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Peripheral blood mononuclear cell viability in response to *in vitro* thermal stress in the Malabari goats of Kerala

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Abstract
Climate change poses a serious risk to livestock production in tropical regions. Among climate-induced stressors, heat stress is one of the most important factors affecting small ruminant production. Heat stress leads to cell death and reduces the number of peripheral blood mononuclear cells (PBMCs). The present study was conducted to investigate the response of PBMCs to heat stress in Malabari goats of Kerala. PBMCs were isolated from peripheral blood and cultured, then exposed *in vitro* to 42 °C for four hours, and their viability was assessed. The results showed a significant decrease in total PBMC count, viable PBMC count, and percentage viability under heat exposure. These findings demonstrated that PBMCs provide a simple and reliable *in vitro* model for assessing cellular responses to heat stress in indigenous goats.

Keywords: Malabari goats, thermal stress, peripheral blood mononuclear cells, *in vitro*, cell viability

1. Introduction
Climate change poses serious risks, including more frequent heat waves and other extreme weather events that affect both human and livestock populations, particularly in tropical regions (UNFCCC, 2014) [1]. The Intergovernmental Panel on Climate Change (IPCC) [2] defines climate change as long-term shifts in climate states resulting from natural processes or external drivers such as anthropogenic emissions and urbanisation. The IPCC Special Report (2018) [3] projects that global temperatures are likely to rise by 1.5 °C between 2030 and 2052 compared with pre-industrial levels.

In India, climate change has increased the risk of flooding and precipitation extremes. These changes adversely affect agriculture, leading to crop losses, reduced reservoir capacity, and depletion of water resources (Ali *et al*., 2019) [4]. The direct effects of climate change include physiological and performance-related responses to heat stress caused by rising ambient temperatures. Among climate-induced stressors, heat stress is one of the most significant environmental factors affecting small ruminant production in tropical and subtropical regions (Dige *et al*., 2024) [5]. Stress refers to environmental factors that disrupt homeostasis and trigger adaptive responses in animals (Mondal *et al*., 2020) [6]. The negative impacts of thermal stress on livestock have been well documented, with reductions in growth, reproduction, production, immune competence, and overall productivity (Silanikove & Koluman, 2015) [7].

Studies have shown that thermal stress causes cell death and a reduction in peripheral blood mononuclear cells (PBMCs) in several cattle breeds (Bhanuprakash *et al*., 2016; Bhattacharya *et al*., 2024; Onasanya *et al*., 2025) [8-10]. The impact of heat varies with temperature and duration, affecting cell viability, integrity, and proliferation. These changes impair immune responses, increase susceptibility to opportunistic infections, and reduce overall production performance (Onasanya *et al*., 2025) [10]. Circulating leukocytes, particularly PBMCs, provide a practical biological system for evaluating livestock responses to temperature variation, especially in tropical environments (Sheikh *et al*., 2017) [11].

PBMCs are widely used in cell culture models to study stressors, including thermal stress (Lacetera *et al*., 2006) [12]. Although cellular responses to heat stress can be assessed using both *in vivo* and *in vitro* models, PBMCs remain the most practical choice because of their accessibility, viability, and immune activity (Kim *et al*., 2020) [13].

With this background, the present study was conducted to assess the effect of *in vitro* thermal stress in PBMCs of Malabari goats.

2. Materials and Methods

2.1 Collection of blood samples

The study was conducted using six healthy, randomly selected female kids, each from Malabari goats, aged between 6 to 9 months, maintained at the University Goat and Sheep Farm, Mannuthy, under KVASU. The animals were managed under a semi-intensive system.

Blood was collected from a total of six Malabari goats. Blood was collected aseptically at the rate of 0.5 mL per kg body weight from the jugular vein of each animal into vials coated with sodium heparin as the anticoagulant (10 IU/mL blood). Samples were immediately brought to the laboratory and processed within one hour of collection. The PBMC isolation and culture procedures were carried out under aseptic conditions in a laminar airflow cabinet (Labline Instruments, Kochi).

2.2 Isolation of PBMCs

The whole blood sample was taken in a 15 mL centrifuge tube (Tarsons, Cat. No 500031) and centrifuged at 3000 rpm for 20 to 30 min at 4 °C. Then the supernatant plasma was discarded, and the buffy coat, along with a small volume of plasma and red blood cells (RBC), was carefully transferred into a new 15 mL centrifuge tube. Then, the buffy coat was diluted with an equal volume of 1X Dulbecco's Phosphate Buffered Saline (DPBS) (Sigma-Aldrich, Cat. No. D8537) and mixed gently. An equal volume of HiSep™ LSM 1077 (HiMedia, Cat. No. LS001) was taken in another 15 mL centrifuge tube. Then, the diluted buffy coat was slowly layered over it without disturbing the interface. Afterwards, these tubes were centrifuged at 2500 rpm for 30 min at 20 °C without brake. After centrifugation, the PBMC layer was visible at the interface between the plasma and HiSep™. This cell layer was aspirated carefully and diluted with an equal volume of 1X DPBS. Then, the cell suspension was centrifuged at 2500 rpm for 15 min at 20 °C. The resulting cell pellet was washed twice by centrifugation to remove residual red blood cells. The final white cell pellet was resuspended in one millilitre of RPMI 1640 culture medium.

The culture medium was prepared by supplementing RPMI 1640 (Sigma-Aldrich, Cat. No. R8758) with 10 percent heat-inactivated foetal bovine serum (Sigma-Aldrich, Cat. No. F7524) and 10 µL/mL of antibiotic-antimycotic solution (Sigma-Aldrich, Cat. No. A5955), containing 100 U of penicillin, 100 µg of streptomycin, and 0.25 µg of amphotericin B per mL.

2.3 Cell Counting, seeding, and culturing of PBMCs

Cell count and cell viability were estimated using the Invitrogen™ Countess™ Automated Cell Counter (Thermo Scientific, Cat. No. C10281) by the Trypan Blue exclusion method. For this, one microlitre of PBMC suspension was diluted with nine microlitres of 1X DPBS. To this, an equal volume of 0.4 percent Trypan Blue (Sigma-Aldrich, Cat. No. T8154) was added and mixed. Ten microlitres of the mixture was loaded onto the counting chamber of Invitrogen™ Countess™ automated cell counter slides, and the cell count and cell viability were recorded.

The volume of PBMC suspension required for seeding was calculated as below:-

- Concentration of viable cells in the suspension (cells/mL), as obtained from the automated cell counter = C_1 viable cells/mL (C_1)
- Desired concentration of viable cells for seeding = 1×10^6 viable cells/mL (C_2)
- Volume of PBMC suspension required for 1 ml of 1×10^6 viable cells/mL = C_1 / C_2 * 1000 µL (V_1)
- Volume of culture medium required to add to make 1ml of 1 $\times10^6$ viable cells/mL = 1000 – V_1 µL (V2)

Therefore, V_1 µL of PBMC suspension was added to V_2 µL of the RPMI 1640 culture medium to obtain a final volume of one microlitre at a concentration of 1 × 10^6 viable cells/mL, which was then used for cell seeding.

The cells were seeded in duplicate for each animal in sterile 6-well plates and incubated at 37 °C for 24 h in a CO_2 incubator (Thermo Scientific) set at five percent CO_2 and 95 percent relative humidity. These cultured cells were used for heat stress treatment.

2.4 Heat stress treatment of the PBMCs

After 24 h of culturing at 37 °C, the PBMC culture from each animal was divided into two sets. One set was maintained at 37 °C for four hours (control) in the CO_2 incubator, while the other was subjected to heat stress at 42 °C for four hours (heat stress treatment-HS treatment) by incubating in another CO_2 incubator. All incubators were set at five percent CO_2 and 95 percent relative humidity (Mondal *et al.*, 2020).

Following heat treatment, both sets of PBMC cultures were removed from the CO_2 incubators. The cell viability was measured using the Trypan Blue dye exclusion method using the Invitrogen™ Countess™ Automated cell counter (Thermo Scientific, Cat. No. C10281).

3. Results and Discussion

The cell count and viability of the PBMCs were estimated after 10-fold dilution in RPMI-1640 culture medium using the Invitrogen™ Countess™ Automated mammalian cell counter with the Trypan Blue exclusion method, and the data are presented in Table 1.

Table 1: Concentration and viability of PBMCs after 10-fold serial dilution in RPMI-1640 culture medium

Sample ID	Total cells/ml	Viable cells/ml	Viability (%)
M1	2.0×10^7	1.9×10^7	95
M2	7.5×10^6	5.9×10^6	78
M3	1.0×10^7	9.5×10^6	95
M4	1.6×10^7	8.3×10^6	52
M5	1.1×10^7	7.0×10^6	64
M6	2.6×10^7	2.5×10^7	96

After 24 h of incubation at 37 °C, PBMCs were divided into two sets. The control and treatment groups were incubated at 37 °C and 42 °C, respectively, for four hours, with both groups maintained at 5 per cent CO_2. The stress temperatures and duration were based on the findings of Mondal *et al.* (2020) [6].

Heat stress treatment at 42 °C for four hours significantly decreased the total and viable PBMC counts of the HS treatment group compared to the control ($p<0.01$). Likewise, the viability percent of the PBMCs also significantly decreased ($p<0.01$) after the HS treatment. The mean viability in the control group was 70.67 percent in Malabari goats. After the heat treatment, the viability decreased to 47 percent. The concentration and viability of PBMCs after four hours of incubation at 37 °C (control) and 42 °C (treatment) are presented in Tables 2 and 3, respectively.

Table 2: Concentration and viability of PBMCs after four hours of incubation at 37 °C (Control)

Sample ID	Total cells/ml	Viable cells/ml	Viability (%)
MC1	1.2×10^6	8.2×10^5	68
MC2	4.0×10^6	2.8×10^6	70
MC3	1.3×10^6	9.8×10^5	75
MC4	1.7×10^6	1×10^6	59
MC5	1.0×10^6	6.9×10^5	69
MC6	8.1×10^5	6.7×10^5	83

MC: Malabari control

Table 3: Concentration and viability of PBMCs after four hours of incubation at 42 °C (HS treatment)

Sample ID	Total cells/ml	Viable cells/ml	Viability (%)
MT1	7.3×10^5	4.0×10^5	55
MT2	7.0×10^5	2.4×10^5	34
MT3	1.1×10^5	4.4×10^4	40
MT4	5.3×10^5	2.8×10^5	53
MT5	1.8×10^5	8.0×10^4	44
MT6	4.0×10^5	2.2×10^5	56

HS: Heat stress; MT: Malabari HS treatment.

Following heat treatment at 42 °C for four hours, total PBMC count, viable count, and percentage viability were decreased significantly compared with respective to control group (37 °C). Similar reduction in the PBMC viability under acute hyperthermia have been reported in several studies. Heat treatment at comparable temperatures affected PBMC morphology (WS Kim, *et al*., 2020) [13], decreased cell viability (Bhanuprakash *et al*., 2016 [8]; Bhattacharya *et al*., 2024 [9]; Kishore *et al*., 2014 [14]; Onasanya *et al*., 2025 [10]), and inhibited PBMC proliferation. (Kim *et al*., 2020) [13]. This was mainly due to cell death as a result of heat stress, which in turn affected cell proliferation (WS Kim *et al*., 2020) [13]. Moreover, heat stress also resulted in decreased DNA and protein synthesis at 42 °C in bovine PBMCs (Lacetera *et al*., 2006) [12]. Similarly, Bhattacharya *et al*. (2024) [9] reported that CBHF showed a greater decline in percentage after heat stress, compared to the native Vechur breed. Breeds that show higher tolerance to heat at the cellular level are generally considered better adapted to hot environments (Lacetera *et al*., 2006) [12].

6. Conclusion

The present study demonstrated that *in vitro* heat treatment at 42 °C, significantly altered the total and viable PBMC count, as well as the viability percent in Malabari goats. Nonetheless, studies involving different goat breeds and their cellular responses to thermal stress are scarce. Thus, similar to previous reports, this study also demonstrated that PBMCs are a suitable *in vitro* model for evaluating cellular responses to thermal stress in livestock. This can be further extended to investigations at cellular and molecular levels, including gene-expression and pathway analyses associated with thermal-stress response.

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9. Conflict of Interest

The authors have no conflict of interest to declare

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