**BASELINE HEMATOLOGICAL PROFILE DURING ESTROUS CYCLE IN BLACK BENGAL DOES OF WEST BENGAL**

**ABSTRACT**

The haematological parameter evaluation offers information on animals and herd health status. Current study was conducted during April and May 2020 with 12 cyclic Black Bengal does in goat farm of NDRI-ERS Kalyani. Blood samples were collected on day 0,3,7,11,14,17,21,24 and on next estrus in order to validate the changing patterns of different blood components (Hb, R.B.C, W.B.C, P.C.V, D.L.C and Erythrocyte indices) as per stage wise during entire estrus cycle in Bengal does. Estrus was detected based on observation of behavioral estrus signs and standstill by aproned buck introduction and further conformed with USG Examination of female reproductive gonad. As per current analysis, Hb concentrations were recorded relatively (P<0.05) higher (9.75±0.12) during estrus period than other stages of estrus cycle. Slight variation in the PCV concentrations was noted during different days of cycle.Erythrocytecount was found to be highest (15.9±0.23) during luteal phase of estrous cycle and leucocytes were found to be maximum (9.18±0.31) during late luteal and proestrus period. Minimal disparity exists between the neutrophil (45±1.0) and lymphocyte (48±0.7) count during the period of receptivity, on contrary substantial difference of neutrophil and lymphocyte count exists during other days of estrus cycle. This results obtained in present study can be used in the realistic evaluation and assessment of herd health status and disease diagnosis.

**Key words:** Buck, semen, freezing-thawing, soybean lecithin, glycerol

**INTRODUCTION**

Rearing of Black Bengal goats is a common practice in the State of West Bengal and also some parts of North-eastern India. The Black Bengal goat rearing has got some added advantages when compared to other goat breeds, as they gain sexual maturity at an earlier age, female goat becomes pregnant twice-a-year and gives birth to one to three kids (twinning and tripling are common), can adapt to any environment easily and had high disease resistance. It produces high quality meat and skin with high prolificacy rate (Miah *et al.,* 2016). Despite of high disease resistance, assessment of hematological parameters is generally followed as routine farm management practice in order to monitor the health status of a herd (Habibu *et al.,* 2017). Alteration in the hematological profile indicates that the animal is affected with some infection (Bamishaiye *et al.,* 2009). The blood components change relatively in accordance with physiological state of animal, whether it is normal or not (Etim *et al.,* 2014). A quantifiable variation was recorded in blood parameters during estrus period in other species (Schalm *et al.,* 1975). There are only a few reports regarding haematological changes during different days and stages of estrous cycle of farm animals. Therefore, the aim of the present study was to evaluate the haematological parameters like blood erythrocytes, leucocytes, haemoglobin, PCV, DLC and erythrocyte indices (MCH, MCV, MCHC) during different days of the estrous cycle in Black Bengal goats.

**MATERIALS AND METHODS**

**Selection of experimental animals and management**

A total of 12 Black Bengal does selected from the Goat farm located at ICAR-National Dairy Research Institute, Eastern Regional Station, Kalyani were used for the present study. All experimental animals were reared under semi- intensive system and housed separately. Prior to final selection, all goats were examined both clinically and physically, treated against both internal and external parasites, and were immunized against various viral diseases. During lactation, kids were kept along with their mothers. During experimentation, all experimental animals were fed and maintained as per standard farm practices followed by the farm. Fresh tap water was made available all the time to the experimental goats. The routine farm operations were not intervened in the experimental animal.

**Detection of estrus**

Estrus was detected through behavioural signs like bleating, flagging of the tail, reddened vulva, vaginal discharges and occasional riding by other does. Goats displayed overt signs of estrus. Further, the estrum was confirmed by introduction aproned buck into a group of housed females and observation of standstill in does and through transabdominal ultrasonography.

**Collection of blood samples and haematological analysis**

Blood samples (1 ml) were collected through jugular veinipuncture with the help of disposable syringe fixed with hypodermic needle from each doe into vacuum tubes containing Na2-EDTA as anticoagulant on day 0, 3, 7, 11, 14, 17, 21 and 24 of the estrous cycle at 11:00 am. The collected samples were then put into an ice-bucket and carried back to the laboratory immediately after collection. Red blood cells (RBCs in $10^{6}$ cell/µl), haemoglobin concentration (Hb in g/dl) , packed cell volume (PCVin %), total white blood cells (WBCs in $10^{3}$ cell/µl), differential leukocyte count (DLC in %), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were estimated as enumerated below:

***Count of RBC***

 Red blood cells were counted using an improved Neubaur hemacytometer (Neubaur improve, Germany). Hayem solution was used as a diluent. A total of five squares were counted, multiplied by 10000 and expressed in $10^{6}$cell/µl.

**Count of total WBC**

 The total white blood cells were counted by Neubaur haemocytometer using Turkey’s solution as diluting fluid. A total of four squares were counted, multiplied by 1000 and expressed in $10^{3}$ cell /µl.

**Estimation of blood haemoglobin**

The haemoglobin was estimated using Sahli’s haemoglobinometer.

**Determination of PCV**

The packed cell volume of erythrocytes was determined by a micro haematocrit- centrifuge method using a special centrifuge tubes (Hetich Germany). The capillary tubes (75mm×1.0 mm) were filled by capillary from a well-mixed blood sample up to ⅔ length of the tube. The outside of the tube was cleaned with absorbent gauze and the opposite end was sealed with soap. The filled tube was centrifuged at 3000 rpm for 20 min. The PCV was measured as percentage.

**Calculation of erythrocytes indices**

The erythrocytes indices were calculated according to (Jain *et al.,* 1993). Mean Corpuscular Volume (MCV) was calculated from the PCV and RBC count as follows: MCV (fl) = PCV÷RBC×10 and mean corpuscular haemoglobin (MCH) was calculated from the RBC and Hb as follows:- MCH (pg) = Hb÷RBC×10. Mean corpuscular haemoglobin concentration (MCHC) was calculated from the PCV and Hb as follows MCHC g/dl =Hb÷PCV×100

***Differential leucocyte count (DLC)***

The blood smears were prepared by placing a drop of blood in the centre line at the end of a clean and grease free slide, then the blood was spread using spreader slide at an angle of 45º. The smear was dried at room temperature and fixed by placing it in absolute methanol for 5 min (Jain, 1993). Smears were stained with diluted Giemsa stain for 45 min; the excess stained was washed under running tap water and air dried.(Jain, 1993). The blood film was examined under the microscope using oil immersion lens (x100). Battlement method was used for the examination of the blood film to give representative sampling of all parts of the film (Jain, 1993).

**Statistical analysis**

All data have been presented as Mean (±SEM). The data were analyzed by an ANOVA for repeated measures technique with a post-test for linear trend to compare the changes in haematological parameters during different days of the estrous cycle. Significance was considered at P<0.05 level if otherwise not stated. GraphPad Prism 4.01 software (San Diego, CA, USA) was used for statistical analysis of the data.

**RESULTS**

The mean (±SEM) values of various haematological parameters as estimated in the present study during different days of the estrous cycle in Black Bengal goats have been tabulated in Table 1. Mean (±SEM) haemoglobin concentrations (g%) was found to be relatively higher (P<0.05) on the day of estrus. As the estrus cycle progresess the concentrations of haemoglobin begins to decline progressively until mid-luteal phase. Relatively lower Hb levels were quantified in luteal phase. As luteal phase advances the concentration tends to rise again in proestrus stage and attained the maximum concentrations on the day of next consecutive estrus.

3.2. Packed cell volume (PCV):

Mean (±SEM) PCV (%) during different days of estrous cycle have been presented in Table 1. The PCV (%) was estimated to be the highest (P<0.01) on the day of estrus which declined to 26.35±0.36% on day 3 and maintained the level throughout the estrous cycle with minor fluctuations thereafter till day 24 and increased to the highest level again on the day of next estrus. The highest values of PCV were noted either on day 0 or in some animals recorded day before onset of estrus.

3.3. Total leucocyte count (TLC)/ WBC count:

Mean (±SEM) WBC (×$10^{3}$/ml) during different days of estrous cycle has been presented in Table 1. The leucocyte count was found to be the maximum on day 0 and lowest number of WBC were recorded during early and mid-luteal phase of estrous cycle. It was also noticed that the number of WBC had decreased progressively during luteal phase, increased abruptly from the day of luteolysis to onset of proestrus.

3.4. Total erythrocyte count (TEC) / RBC count:

Mean (±SEM) RBC (×$10^{6}$/ml) during different days of estrous cycle have been presented in Table 1. The erythrocyte count was found to increase progressively from the day 3 to day7 and abrupt elevation of RBC count was observed in the luteal phase, especially during diestrus period. Maximum concentrations of RBC count was recorded at day 14 (15.9±0.23 ×$10^{6}$ cells /ml). From day 14, the concentrations were decreased with minor fluctuating till onset of next estrus.

Table 1. Mean (±SEM) of different Haematological parameters during different days of estrous cycle in Black Bengal Goats (n=12).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Day of estrous cycle**  | **Haemoglobin (g%)** | **PCV (%)** | **WBC (×**$10^{3}$**/ml)** | **RBC (×**$10^{6}$**/ml)** | **MCV** | **MCH** | **MCHC** |
| Day 0 | 9.75±0.12a | 29.12±0.34 | 8.07±0.22 | 11.52±0.3 | 22.45±0.69 | 8.51±0.21 | 33.5±0.3 |
| Day 3 | 8.74±0.12b | 26.35±0.36 | 8.94±0.15 | 11.81±0.16 | 22.38±0.24 | 7.43±0.09 | 33.19±0.26 |
| Day 7 | 8.38±0.14b | 25.87±0.49 | 7.24±0.2 | 11.38±0.24 | 22.44±0.6 | 7.32±0.18 | 32.64±0.21 |
| Day 11 | 8.5±0.18b | 26.51±0.5 | 6.88±0.11 | 14.4±0.55 | 18.37±0.52 | 5.86±0.24 | 31.8±0.45 |
| Day 14 | 8.42±0.15b | 25.51±0.38 | 6.78±0.12 | 15.9±0.23 | 16.18±0.35 | 5.31±0.15 | 33.01±0.33 |
| Day 17 | 8.5±0.16b | 25.9±0.38 | 6.68±0.11 | 13.94±0.16 | 18.58±0.27 | 6.1±0.11 | 32.81±0.35 |
| Day 21 | 8.76±0.3b | 27.07±0.79 | 9.18±0.31 | 14.75±0.29 | 18.49±0.81 | 5.99±0.29 | 32.35±0.37 |
| Day 24 | 8.73±0.03b | 26.47±0.03 | 9.1±0.03 | 12.94±0.52 | 20.73±0.91 | 6.83±0.26 | 33±0.17 |
| Day 0 | 9.60±0.17a | 29.64±0.28 | 9.71±0.21 | 13.24±0.24 | 22.49±0.4 | 7.2±0.15 | 32.02±0.5 |

3.5. Erythrocyte indices:

Mean (±SEM) erythrocyte indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentrations (MCHC) during different days of estrous cycle have been presented in Table 1. It was noted that there has been a gradual decrease in MCV from day 0 to day 14 and a gradual increase from day 14 to the next estrus. It was recorded that concentrations of MCH had been found to decrease slowly from day 0 to day 14 and increased thereafter from day 14 to the next estrus. On the other hand, no substantial differences in the concentrations of MCHC were recorded throughout the estrous cycle.

3.6. Differential leucocyte count:

Mean (±SEM) DLC (%) during different days of estrous cycle have been presented in Table 2. Percentage of neutrophils was found to be highest on day 0, from day 0 it decreased progressively till day 11 and from day11 it gradually increased till next estrus. Percentage of lymphocyte was found to increase from day 0 to day 14 with minor fluctuations thereafter. Then, lymphocyte (%) was found to decrease till onset of next estrus. Eosinophil (%) was found to be highest on day 0. The highest number of monocytes (% ) was recorded on day 11 i.e. luteal phase of estrous cycle. Ratio of lymphocyte to neutrophil was found to be less during estrus period; it increased as estrous cycle advances and luteal phase onsets.

Table 2. Mean (±SEM) Differential Leucocyte Count (DLC) during different days of estrous cycle in Black Bengal goats (n=12).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Day of estrous cycle** | **Neutrophils** | **Lymphocyte** | **Eosinophil** | **Monocyte** | **Basophil** |
| Day 0 | 45±1.0 | 48±0.7 | 4.4±0.4 | 2.3±0.4 | 0±0 |
| Day 3 | 39±1.0 | 56±0.9 | 1.9±0.5 | 3.1±0.6 | 0±0 |
| Day 7 | 39±1.5 | 55±1.8 | 2.6±0.5 | 3±0.8 | 0±0 |
| Day 11 | 39±1.4 | 55±0.2 | 1.5±0.2 | 4.5±1.0 | 0±0 |
| Day 14 | 40±1.6 | 57±1.9 | 2±0.6 | 1.8±0.3 | 0±0 |
| Day 17 | 42±1.9 | 52±1.7 | 2±0.8 | 3.8±0.7 | 0±0 |
| Day 21 | 43±2.6 | 52±2.2 | 1.5±0.5 | 3.8±0.7 | 0±0 |
| Day 24 | 45±0.0 | 52±1.3 | 1.3±0.7 | 1.3±0.7 | 0±0 |
| Day 0 | 46±0.9 | 49±0.8 | 3±0.5 | 2.2±0.4 | 0±0 |

**DISCUSSION**

In the current study our results that were postulated has been compared with other validated results. Haemoglobin values (g%) that were obtained in the present study are within the range of reference value for Black Bengal goats (Sharmin *et al.,* 2004). During estrus stage, increase in hemoglobin was attributed to the increased blood supply to the peripheral organs (Fazio *et al.,* 2017) which can be evidenced as vulval hyperemia during estrus behavioral observation. Inappreciable elevation of PCV values on day 0 could be due to haemodilution. And concentrations of haematocrict values obtained were found to be in tandem with other investigations (Sharmin *et al.,* 2004).

Haemodilution could be the probable reason for elevated concentrations of PCV and Erythrocyte count during day 11-12 and on day 16 which can be attributed as the transient elevation of plasma volume that led to increase in RBC count apparently but not absolutely (Fazio *et al.,* 2017). In particular, the reduced number of leucocytes during the luteal phase especially from 5th to 17th day might be due to persistence of local defensive mechanisms against cellular transformation for a period of time during luteal phase (Fazio *et al.,* 2017). Over scoring of neutrophils on the day of estrus ensures the local protective mechanism which was established against inflammation that was developed during first few hours of ovulation (Fazio *et al.,* 2017). On the contrary, lowest monocyte count on day of estrus could be due to local protection.

**Conclusion:**

The results of our study indicate that heamatological parameters during different stages of estrous cycle would be helpful for realistic evaluation and assessment of herd health status and diagnosis of infectious disease in black Bengal does.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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