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Haemato-biochemical profile of repeat breeding buffalo in relation to different phases of oestrus cycle under field conditions

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Abstract

This study was conducted with an aim to compare the haematological and biochemical profile in normal and repeat breeding buffaloes under field condition during 0 (day of oestrus), 11th day (mid luteal phase) and 16th day (Early follicular phase) of the oestrus cycle. A total of sixteen buffaloes were selected from the cases presented for A.I. to various Veterinary Dispensaries in and around Bidar. Buffaloes were grouped based on clinical examination and uterine infection was ruled out by white side test. Blood sampling was carried out on corresponding days for analysis. Result of present study elucidated that mean levels of TEC, Hb and PCV were significantly ($p \leq 0.05$) lower in repeat breeders whereas, no difference was found with TLC and DLC levels. Repeat breeders had significantly ($p \leq 0.05$) lower cholesterol than normal breeders however, no significant difference was observed between two groups in glucose, alkaline phosphatase, triglycerides, total protein and albumin levels. No significant difference was observed among the aforementioned phases for these haemato-biochemical parameters in both groups.

Keywords: Buffalo, repeat breeder, phases of oestrus cycle, haematological, biochemical parameters

Introduction

Under field conditions in India, the major infertility or sub-fertility problems in buffalo are due to anoestrus and repeat breeding syndrome (RBS). Sub-fertility is matter of actual concern than infertility and one of the most important and commonly encountered sub-fertility is repeat breeding syndrome. The repeat breeding syndrome is defined as a condition in which dairy animal have regular oestrus cycle and appear normal on superficial clinical examination but fails to become pregnant following three or more breeding (Bartlett *et al.*, 1986) [2]. The causes of RBS can be divided into two major categories mainly fertilization failure and embryonic death. Other important causes of repeat breeding are delayed ovulation, anovulation, endocrine dysfunction, chromosomal aberrations, congenital or acquired defects of ovum or spermatozoa, anti-sperm antibodies, uterine infection, anatomical defects of the reproductive tract etc. However, in field conditions poor management practices fallout as, nutritional deficiencies and environmental stress etc. plays a much more important role in causing repeat breeding.

From the economic point of view, the repeat breeding syndrome impacts the buffalo production under field conditions as it causes reduced milk production, increased culling and reduces value of animal upon sale. On the other side, increases the indirect costs of sound diagnosis, treatment trials and repeated artificial inseminations. Jeyakumari *et al.* (2003) [11] estimated annual losses incurred as a result of repeat breeding ranged between Rs. 2902.32 to Rs. 3101.70 per animal under Indian conditions. Therefore, the management of reproductive health for optimization of reproductive performance warrants comprehensive evaluation and comparative analysis of the baseline information regarding various haemato-biochemical determinants during different phases of oestrus cycle in repeat breeding buffalo so as to improve buffalo production under field/ rural conditions and hence the current study was planned.

Materials and Methods

Buffaloes presented to veterinary dispensaries for Artificial Insemination (A.I.) were clinically examined for their reproductive health; and ruled out for any uterine infection by performing

white side test as described by Pateria and Rawal (1990) [20]. Breeding history of the buffaloes was collected and thorough per-rectal examination was performed to ascertain reproductive status. Based on the above observations on reproductive history and clinical examination the buffaloes were grouped as normal breeder buffalo (8 numbers) and repeat breeder buffalo (8 numbers). For the estimation of various hematological and blood biochemical parameters blood samples were collected from jugular vein i.e. on 0 (day of estrus), 11th day (mid luteal phase) and 16th day (Early follicular phase) of the oestrus cycle from normal breeding as well as repeat breeding buffaloes. A total of 20ml of blood was collected and out of that 10ml of blood was transferred to EDTA coated vials for hematological analysis. Another 10ml blood was stored in vials coated with clot-activator and serum was separated after one hour and stored at -20°C until analysis of biochemical profile.

Hematological parameters viz., total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV), which were analyzed using veterinary auto-hematology analyzer (ARTOCELL-200VET make from by SWEMED BIOMEDIALS PVT.LTD). Differential leukocyte count (DLC) was done manually as described by Weiss and Wardop (2011) [25].

The blood biochemical parameters viz., glucose, triglycerides, cholesterol, total protein, albumin, and alkaline phosphatase were estimated using semi automated biochemistry analyzer (ARTOS® make from SWEMED BIOMEDIALS PVT.LTD) using SWEMED® Diagnostic Kits, Bangalore following the instructions and procedure supplied with the diagnostic kits. The data obtained from the present study was subjected to statistical analysis using one way ANOVA and Student 't' test as per the standard procedure described by Snedecor and Cochran (1994) [24].

Result and Discussion

Haematological Parameters

Total erythrocyte count, Packed Cell Volume and Hemoglobin

The mean levels of TEC, PCV and Hb were significantly ($p \leq 0.05$) lower in repeat breeding buffaloes as compared to normal breeding buffaloes throughout the oestrus cycle (Table.1). There was no significant ($p \leq 0.05$) difference in the TEC, PCV and Hb values within the group during oestrus, mid luteal and early follicular phase of oestrus cycle in both the groups. In agreement to these findings, the lower TEC, PCV and Hb values during repeat breeding condition are reported in buffaloes (Sabasthin *et al.*, 2012) [21]. In contrast, Jani *et al.* (1995) [9] have reported only the significantly lower Hb in repeat breeder buffaloes without any significant difference in TEC and PCV values. In similar to the results of the present study, Kekan *et al.* (2005) [13] have reported that values of Hb, PCV and TEC were not significantly different on 0, 7th and 15th days of oestrus cycle in repeat breeding cows.

The TEC, PCV and Hb values of an animal are the indicators of oxygen carrying capacity of the RBC, which is the major physiological function of blood. Any significant reduction in their values would severely affect tissue oxygenation process. Though TEC, PCV and Hb values were in normal physiological range in both the groups, these values were significantly on lower end of that range in repeat breeding

buffaloes, which might have forced the gonadal cells to underperform in hypoxic conditions. Therefore, the lower TEC, PCV and Hb values could be the one of the contributing factors in predisposing the buffaloes to repeat breeding.

Erythrocyte Indices

In this study, significantly ($p \leq 0.05$) higher mean corpuscular volume was recorded in repeat breeding group as compared to normal breeding group in all the phases of oestrus cycle whereas, no such significant variation was observed with respect to MCH and MCHC between two groups (Table. 2). No significant ($p \leq 0.05$) difference was observed in MCV, MCH and MCHC values on 0, 11th and 16th day of the oestrus cycle within each group in repeat breeding as well as in normal breeding buffaloes. Kumar *et al.* (1986) [15] and Kekan *et al.* (2005) [13] have reported that MCV and MCH were significantly higher in repeat breeder than normal breeder cows and MCHC was non-significantly higher in normal breeder than repeat breeder. Kumar *et al.* (1991) [14] observed significantly higher MCV values in repeat breeder with no significant difference in MCH and MCHC between normal and repeat breeder buffaloes. The erythrocyte indices are basically the derived values that are calculated from the TEC, PCV and Hb values. Therefore, the MCV, MCH and MCHC values not only depend on absolute values of TEC, PCV and Hb but also on their relative differences among the values of each other. This above fact can be attributed for the wide discrepancy in erythrocyte indices reported by various workers in normal and repeat breeding animals.

Total leukocyte count and Differential Leukocyte Count

The present study documented that there was no significant ($p \leq 0.05$) difference either in the total leukocyte count or in the differential leukocyte count i.e. percentages of neutrophils, lymphocytes, monocytes, eosinophils, and basophils between repeat breeding and normal breeding buffaloes in any of the phases of oestrus cycle under study. No significant ($p \leq 0.05$) difference was also observed within the group for TLC and DLC values on 0, 11th and 16th day of the oestrus cycle in normal as well as in repeat breeding group. These findings are very much in contrast to the observations of Sabasthin *et al.* (2012) [21], Mondal and Paul (2012) [18] who have reported significantly greater TLC in repeat breeder than normal breeding animals. Significant neutrophilia and lymphopenia was documented in repeat breeding buffaloes by Kekan *et al.* (2005) [13]. The discrepancy may be ascribed to the fact that in the present study, buffaloes were ruled out for any uterine infection by performing wide side test and hence only the repeat breeding buffaloes of non infectious origin were selected.

Biochemical Parameters

Glucose

Repeating breeding buffaloes had apparently lower glucose concentration than the normal breeders at all the phases of oestrus cycle, though the difference was statistically non significant. No significant ($p \leq 0.05$) difference in glucose concentration was observed between different phase of oestrus cycle either in normal breeding or in repeat breeding buffaloes. Optimum blood glucose is necessary for progesterone production by the steroidogenic cells (Zaker and Hertelendy, 1980) [26] and the growth of developing embryo (Gao *et al.*, 2007) [8]. El- Belely, (1993) [6] suggested that altered level of glucose might be the reason for reduced luteal

functions in repeat breeding cows. Lower glucose concentration may result into lack of energy to sperm, ovum and embryo. Arosh *et al.* (1998) [1] opined poor energy status in repeat breeders due to hypoglycemia could be the reason for impaired hypothalamic-hypophyseal-ovarian axis and reduced ovarian activities.

Triglycerides

The concentration of triglycerides on different phases of the oestrus cycle did not differ significantly ($p \leq 0.05$) between normal and repeat breeding buffaloes. No significant ($p \leq 0.05$) difference was also observed in triglyceride concentration among 0, 11th and 16th day of the oestrus cycle within group in both normal and repeat breeding buffaloes.

Cholesterol

Cholesterol concentration was significantly ($p \leq 0.05$) lower in repeat breeding group as compared to normal breeding group in all the phases of the oestrus cycle. Chauhan *et al.* (1981) [5] opined that the low levels of cholesterol in repeat breeder cows are indicative of subnormal energy status which is known to influence the pituitary function and reduce the secretion of gonadotropins due to which follicles fail to develop and undergo atresia, and subsequent development of early embryos is affected. Nair *et al.* (1987) [19] positively correlated cholesterol levels to better reproductive performance in repeat breeder cows. Serum cholesterol acts as a precursor for steroid hormone synthesis and its availability in micro circulation determines the quantum of steroid hormones secreted (Sabasthin *et al.*, 2012) [21]. Hence, a decrease in the cholesterol level could be one of important the causes for repeat breeding problem being prevalent in the field conditions. In the present study, there was no significant ($p \leq 0.05$) difference in cholesterol concentration among oestrus, mid luteal and early follicular phases of the oestrus cycle in both normal and repeat breeding buffaloes. In contrary, Burle *et al.* (1995) [3] have reported that the cholesterol levels at oestrus phase were higher than luteal

phase in normal and repeat breeding groups.

Total Protein and Albumin

The mean total protein and albumin concentrations on 0, 11th, 16th day of the oestrus cycle did not differ significantly ($p \leq 0.05$) between normal and repeat breeding buffaloes. Within each group also there was no significant ($p \leq 0.05$) difference in the concentration of these parameters between oestrus, mid luteal and early follicular phase of oestrus cycle in normal as well as in repeat breeding group. These results are in conformity with the findings of Ghandotra *et al.* (1993) [7], Jaychandran *et al.* (2007) [10] and Shukla *et al.* (2007) [23] in cows. In contrast, Butani *et al.* (2011) [4] and Sabasthin *et al.* (2012) [21] have documented significantly lower plasma protein values in repeat breeding buffaloes as compared to normal buffaloes. Similarly, Marai *et al.* (1992) [17] have documented lower albumin levels in repeat breeding buffaloes. Shiraz *et al.* (2010) [22] reported lowest level of albumin on day 15th day of oestrus cycle. The discrepancy in the results of various workers with respect to total protein and albumin concentration in normal and repeat breeding animals may be attributed to considerable differences in the composition of feed, quantity and frequency of feeding to the experimental buffaloes under field condition.

Alkaline Phosphatase

The activity of alkaline phosphatase (IU/L) in the serum of normal breeding and repeat breeding groups were statistically insignificant. However, repeat breeding buffaloes showed marginally elevated levels of serum alkaline phosphatase activity throughout the oestrus cycle. Increased activity of alkaline phosphatase in repeat breeders may help in release more phosphorus to maintain calcium-phosphorus ratio in the blood as the concentration of phosphorus in such animals would be low (Lehinger, 1990) [16]. Significantly higher concentration of alkaline phosphatase in repeat breeders as compared to normal breeders was documented by Kalita and Sarmah, (2006) [12] in cows.

Table 1: TEC, Hb and PCV (Mean± SE) at different phases of oestrus cycle in normal and repeat breeding buffaloes

Day of oestrus cycle	TEC (10 ⁶ /μl)		Hb (g/dl)		PCV (%)	
	Normal breeder	Repeat breeder	Normal breeder	Repeat breeder	Normal breeder	Repeat breeder
0 day	7.06±0.05	5.90±0.14*	11.60±0.36	10.44±0.18*	34.73±0.62	31.01±0.61*
11 th day	7.05±0.06	5.90±0.17*	11.49±0.22	10.28±0.25*	34.94±0.58	31.19±0.48*
16 th day	6.94±0.08	5.80±0.11*	11.51±0.29	10.38±0.16*	34.59±0.64	31.02±0.40*

* Significant difference ($p \leq 0.05$) between groups for each parameter on respective day of oestrus cycle.

Table 2: Erythrocyte indices at different phases of oestrus cycle in normal and repeat breeding buffaloes

Day of oestrus cycle	MCV (fl)		MCHC (g/dl)		MCH (pg)	
	Normal breeder	Repeat breeder	Normal breeder	Repeat breeder	Normal breeder	Repeat breeder
0 day	49.14±1.03	52.57±0.56*	33.49±1.25	33.74±0.85	16.45±0.61	17.73±0.45
11 th day	49.58±1.01	52.78±1.57*	32.99±1.07	33.06±1.10	16.29±0.30	17.40±0.57
16 th day	49.81±0.90	53.33±1.27*	33.45±1.34	33.52±0.77	16.60±0.51	17.84±0.40

* Significant difference ($p \leq 0.05$) between groups for each parameter on respective day of oestrus cycle.

Table 3: Serum concentrations (Mean± SE) of glucose, triglycerides and cholesterol at different phases of oestrus cycle in normal and repeat breeding buffaloes

Day of oestrus cycle	Glucose (mg/dl)		Triglycerides (mg/dl)		Cholesterol (mg/dl)	
	Normal breeder	Repeat breeder	Normal breeder	Repeat breeder	Normal breeder	Repeat breeder
0 day	58.26±3.63	50.57±3.79	36.36±1.65	33.28±2.34	62.04±1.57	53.51±0.74*
11 th day	54.94±2.48	50.38±2.25	35.78±1.26	31.13±1.27	61.84±1.44	53.52±1.32*
16 th day	52.42±1.61	50.05±1.63	37.44±1.68	32.42±1.69	61.80±2.09	53.93±0.92*

* Significant difference ($p \leq 0.05$) between groups for each parameter on respective day of oestrus cycle.

Table 4: Serum concentrations (Mean± SE) of total protein, albumin and alkaline phosphatase at different phases of oestrus cycle in normal and repeat breeding buffaloes.

Day of oestrus cycle	Total protein(g/dl)		Albumin (g/dl)		Alkaline phosphatase(IU/L)	
	Normal breeder	Repeat breeder	Normal breeder	Repeat breeder	Normal breeder	Repeat breeder
0 day	7.15±0.16	6.70±0.20	3.16±0.04	3.15±0.09	185.60±9.77	210.90±13.77
11 th day	7.10±0.18	6.81±0.45	3.15±0.09	3.15±0.13	193.80±14.11	209.70±20.85
16 th day	7.12±0.28	6.77±0.18	3.17±0.10	3.16±0.18	191.50±12.38	208.50±9.54

Conclusion

It can be concluded from the study that repeat breeding buffaloes had significantly ($p \leq 0.05$) lower values of TEC, Hb, PCV, cholesterol, apparently lower glucose concentration, elevated levels of serum alkaline phosphatase activity as compared to normal breeding buffaloes during all phases of oestrus cycle. No significant difference was observed among oestrus, mid luteal and early follicular phases of the oestrus cycle for these parameters in both normal and repeat breeding buffaloes. Therefore, nutritional and other managemental practices needs to be looked into while developing the strategies to overcome the repeat breeding problems in buffaloes under field conditions.

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