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# Blood haematological and biochemical parameters in normal cycling, pregnant and repeat breeding buffaloes (*Bubalus bubalis*) maintained in isothermic and isonutritional conditions

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# ABSTRACT

Objective: The present study was envisaged to examine the hematological and biochemical, parameters in three different groups of buffaloes (regularly cycling, pregnant and repeat breeding) maintained in isothermic and isonutritional conditions to establish the variations in blood and/or serum components in these groups. Methods: Blood samples were analyzed for hemoglobin, PCV, TLC, neutophil, lymphocyte, eosinophil, and monocyte count, glucose, total protein, albumin, globulin, urea and cholesterol. Results: The results revealed significantly lower haemoglobin and packed cell volume value in repeat breeding when compared to pregnant and regularly cycling animals. The WBC value significantly higher in repeat breeding when compared to regularly cycling animals. The average mean values of neutrophils, lymphocyte and eosinophils revealed a no significant difference in the neutrophils, lymphocyte and eosinophils in between the group. The mean values of monocytes revealed a significantly higher value in repeat breeding animals when compared to pregnant animals. The serum glucose, total protein, cholesterol and urea levels were significantly lower in repeat breeding compared to pregnant and regularly cycling animals. The levels of albumin and globulin revealed non-significant difference among the groups. Conclusions: A significant decrease in the hemoglobin, PCV, glucose, total protein, cholesterol and urea was observed in the repeat breeding animals when compared to pregnant and regularly cycling animals.

# **1. Introduction**

Reproductive disorders are often multifaceted and multifactorial, which depend on their nature and severity culminating in varying degrees of suboptimal reproductive performance; one such severe problem is repeat breeding<sup>[1]</sup>. Normal levels of vital haemato-biochemical constituents are of utmost importance for maintaining functional integrity of the reproductive system. Any change in hematological and biochemical parameters may adversely affect the reproductive efficiency of livestock. The present work was undertaken to compare the haematological and biochemical parameters in repeat breeding, pregnant and regularly cycling buffaloes maintained in isonutritional and isothermal conditions in a dairy farm.

### 2. Materials and methods

Selected buffaloes (n=18) maintained in Vinayaka Dairy Farm, Dharwad, Karnataka, India were on isonutritional condition under regular veterinary care and management. The ambient temperature ranged from 26–28 °C during the experiment period. The buffaloes were categorized into three groups for the present study, viz., repeat breeding group, pregnant group and regularly cycling group, on the basis of the available records maintained in the dairy farm as well as by rectal examination. The blood collections

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and all estimations were done in quadruplicates. Blood samples collected from all the eighteen animals were analyzed for hemoglobin, packed cell volume (PCV), total leucocyte count (TLC), neutophil, lymphocyte, eosinophil, and monocyte count by following standard procedures<sup>[2]</sup>. The serum samples were analyzed for the biochemical parameters (glucose, total protein, albumin, globulin, urea and cholesterol) as per AOAC<sup>[3]</sup>.

The data obtained were subjected to statistical analysis using computerized Graph pad prism software<sup>[4]</sup>. All hematological and biochemical values obtained were subjected to one-way ANOVA with Tukey's post test. The statistical significance of all the results are expressed at  $P \leq 0.05$ .

# 3. Results

The haemoglobin and PCV values were significantly  $(P \leq 0.05)$  lower in the repeat breeding buffaloes than in the pregnant and regularly cycling ones (Table 1). Further, the values were also found to be significantly ( $P \le 0.05$ ) lower in the pregnant buffaloes than in the regularly cycling ones. The TLC value was significantly ( $P \le 0.05$ ) higher in the repeat breeding buffaloes than in the regularly cycling animals, however, no significant difference was observed in the values between the repeat breeding buffaloes and pregnant ones. Furthermore, the TLC value was also found to be significantly ( $P \leq 0.05$ ) higher in the pregnant buffaloes than in the regularly cycling ones. The average mean values of neutrophils, lymphocytes and eosinophils were not significantly different between the repeat breeding, pregnant and regularly cycling buffaloes, however, significant  $(P \leq 0.05)$  difference was observed in the values between pregnant and regularly cycling animals. The average mean value of monocytes was significantly ( $P \le 0.05$ ) higher in

### Table 1

Blood haematological and biochemical parameters (mean±SE) of repeat breeding, pregnant and regularly cycling buffaloes.			
Blood parameter	Repeat breeding animals	Pregnant animals	Regularly cycling animals
Haemoglobin (g/dL)	$9.7 \pm 0.16^{a}$	$11.0\pm0.22^{b}$	12.0±0.13°
PCV (%)	$29.0\pm0.49^{a}$	$31.0\pm0.66^{b}$	36.0±0.39°
TLC $(\times 10^3)$	$15.0\pm0.07^{a}$	$13.0\pm0.28^{a}$	$9.0 \pm 0.28^{b}$
Lymphocyte (%)	$60.0 \pm 4.5^{a}$	$66.0 \pm 2.4^{ab}$	$54.0 \pm 1.8^{\rm ac}$
Neutrophil count (%)	$36.0 \pm 4.0^{a}$	30.0±1.5 <sup>ab</sup>	$45.0 \pm 1.7^{\rm ac}$
Eosinophil (%)	$2.3\pm0.71^{a}$	$1.8{\pm}0.48^{\rm ab}$	$4.5\pm0.89^{\mathrm{ac}}$
Monocyte (%)	$3.2 \pm 0.60^{a}$	1.3±0.21 <sup>b</sup>	$2.5\pm0.22^{\mathrm{ab}}$
Glucose (mg/dL)	$38.05 \pm 0.67^{a}$	$57.93 \pm 0.50^{b}$	63.48±1.61°
Total protein (g/dL)	$6.5 \pm 0.09^{a}$	$6.8\pm0.08^{\mathrm{ab}}$	$7.0\pm0.12^{\rm b}$
Albumin (g/dL)	3.0±0.10	3.1±0.04	3.2±0.05
Globulin (g/dL)	3.5±0.14	3.6±0.09	3.8±0.15
Cholesterol (mg/dL)	$83.70 \pm 0.97^{a}$	87.12±0.55 <sup>a</sup>	$116.60 \pm 1.57^{\rm b}$
Urea (mg/dL)	$18.0\pm0.76^{a}$	$26.0\pm0.67^{\rm b}$	$24.0\pm1.1^{ m b}$

Values with different superscripts (lower case alphabets) within a row are significantly different (P≤0.05).

the repeat breeding buffaloes than in the pregnant ones, however, no significant difference was observed in the values between the regularly cycling and pregnant as well as the repeat breeding buffaloes.

The glucose level was significantly ( $P \leq 0.05$ ) lower in the repeat breeding buffaloes than in the pregnant and regularly cycling ones (Table 1). Further, the glucose level was also found to be significantly ( $P \le 0.05$ ) lower in the pregnant buffaloes than in the regularly cycling ones. No significant difference in the albumin and globulin levels between the repeat breeding, pregnant and regularly cycling buffaloes were observed. The cholesterol level was significantly  $(P \leq 0.05)$  lower in the repeat breeding buffaloes than in the regularly cycling ones. Further, the cholesterol level was also found to be significantly ( $P \leq 0.05$ ) lower in the pregnant buffaloes than in the regularly cycling ones. No significant difference in the cholesterol level between the repeat breeding and pregnant animals was observed. The urea level was significantly ( $P \leq 0.05$ ) lower in the repeat breeding buffaloes than in the regularly cycling and pregnant ones. However, no significant difference in the urea level was observed between the pregnant and regularly cycling buffaloes.

# 4. Discussion

Tissue oxygenation of reproductive tract and in turn the normal cyclicity are dependent on the hemoglobin level in the circulation. During oestrus, sufficient concentration of haemoglobin in blood is required for the proper transportation of oxygen and nutrients to the vital organs, as the same is required for metabolic activities of the gonadal cells. Hence, a decrease in the hemoglobin level could be a cause for repeat breeding buffaloes in the present study. The trend of variation in the PCV values was found to be

similar to the values of haemoglobin in all the three groups of buffaloes. Significant increase in TLC count in animals could be due to increased enzymatic activity in the body. The values were found to be significantly ( $P \leq 0.05$ ) higher in the pregnant buffaloes than in the regularly cycling ones. Roy et al. reported an increase in the total TLC count in pregnant animals and also reported that the same could be due to endogenous adrenaline release resulting in increase in TLC<sup>[5]</sup>. In the present study, there was no significant difference in the lymphocytes, neutrophils and eosinophils values between the repeat breeding and pregnant or regularly cycling buffaloes. The results indicated that lymphocytes or neutrophils or eosinophils values cannot be used as a marker to identify the incidence of repeat breeding in a herd. Further, the reason for an increase in the monocyte value in the repeat breeding buffaloes when compared to the pregnant ones is suggestive of increased phagocyte activity as well as increased exhibition of the function of macrophage.

The serum glucose was reported to be an important factor which modulates reproduction and the same at lower level is postulated as the cause for decreased fertility rate as well as for non cyclicity and reduced fertility[6]. In contrast, Awasthi and Kharche reported an increase in glucose concentration in repeat breading cows when compared to regularly cycling cows[7]. Further, the glucose levels were also found to be significantly ( $P \leq 0.05$ ) lower in the pregnant buffaloes than in the regularly cycling ones. Cows generally go ketotic during the third trimester, suggesting increasing demand for glucose in pregnant animals. In the present study, the blood samples were from animals that were in the first trimester of pregnancy. The glucose levels as observed in the present study though lower than normal levels are found to be within the physiological range of 45–75 mg/dL; therefore, the significant decrease in the pregnant animal is only suggestive of the demand by the growing fetus. The total protein values observed in the present study in all the three groups of animals were found to be within the normal physiological range. However, significantly ( $P \leq 0.05$ ) lower protein value in the repeat breeding buffaloes was observed compared to the regularly cycling cows. Shiraz et al. reported a decreased concentration of plasma total protein in the repeat breeding cows in comparison with the regularly cycling animals<sup>[8]</sup>. Further, the total protein in the circulation represents the balance between the biosynthesis and catabolism or mechanical loss, and the decrease level of serum proteins may cause deficiency of certain amino acids required for the synthesis of proteins in the body. Shiraz et al. also observed a decreased globulin level in repeat breading cows, suggesting that globulin which functions as a carrier protein for copper was found to alter the biosynthesis

of specific coenzymes and thus the steroidogenesis during early luteal phase of the cycle in repeat breeding cows<sup>[8]</sup>. Serum cholesterol acts as a precursor for steroid hormone synthesis and its availability in micro circulation determines the quantum of steroid hormones secreted. Hence, a decrease in the cholesterol level in the repeat breeding buffaloes when compared to the pregnant and regularly cycling ones could be the cause for repeat breeding being prevalent in the herd. The reason for the decreased urea level in the repeat breeding buffaloes when compared to pregnant and/or regularly cycling ones could be due to the decreased protein level as observed in the present study.

In conclusions, a significant ( $P \le 0.05$ ) decrease in the hemoglobin, PCV, glucose, total protein, cholesterol and urea was observed in the repeat breeding buffaloes when compared to the pregnant and regularly cycling ones. Likewise, a significant ( $P \le 0.05$ ) increase in the monocytes and TLC was observed in the repeat breeding buffaloes when compared to the pregnant and regularly cycling ones.

# **Conflict of interest statement**

We declare that we have no conflict of interest.

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