

Haematological study on peripheral blood of prenatal black Bengal goat with special reference to its phagocytic activity

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Abstract

The present investigation was conducted on the six goat foeti each of 50,62,73,80,87,94,99,106,112,120,130,142 day of gestation age and day old neonates to reveal the age dependent load of the lymphocytes, monocytes, heterophills in the peripheral blood and their phagocytic activity. Total leucocyte count (TLC), differential leucocyte count (DLC), Absolute leucocyte count (ALC), Absolute heterophil count (AHC) and Absolute monocyte count (AMC) increased gradually with increasing foetal age of goat. Intracytoplasmic carbon particles were first observed on day 120 of gestation and recorded 2.10 % and its maximum concentration was seen in day old neonates *i.e* 18.50 %.

Key words:-Phagocytic activity, haematology, prenatal goat

Introduction

Age dependent load of the lymphocytes, monocytes, heterophills in the peripheral blood and their phagocytic activity provides supplementary support to the immune response. Developmentally the first blood cell appears in blood islands which are aggregates of splanchnic mesoderm on the surface of yolk sac and allantois. The peripheral mesenchymal cells of blood islands joint together to form vesicles, the lining of which becomes vascular endothelium. These vesicles coalase to form vascular channels and subsequently establish the vitelline and allantoic vessels (Noden and De Lahunta, 1985). Morphologically, the mature neutrophil are not consistently observed until 123 day in ovine foetus. Number of each class of leukocyte increased from the time of their appearance until just prior to birth when they decline (Sawyer *et al.*, 1978). The literature is very scanty on this aspect in prenatal goat. Hence the present investigation was undertaken to know the phagocytic activity of peripheral heterophil in prenatal black Bengal goat.

Materials and Methods

The umbilical cord of the goat foeti was ligated and severed and then blood samples were collected by heart puncture for hematological study and macrophage function test. Six goat foeti each of 50,62,73,80,87,94,99,106,112,120,130,142 day of gestation age and day old neonates were used. The approximate age was calculated using the "CRL-Gestation Age" correlation of Norden and De Lahunta (1985) in sheep. In present observation following parameters were observed.

a. Total Leucocyte Count (TLC): TLC (mm^3) was estimated as per the method of Coffin (1953) using a diluent containing 0.1M NaCl, 0.008M $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$, 0.002M KH_2PO_4 , 7.5ml formaldehyde and 0.1g methyl violet 2B.

b. Differential Leucocyte Count (DLC): Thin blood smear was made using a drop of blood without anticoagulant. The smear was air dried, fixed in methanol for 2min. and stained with 1:10 diluted Giemsa stain for 30min. The different leucocytes were counted by random method as described by Coffin (1953) and recorded in percent.

c. Absolute Lymphocyte Count (ALC): The ALC values of goat foeti were calculated by using the formula: ALC/mm^3 of blood = (% Lymphocyte X Total leucocyte count)/100.

d. Absolute Heterophil Count (AHC): The AHC values for goat foetus were calculated by using the formula: AHC/mm^3 of blood = (% Heterophil X Total leucocyte count)/100.

e. Absolute Monocyte Count (AMC): The AMC values for the goat foeti were calculated by using the formula: AMC/mm^3 of blood = (% Monocyte X Total leucocyte count)/100.

d. Opsonin activated charcoal assay for macrophage function: To assess the function of peripheral blood macrophages 0.5ml heparinized blood was mixed with 0.5ml PBS (pH 7.2) in a vial. The 0.5ml of carbon particle suspension (0.02% in PBS, pH 7.2) was added to the vial and then 0.1 ml of *E.coli* whole cell culture (10^7 cells/ml PBS) was added to the vial and mixed well. The vials were incubated at 37°C for 30 minutes. The vials were then centrifuged at 12000 rpm for 5 minutes. The supernatant was discarded. Then the blood cells were washed 3 times using 1 ml PBS following centrifuge. Finally after discarding the supernatant, a

drop of the sediment was used to prepare a thin smear on a glass slide. The smears are air-dried, fixed in methanol for 2 minutes and stained with wright's stain. The positive cells carrying carbon particles in their cytoplasm were counted using every 5th field and the percent macrophage was estimated.

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Result And Discussion

Total leucocyte count (TLC)

The mean TLC value in goat foeti of day in 50, 99 and 142 old was 0.99×10^3 , 4.80×10^3 and 8.65×10^3 per ml blood while the value was 9.25×10^3 per ml blood in day old neonates (Table.1). The finding on neonatal kids is in agreement with reports of Coffin (1953) and Banks (1993). However fetal age dependent TLC values are not available in the literature for

comparison. Oduge (1976) opined that TLC value is considerably higher in Nigerian sheep and goat, lower in temperate breeds of animals, young and pregnant goats. According to the author, age and sex have no influence on TLC value which contradicts the present observations. An age dependent impound in TLC value in fetal goat reflects the higher rate of granulopoiesis and thus preparing the animal to achieve immunocompetence by the time it is delivered and thus the kids can face the challenges put forth by the hostile environment.

Differential leucocyte count (DLC)

The mean DLC value on 50th day of gestation was 36.20% lymphocytes, 63.65% heterophils, 0% monocytes, 0.15% eosinophils and basophils. On 99th day 142nd day and in day old neonate the corresponding DLC values were 45.00, 52.55, 1.00 and 1.45%; 56.25, 38.50, 2.10, and 3.15% ; and 58.75, 35.50, 2.00 and 3.75% respectively (Table.1).. As per Coffin (1953) and Banks (1993) the DLC value in adult goat registers 36% neutrophils, 56% lymphocytes, 2.5% monocytes, 5% eosinophils and 0.5% basophils. The age related increase in the percentage of lymphocytes probably supports the view that numerous lymphocytes are required to enter into the primary lymphoid organ for differentiation into T-cell, B-cell or NK-cells which subsequently seed the secondary lymphoid organ. Thus the goat achieves specific and paraspecific immunity.

Absolute lymphocyte count (ALC)

The mean ALC values in day 50, 99, 142 old goat foeti and in day old neonates were 0.35×10^3 , 2.16×10^3 , 4.86×10^3 and $5.43 \times 10^3 / \mu\text{l}$ of blood respectively (Table.1).. Wagner (1976) revealed the absolute lymphocyte count to decrease sharply until 20 weeks of age in rats and falls slowly until 40 weeks of age, and after 70 weeks of age it rises slowly until death. The present findings could not be compared with literature because of paucity of information. The study however signifies an age dependent loading of lymphocytes in the peripheral blood and probably it steps ahead the development of humoral and cell mediated immunity in the developing fetus.

Absolute heterophil count (AHC)

The mean AHC values were 0.63×10^3 , 2.52×10^3 , 3.33×10^3 and $3.28 \times 10^3 / \mu\text{l}$ of blood in the 50, 99, and 142 day old goat foeti and in the day old neonates respectively (Table.1).. The AHC revealed a continuous increase upto the neonatal age. The heterophils perform a wide range of functions including peripheral blood phagocytosis. Thus increased heterophil count might suggest a age dependent readiness of foetal goat to phagocitised foreign particle soon the challenge posted by the hostile environment in the perinatal/postnatal age.

Absolute monocyte count (AMC)

Monocytes could not be traced in the peripheral blood of fetal goat upto the age of 50 days of gestation . However by day 87 a small fraction of monocytes ($0.01 \times 10^3 / \mu\text{l}$) was evident . Thereafter the rise in the AMC was slow and steady and attained to $0.18 \times 10^3 / \mu\text{l}$ of blood by day 142 of gestation and $0.19 \times 10^3 / \mu\text{l}$ of blood in the neonates (Table.1). Monocytes also contribute to the phagocytosis in the peripheral blood. Their progressively increasing appearance in the peripheral blood vouches attainment of cellular competence to engulf foreign particles in the perinatal kids.

Opsonin activated charcoal assay for the macrophage function

When the peripheral blood macrophages were stimulated by *E.coli* whole cell culture and were then subjected to carbon particles, no phagocytosis of these particles was observed until 112 days of gestation. On day 120, 130 and 142 of gestation and in day old neonates the macrophages recorded a count of 2.10%, 8.75%, 13.10% and 18.50% respectively (Table.1). Kumar (2001) recorded about 11.3% macrophages in the peripheral blood of chicks on the day of hatching. However the present finding contradicts the reports of Janse and Jeurisen (1991) who opined that macrophages start to absorb the antigens a few days after hatching in chicks. The present findings hypothesize that fetal goat during the perinatal age can handle the foreign particles through phagocytosis.

Table 1 Showing the total leucocyte count (TLC), differential leucocyte count (DLC), absolute leucocyte count (ALC) and Macrophage function test (MFT) in prenatal goat

| Sl. No. | Calculated Age (days) | TLC ($10^3 \mu\text{l}^{-1}$) | DLC : % | | | | ALC : $10^3 \mu\text{l}^{-1}$ | | | MFT (%) |
|---------|-----------------------|---------------------------------|------------------|------------------|---|-----------------|-------------------------------|-----------------|---|---------|
| | | | *L | H | M | E+B | L | H | M | |
| 1. | 50 | 0.99 ± 0.92 | 36.20 ± 1.27 | 63.65 ± 2.16 | - | 0.15 ± 0.01 | 0.35 ± 0.2 | 0.63 ± 0.11 | - | - |
| 2. | 62.5 | 1.03 ± 0.73 | 38.50 ± 1.00 | 61.25 ± 2.11 | - | 0.25 ± 0.02 | 0.39 ± 0.21 | 0.63 ± 0.08 | - | - |

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|-----|--------------------|---------------|----------------|----------------|---------------|---------------|---------------|---------------|---------------|-------|
| 3. | 73 | 1.20± 0.63 | 39.25± 1.05 | 60.20± 2.00 | 0.20± 0.01 | 0.35± 0.01 | 0.47± 0.37 | 0.72± 0.09 | - | - |
| 4. | 80 | 1.50± 0.58 | 40.90± 0.91 | 58.05± 1.91 | 0.3± 0.02 | 0.75± 0.06 | 0.61± 0.5 | 0.87± 0.05 | - | - |
| 5. | 87 | 2.10± 1.01 | 42.10± 0.89 | 56.20v 1.50 | 0.60± 0.02 | 1.10± 0.09 | 0.88± 0.09 | 1.18± 0.12 | 0.01± 0.01 | - |
| 6. | 94 | 3.05± 0.97 | 43.75± 1.10 | 54.30± 1.25 | 0.80± 0.03 | 1.15± 0.11 | 1.33± 0.31 | 1.65± 0.99 | 0.02± 0.01 | - |
| 7. | 99 | 4.80± 0.41 | 45.00± 1.15 | 52.55± 1.75 | 1.00± 0.09 | 1.45± 0.10 | 2.16± 0.45 | 2.52± 0.79 | 0.04± 0.01 | - |
| 8. | 106 | 5.21± 0.68 | 47.95± 2.76 | 49.05± 1.80 | 1.25± 0.10 | 1.75± 0.07 | 2.49± 0.68 | 2.55± 0.29 | 0.06± 0.01 | - |
| 9. | 112 | 6.37± 0.37 | 49.75± 2.11 | 46.50± 1.76 | 1.75± 0.2 | 2.00± 0.05 | 3.16± 0.79 | 2.96± 0.67 | 0.11± 0.01 | - |
| 10. | 120 | 7.05± 0.18 | 51.55± 0.75 | 44.80± 1.11 | 1.30± 0.30 | 2.35± 0.91 | 3.63± 0.91 | 3.15± 0.91 | 0.09± 0.01 | 2.10 |
| 11. | 130 | 7.95± 0.18 | 54.00± 0.19 | 41.15± 1.92 | 1.90± 0.11 | 2.95± 0.75 | 4.29± 0.92 | 3.27± 0.93 | 0.15± 0.01 | 8.75 |
| 12. | 142 | 8.65± 0.57 | 56.25± 1.01 | 38.50± 3.01 | 2.10± 0.97 | 3.15± 0.67 | 4.86± 0.79 | 3.33± 0.71 | 0.18± 0.11 | 13.10 |
| 13. | Day old neonate | 9.25± 0.90 | 58.75± 1.15 | 35.50± 1.75 | 2.00± 0.75 | 3.75± 0.56 | 5.43± 0.02 | 3.28± 0.90 | 0.19± 0.12 | 18.50 |

*L: lymphocyte, H: heterophil, M: monocyte, E: eosinophil, B: basophil

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