrus in buffaloes.

Micturition

Frequent micturition was observed in 3 (50.00%), 4 (66.66%), 3 (50.00%) and 5 (83.33%), in Group 1, 2, 3 and 4 buffaloes, respectively (Table 1). Exhibition of micturition in treated buffaloes is in close proximity with the findings of earlier workers, Vale (1983); Danell *et al.* (1984); Singh *et al.* (1984); Jindal *et al.* (1988); Chohan *et al.* (1992) who reported frequent micturition in 54, 27.6, 34.78, 57.1 and 47.72% buffaloes. The earlier observations and present finding for frequent micturition in buffaloes are suggestive for important signs for estrus detection in buffaloes.

Homosexual behavior

Frequent and intermittent homosexual behavior in Group 1 and 4, was seen in 4 (66.66%) and 2 (33.33%) buffaloes, respectively. In Group 2, frequent in 5 (83.33%) buffaloes and intermittent in 1(16.66%) buffalo. Similarly, in Group 3 frequent and intermittent in 3 (50.00%) and 3 (50.00%) buffaloes, respectively. Homosexual behavior though not very common feature seen in buffaloes but in this study, it may possibly be expressed due to hormonal treatment. Vale (1983); Danell *et al.* (1984); Ullah and Usmani (1985); Alyas *et al*. (2015); Wani *et al.* (2018) reported homosexual behavior in 19.3, 20.7, 24, 16.67, and 25% buffaloes, respectively.

Swelling of vulva

Vulval swelling (mild to severe) in hormonally treated buffaloes is due to high estradiol concentration in the circulation which subsides after estrus indicative of positive response to the treatment. Severe swelling of vulva lips was observed in 4 (66.66), 3 (50.00), 2 (33.33) and 3 (50.00) in Group 1, Group 2, Group 3 and Group 4 buffaloes (Table 1). Srivastava and Kharche (1985) reported swelling of vulval lips were intense, intermediate and slight in 47.05, 29.41 and 23.52% buffaloes, respectively.

Estrus discharge

The quantity of estrus discharge was

categorized copious, moderate and absent (Table 1). Out of 24 buffaloes 14 buffaloes had copious estrus discharge, 6 had moderate discharge and 4 buffaloes did not have estrus discharge. During peri-estrual period circulator estrogen level is high, which had its influence on the secretory activity of the cervical glands also there is increase electrolyte concentration in the cervical mucus which alters the glycoprotein: water ratio, leading to flow of copious discharge (Layek *et al*., 2013). Singh *et al.* (1984) reported that in 46.73% and 53.26% buffaloes, discharge was copious and small in quantity, respectively. Joshi *et al.* (2017) reported copious, moderate and scanty cervical mucus discharge in 70, 23.33 and 6.67% buffaloes, respectively. Wani *et al*. (2018); Mujawar *et al*. (2019) in synchronized buffaloes treated with used CIDR plus vit. E and Se protocols. Buffaloes in the various treatment groups showed variable patterns of intensity of estrus (Table 1).

Haemato-biochemical profile

Haemato-biochemical parameters were assessed by collecting blood on different days of treatment i.e. 0, 9 and 27th day (Table 2).

Haemoglobin (Hb)

ANOVA for Hb on day 0, 9 and 27 of the treatment do not differ significantly between the treatment groups. However, Hb in Group 2 was non-significantly higher 9.08±0.40 and 9.51±0.50 g/dl on day 9 and day 27, respectively as compared to Group 1. While Hb concentration in Group 4 did not increase as compared to Group 3 on day 9, however Hb concentration 9.59±0.26 g/dl on day 27 was non-significantly higher than 9.26±0.40 g/dl in Group 3. In present finding for Hb concentration in treated buffaloes is in line with the earlier reports of Mondal and Paul (2012) in cows, Venkateswarlu *et al.* (2019); Sabasthin *et al*. (2012) who reported the Hb concentration as 9.29 g/dl, 8.61±0.20 gm percent and 9.7±0.16 g/dl in repeat breeding buffaloes. Similarly, Mujawar (2019) in anestrus buffaloes. Patil *et al.* (2015) reported significant increase in Hb concentration as the treatment progressed. Low Hb concentration though within the normal range recorded in repeat breeding buffaloes before treatment reflects protein deficiency which affects tissue oxygenation of the genitals resulting in adverse effect on the cyclicity of the animal (Ramakrishna, 1997) and failure to conceive. Low Hb level affects oxygen transport to vital tissues causing reduced oxidation, ultimately affecting the cellular metabolism of the gonadal cells (Swenson and Reece, 1993).

Packed Cell Volume (PCV)

Packed Cell Volume in four treatment groups do not differ significantly. However, PCV (27.17±1.18 and 27.30±1.53%) in Group 2 was non-significantly higher as compared to (25.07±0.99 and 27.91±1.98%) in Group 1 on day 9 and day 27, respectively. While PCV (27.70±1.21 and 27.94±1.11%) in Group 4 was higher on day 9 and day 27, respectively as compared to (26.54±1.65 and 25.52±1.18%) in Group 3 (non-significant). Sabasthin *et al.* (2012) reported PCV as 29.0±0.49% in buffaloes and Mondal and Paul (2012) reported 28.4% in repeat breeding cows. Venkateswarlu *et al.* (2019) reported mean packed cell volume 35.21±0.99 and 39.13±0.48% in repeat breeder and normal cycling buffaloes, respectively. Higher PCV may be due to better nutrition, health and state of the cycle, which are supposed to have anabolic activity. Low PCV in buffaloes reflect malnutrition. (Islam *et al*., 1999; Ghani *et al*., 2017).

Total protein

Mean total protein values at different interval within Group 1, 2 and 3 do not differ significantly with the values on day 0 (Table 2). In Group 4 mean protein values significantly increased (P<0.05) on day 9 (6.27±0.12) and day 27 (6.43±0.12) as compared to day 0 (5.91±0.10). Mean total protein 6.20±0.14, 6.60±0.10, 6.51±0.07 and 5.91±0.10 g/dl on day 0 between treatment groups differ significant (P<0.01). However, total protein concentration (g/dl) is on day 9 and day 27. Dutta *et al.* (1991); Mondal and Paul (2012) reported serum total protein as 5.30±0.06 mg/100 ml in repeat breeder and 6.54±0.07 mg/100 ml, in normal cows and 5.71 g/dl and 5.52 g/dl. in cycling and repeat breeding cows.

Albumin

Mean Albumin concentration (g/dl) between interval in treatment groups do not differ significantly. In Group 1, within interval albumin level increased on day 27. However, in Group 2, 3 and 4 albumin concentration decreased on day 27. ANOVA for albumin concentration between different treatment groups on day 9 and 27 do not differ significantly, however albumin concentration on day 9 in Group 1 and 2 was significantly lower (P<0.05) as compared to values of Group 3 and 4. Mondal and Paul (2012); Zaman *et al.* (2014) reported 2.81 and 2.71 g/dl; 2.66±0.09 and 2.70±0.14 g/dl in normal cyclic and repeat breeding cows, respectively.

Globulin

Mean total globulin concentration (g/dl) between interval within Group 1, 2 and 3 on day 0, 9 and on day 27 do not differ significantly. However, in Group 4 mean globulin concentration increased significantly (P<0.05) on day 9 (3.34±0.08) and day 27 (3.77±0.16) as compared to day 0 (3.21±0.12). Mean globulin concentration (g/dl) between the groups on day 0 differ significantly (P<0.01). However, globulin concentration (g/dl) on day 9 and day 27 do not differ significantly between Group 1, 2, 3 and 4, respectively. Mondal and Paul (2012) reported significantly higher (P<0.05) values of serum globulin (2.89 g/dl) in cycling as compared to (2.82 g/dl) in repeat breeding cows. Mujawar (2018) reported globulin (g/dl) concentration on day 0, and on 2nd administration of vitamin E and Selenium and at the time of AI as 2.97±0.08, 3.05±0.06 and 3.36±0.06, respectively; 2.86±0.04, 2.85±0.08, 3.12±0.04, respectively treated with two different protocols.

Intensity of estrus

All the buffaloes in different Treatment groups showed variable pattern of intensity as intense, intermediate and weak in Group 1, 2, 3 and 4 was 1 (16.66%), 4 (66.66%) and 1 (16.66%); 2 (33.33%), 3 (50.00%) and 1 (16.66%); 1 (16.66%), 5 (83.33%) and 0 (00.00%); 3 (50.00%), 2 (33.33%) and 1 (16.66%), respectively. Alyas *et al.* (2015); Kaliannan *et al.* (2018); Mujawar *et al.* (2019) reported variable pattern of estrus intensity, which is due to individual hormonal orchestra and individual response to the hormonal treatment.

CONCLUSIONS

White side test is an important test and can be used effectively for differential diagnosis of infectious and non-infectious repeat breeding buffaloes. Repeat breeding buffaloes respond well to hormonal treatment with and without antioxidant. Buffaloes treated with hormonal protocols plus

Figure 1. White side test.

Figure 2. Frequent micturition.

Figure 3. Swelling of vulval lips.

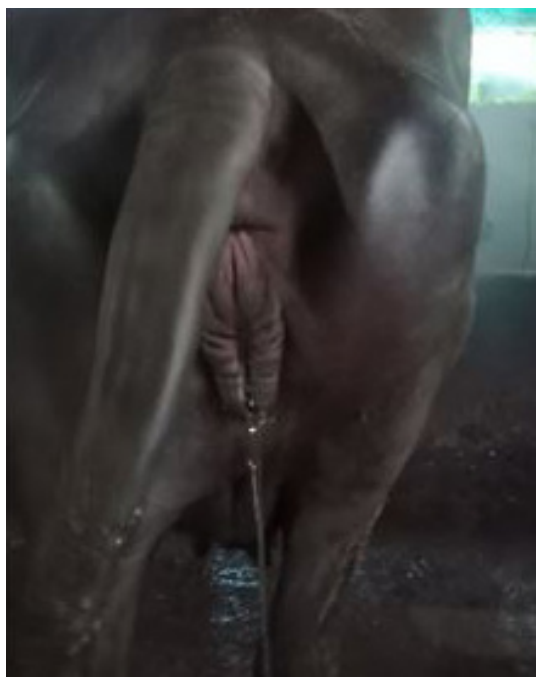

Figure 4. Cervical discharge.

Table 1. Behavioural symptoms during estrus in repeat breeding buffaloes.

Behavioural Symptoms		Group 1	Group 2	Group 3	Group 4
Excitement (%)	Intense	2 (33.33)	1 (16.66)	0 (00.00)	2 (33.33)
	Intermediate	1 (16.66)	3 (50.00)	2 (33.33)	1 (16.66)
	Absent	3 (50.00)	2 (33.33)	4 (66.66)	3 (50.00)
Bellowing (%)	Frequent	2 (33.33)	4 (66.66)	2 (33.33)	3 (50.00)
	Intermediate	3 (50.00)	1 (16.66)	4 (66.66)	3 (50.00)
	Absent	1 (16.66)	1 (16.66)	0 (00.00)	0 (00.00)
Micturition (%)	Frequent	3 (50.00)	4 (66.66)	3 (50.00)	5 (83.33)
	Intermittent	1 (16.66)	0 (00.00)	2 (33.33)	1 (16.66)
	Absent	2 (33.33)	2 (33.33)	1 (16.66)	0 (00.00)
Homosexual behaviour (%)	Frequent	4 (66.66)	5 (83.33)	3 (50.00)	4 (66.66)
	Intermittent	2 (33.33)	1 (16.66)	3 (50.00)	2 (33.33)
Swelling of vulva lips (%)	Mild	1 (16.66)	1 (16.66)	0 (00.00)	0 (00.00)
	Moderate	1 (16.66)	2 (33.33)	4 (66.66)	3 (50.00)
	Severe	4 (66.66)	3 (50.00)	2 (33.33)	3 (50.00)
Estrus discharge (%)	Copious	3 (50.00)	5 (83.33)	2 (33.33)	4 (66.66)
	Moderate	2 (33.33)	1 (16.66)	2 (33.33)	1 (16.66)
	Absent	1 (16.66)	0 (00.00)	2 (33.33)	1 (16.66)

Table 2. Haemato-biochemical parameters estimated on day 0, 9 and 27.

Sr. No.	Groups	Haemoglobin (g/dl) (Mean±SE)			ANOVA (between interval)	
		Day 0	Day 9	Day 27	F Cal	P value
1.	I	8.87±0.33	8.57±0.22	9.31±0.56	0.855^{NS}	0.445
2.	II	8.52±0.27	9.08±0.40	9.51±0.50	1.508^{NS}	0.253
3.	III	8.78±0.60	9.08±0.51	9.26±0.40	0.224^{NS}	0.802
4.	IV	9.16±0.35	8.77±0.42	9.59±0.26	1.352^{NS}	0.288
ANOVA (between group)	F Cal	0.413^{NS}	0.384^{NS}	0.128^{NS}		
	P value	0.746	0.766	0.942		
Sr. No.	**Groups**	**Packed Cell Volume (%) (Mean±SE)**			**ANOVA (between interval)**	
		Day 0	Day 9	Day 27	F Cal	P value
1.	I	25.88±1.16	25.07±0.99	27.91±1.98	1.018^{NS}	0.385
2.	II	25.05±0.83	27.17±1.18	27.30±1.53	1.078^{NS}	0.365
3.	III	25.99±1.55	26.54±1.65	25.52±1.18	0.119^{NS}	0.888
4.	IV	26.53±1.36	27.70±1.21	27.94±1.11	0.375^{NS}	0.694
ANOVA (between group)	F Cal	0.235^{NS}	0.780^{NS}	0.581^{NS}		
	P value	0.871	0.519	0.634		
Sr. No.	**Groups**	**Total Protein (g/dl) (Mean±SE)**			**ANOVA (between interval)**	
		Day 0	Day 9	Day 27	F Cal	P value
1.	I	6.20 ± 0.14^{PQ}	5.97±0.10	6.48±0.17	3.058^{NS}	0.077
2.	II	6.60 ± 0.11^{R}	6.44±0.24	5.99±0.19	2.763^{NS}	0.095
3.	III	6.51 ± 0.08^{QR}	6.01±0.18	6.25±0.09	3.090^{NS}	0.075
4.	IV	$5.91^{A}\pm0.11^{P}$	$6.27^{AB}\pm0.12$	$6.43^{B}\pm0.12$	4.439^{*}	0.031
ANOVA (between group)	F Cal	7.708**	1.579^{NS}	1.899^{NS}		
	P value	0.001	0.226	0.162		

The values having different superscript ABCD between intervals and PQRS between groups differ significantly **(P<0.01), *(P<0.05).

Table 2. Haemato-biochemical parameters estimated on day 0, 9 and 27. (Continue).

Sr. No.	Groups	Globulin (g/dl) (Mean±SE)			ANOVA (between interval)	
		Day 0	Day 9	Day 27	F Cal	P value
1.	I	3.46 ± 0.09^{PQ}	3.33 ± 0.09	3.66 ± 0.19	0.945^{NS}	0.411
2.	II	3.78 ± 0.14^{Q}	3.76 ± 0.29	3.34 ± 0.16	1.394^{NS}	0.278
3.	III	3.54 ± 0.12^{PQ}	2.99 ± 0.23	3.53 ± 0.11	3.539^{NS}	0.055
4.	IV	$3.21^{A}\pm0.12^{P}$	$3.34^{A}\pm0.08$	$3.77^{B}\pm0.16$	5.289*	0.018
ANOVA (between group)	F Cal	3.745*	2.518^{NS}	1.287^{NS}		
	P value	0.028	0.087	0.306		

The values having different superscript ABCD between intervals and PQRS between groups differ significantly **(P<0.01), *(P<0.05).

vitamin E and Selenium express more pronounced signs of estrus. There was no significant effect on haematobio-chemical parameters when treated with or without antioxidants.

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