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Exploring the intricate relationship: maternal parity's effect on hematological parameters in lamb offspring

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Abstract

The influence of ewe parity on maternal and lamb health has long been recognized in the domain of animal and veterinary sciences. Understanding the intricate interplay between these factors, which directly or indirectly impact maternal and offspring well-being, is imperative for optimizing the health of both the mother and the offspring. In this context a study was designed that aimed to investigate the variations in hematological parameters among lambs born to ewes of different parities. Twelve healthy pregnant ewes were selected and categorized into two groups based on parity: Primiparous (PP) and Multiparous (MP). Subsequently, hematological parameters of the lambs born to these ewes were analyzed at various time points, on Day 0 (birth), 7, 14, 28, 60, and 90 post-birth. The parameters under investigation included Absolute Lymphocyte Count (ALC), Absolute Eosinophil Count (AEC), Absolute Neutrophil Count (ANC), Absolute Monocyte Count (AMC), Total Leukocyte Count (TLC), Total Erythrocytic Count (TEC), and Hemoglobin (Hb). Significant disparities in the absolute counts of lymphocytes, neutrophils and eosinophils were observed between the two parity groups (p -value < 0.05), with higher levels noted in the primiparous group lambs. Additionally, a significant time * group interaction was identified for all these absolute counts (p -value < 0.05). Notably, Total Leukocyte Count (TLC) exhibited a significant difference between the two groups (p -value < 0.05), with markedly higher levels in the primiparous group lambs. A significant time * group interaction was evident for TLC in both groups ($p < 0.05$). Conversely, Absolute Monocyte Count (AMC), Total Erythrocyte Count (TEC) and Hemoglobin levels (Hb) displayed no significant differences between the two groups (p -value > 0.05), and no time * group interaction was observed. These findings underscore the hematological distinctions resulting from variations in maternal parity, shedding light on the intricate dynamics influencing the health of lambs born to primiparous ewes.

Keywords: Parity, primiparous, multiparous, ewes, lambs, hematology

Introduction

Developmental / fetal programming, a concept that revolves around the fact that exposure to any factor/environment during the pre-natal life affects the growth and development of the fetus, which in turn manifests changes in the physiological system including the metabolic functioning of the fetus that can affect its life during adulthood (Wu *et al.*, 2006) [1], the most important of them being the maternal factors as they constitute the immediate most environment of the fetus. Comprehending those maternal factors that impact the lamb in any manner is essential for the newborn's survival as well as its long-term well-being (Godfrey and Barker, 2001) [2] as they affect the fetal development, its physiological metabolisms and regulations, the disease incidence and in turn the overall wellbeing of the new born (Barker, 2007; Cottrell and Ozanne, 2008) [3-4]. Human and animal studies have given insight into the impact that maternal factors like nutrition, body condition score etc. have on the physiological programming of fetus during pre-natal life as the process of fetal development is highly plastic (Cottrell and Ozanne, 2008; Symonds *et al.*, 2009; Paliy *et al.*, 2014) [4-6]. Prenatal programming because of maternal factors substantially impacts the growth, development, and productive performance of lamb (Gluckman and Hanson, 2004; Gardener *et al.*, 2007) [7-8].

Parity, an important maternal characteristic has also been known to affect a lamb's capacity to cater to its metabolic demands and also affects its growth and development. The impact of parity on lamb health can be multifaceted as evidenced by various studies that have revealed that in primiparous ewes, the ewe and fetus compete for the resources required for the development of the maternal body, and in multiparous ewes, for the purpose of lactation which in turn will alter the amount of nutrition available for the fetal growth as well as maternal and lamb health.

Also, primiparous ewes and multiparous ewes also have different body condition scores which may initiate alterations in lamb health (Gluckman and Hanson, 2004) [7]. Studies from dairy cows also support the fact that parity affects the health and wellbeing of offspring. Also, the fact that younger (1st/2nd parity) cows give birth to light weight calves has been well accepted (Cushman *et al.*, 2020) [9]. It has been also seen that cows of first parity (primiparous cows) produce low-quality colostrum (Morin *et al.*, 2001; Shivley *et al.*, 2018) [10-11], and the offsprings that receive this colostrum have a high incidence of respiratory as well as digestive issues (Svensson *et al.*, 2003) [12]. Also, studies in cattle reveal that as maternal age and parity increases, cows encounter various diseases and environmental stresses which leads to change their physiological functioning and productivity, and these affect the growing fetus in their postnatal health (Collier *et al.*, 2006; Gao *et al.*, 2012; Ling, *et al.*, 2018; Evans *et al.*, 2012) [13-16].

The estimation of hematological parameters of new born lambs provide a valuable insight into their overall health and wellbeing as the blood composition is affected by various factors including maternal health, nutrition, body weight etc. during the entire gestational period. Also, any change in these parameters essentially indicate any derangement in the physiological status of the animal. Hence, the present study was designed and aimed to evaluate the effect of maternal parity on the hematological parameters of new born lambs.

Materials and Methods

Experimental animals

The study was conducted from February to June, 2019 at Mountain Research Centre for Sheep and Goat (MRCSG), SKUAST-Kashmir. Twelve healthy pregnant ewes were selected and housed separately 4 weeks before the expected date of lambing. All the selected ewes were stall fed and provided daily ration to meet their maintenance requirements and the demands of growing fetus. Water was provided ad lib to all the ewes.

Experimental design

4 weeks prior to lambing 12 healthy pregnant ewes were selected and on the basis of parity were divided into two groups of 6 ewes each.

Group-I: 6 Primiparous ewes

Group-II: 6 Multiparous ewes

Blood Sampling

Blood was collected from jugular vein from the lambs born to these ewes on the day of birth, 7, 14 and 28 days after birth and then on monthly basis up to weaning for estimation of different hematological parameters.

Parameters analyzed monthly include:

- Differential Leukocyte Count (Absolute Neutrophil Count, Absolute Lymphocyte Count, Absolute Eosinophil Count, Absolute Monocyte Count)
- Total Leukocyte Count
- Total Erythrocyte Count
- Hemoglobin

Statistical Analysis

The data was checked for normalcy and was found to be normally distributed. Two-way analysis of variance for repeated measures was applied to assess the statistically

significant parameters between the two groups and effect of the sampling time on the studied parameters.

Results

Absolute Lymphocyte Count (ALC)

The Absolute Lymphocyte Count (ALC) showed a significant change along the time (p-value= 0.01) in both the groups (Table 1 and Figure 1) with significantly higher count seen at day 28 in case of lambs born to primiparous ewes and at day 90 after lambing in case of lambs of multiparous ewes. Significant group differences were observed between the lambs of primiparous and multiparous group with significantly higher count in the lambs of primiparous group. Also, the time X group interaction revealed significantly higher count in the primiparous group (p-value= 0.003) at Day 60.

Absolute Eosinophil Count (AEC)

Along the time, the Absolute Eosinophil Count (AEC) in both the groups showed a significant variation (p= 0.028) (Table 1 and Figure 2) with significantly lower count seen in lambs of primiparous group at Day 90 and in multiparous group lambs at Day 0. Significant group differences were observed with significantly higher count seen in the primiparous group lambs (p= 0.013). Time X Group interaction revealed significantly lower eosinophil count in lambs of multiparous group at Day 0 and Day 60 (p-value= 0.01).

Absolute Neutrophil Count (ANC)

Significant changes were seen along the time in the absolute neutrophil count of both the groups with significantly higher counts observed at Day 28 in case of lambs of primiparous group and Day 7 in case of the multiparous ones (Table 1 and Figure 3). There was a significant group difference with higher count seen in case of lambs of primiparous group ewes (p-value= 0.002). A significant Time X Group interaction was observed with significantly higher count seen in primiparous ewes at Day 0, 14, 28 and 60 (p-value= 0.00).

Absolute Monocyte Count (AMC)

The Absolute Monocyte Count showed no significant difference along the time in both the groups (p-value= 0.06) (Table 1 and Figure 4). No significant group difference (p-value= 0.123) was observed. Also, there was no significant time X group interaction (p-value= 0.283).

Total Leukocyte Count (TLC)

A significant difference was seen along the time in the Total Leukocyte Count (TLC) in both the groups with significantly higher counts seen at Day 28 in primiparous group and at Day 7 in case of multiparous group (p-value= 0.008) (Table 1 and Figure 5). Significant group differences were seen (p-value= 0.001) with significantly higher counts seen in primiparous group. A significant time X group interaction was also seen with significantly higher count at Day 0, 28 and 60 (p-value= 0.040) in lambs of primiparous group.

Total erythrocyte count (TEC)

No significant difference was seen in the total erythrocytic count (TEC) along the time in any of the two groups (p-value= 0.847) (Table 1 and Figure 6). There were no significant group differences (p-value= 0.082). Also, no significant time X group interaction was seen (p-value= 0.304).

Hemoglobin levels (Hb)

Hemoglobin (Hb) levels showed no significant difference along the time between the two groups (p-value= 0.460) (Table 1 and Figure 7). No significant group differences were seen (p-value= 0.960). Also, there was no significant time x group interaction (p-value= 0.695).

Discussions

Development of fetus is affected by factors encompassing genetic and maternal ones and these affect the development individually as well as by interacting with each other. Physiological and nutritional status of the mother exerts a significant effect on the fetal growth and development as supported by the fact that the physiological status of the mother accounts for almost 30% of the variability seen in the body weight of the newborn (Johnston *et al.*, 2002) [17]. The maternal environment/factor affects the lamb health intensely as it constitutes the immediate most environment of the growing and developing fetus. Factors like maternal age, rank of pregnancy, nutritional and health status of the ewe play an important role in the physiological development of the fetus. Other than these another important maternal factor that affects the lamb health, viability and physiological wellbeing is parity as ewes of different parities have differences in many aspects so they can affect the fetal physiology accordingly as evidenced by the fact that the primiparous ewes in comparison to the multiparous ewes have to face the challenge of sustaining their own growth and development in addition to fulfilling the nutritional demands of the growing fetus (Gardener *et al.*, 2007) [8] which can pose a nutritional threat to the growing fetus. It is well known that multiparous ewes are better able to fulfill the nutritional demands of the fetus in comparison to the primiparous ones. Also, the susceptibility to stress during the periparturient period also varies with the varying parity (Wathes *et al.*, 2007, Morales Piñeyría *et al.*, 2018) [18-19]. Hence, the primiparous ewes face a greater metabolic challenge in comparison to the multiparous ones and thus can affect the neonatal health (Taylor *et al.*, 2003) [20]. Since assessment of hematological parameters in veterinary medicine is an essential tool for judging the physiological changes and evaluating the health, nutritional and metabolic status of the animals, a study was designed to evaluate the effect of maternal parity on the hematological parameters of the newborn lambs (Gupta *et al.*, 2007) [21].

The Absolute Lymphocytic count (ALC) exhibited an ascending trajectory from the day of birth (Day 0) through the entire period (Day 90). This pattern aligns with the established understanding in ruminants, where the leukocytic profile undergoes a predominant shift towards the lymphocytic side as the animals age, reflecting heightened demand and production of these cells with advancing age (Jones and Allison, 2007) [22].

Moreover, the ALC was notably higher in lambs from the primiparous group compared to their multiparous counterparts. This discrepancy in lymphocytic count (recognized as an indicator of stress/infection), may be attributed to the increased susceptibility to stress or infections in lambs born to primiparous ewes. As is already known, the primiparous ruminants have been observed to produce colostrum of lower quality than multiparous ones (Cappel *et al.*, 1998; Morin *et al.*, 2001; Svensson *et al.*, 2003; Shivley *et al.*, 2018) [23, 10-12]. Additionally, IgG levels in lambs born to primiparous ewes tend to be insignificantly lower than those born to multiparous ewes (Kaymaz *et al.*, 2000; Khan *et al.*, 2006; Brujeni *et al.*, 2010; Turquino *et al.*, 2011) [24-27] which

is consistent with reports of primiparous ewes having lower volumes, concentrations, or quality (lower IgG levels) of colostrum and displaying poorer maternal ability compared to mature ewes (Mellor and Stafford, 2004; Dwyer *et al.*, 2005; Waldner and, Rosengren, 2009) [28-30]. Consequently, the heightened susceptibility to infections in lambs born to primiparous ewes is plausible. Furthermore, the elevated Absolute Lymphocytic Count (ALC) in lambs from primiparous ewes, signifying potential susceptibility to stress or infections, warrants deeper exploration into the underlying stress response mechanisms during the neonatal period.

The Absolute Eosinophilic Count (AEC) exhibited a variable trend in both groups over time, with significantly elevated levels observed at Day 0 and Day 60 in lambs born to primiparous ewes. Furthermore, the overall group difference revealed consistently higher eosinophil levels in lambs from the primiparous group. Eosinophils, recognized as key players in defense against parasitic infections (such as helminthic), allergic diseases, and the modulation of inflammatory responses in hypersensitivity reactions (Young and Meadows, 2010) [31], are produced in response to these mechanisms. Notably, a robust correlation exists between the rise in eosinophil counts and age progression during the initial growth phase. Therefore, the heightened eosinophilic count observed in lambs born to primiparous ewes may be attributed to their increased susceptibility to parasitic infections. This underscores the importance of considering maternal factors, such as parity, in understanding variations in immune response parameters among neonates.

The Absolute Neutrophil Count (ANC) exhibited a consistently higher level in lambs from the primiparous group across various sampling times. Neutrophils play a crucial role in guiding and supporting both innate and adaptive immune responses (Mantovani *et al.*, 2011) [32]. These cells release proinflammatory mediators that amplify the inflammatory process, indicating a significant role in modulating immune responses (Bowdridge *et al.*, 2015; Weiss & Wardrop, 2010) [33-34], and serve as indicators of an effective host response against infections (Ortolani *et al.*, 2013) [35]. Both neutrophils and lymphocytes are potential indicators of stress or infection (Cappel *et al.*, 1998) [23]. The elevated levels in lambs born to primiparous ewes suggest increased stress susceptibility and potential for infections compared to those born to multiparous ewes. This may lead to heightened adrenal gland activity, resulting in increased production of corticosteroids, promoting neutrophil endothelial demargination and subsequently elevating the neutrophil count (Byers and Kramer, 2010; Fontequé *et al.*, 2013) [36-37].

In the present study significantly higher Total leukocyte count (TLC) was seen in case of the primiparous group lambs. In both the groups, there was a variation that was seen in the total leukocyte count with advance in age. This reflects the adaptability process of the hematopoietic system of the growing lamb to the extra uterine life, which, with the advance in age, brings the total leukocyte values of young animals closer to the ideal levels (Jain, 1993) [38]. Stressors, e.g., cold stress, Infections, poor nutritional status might activate the hypothalamic-pituitary-adrenal axis, resulting in increased cortisol levels (Dantzer and Mormède 1983) [39]. The stress hormones, particularly cortisol and adrenaline, enhance the increase in leukocyte count (Cupps and Fauci 1982) [40] which might possibly explain the comparatively higher levels in case of primiparous group lambs.

The higher levels of Absolute Neutrophil Count (ANC) and

Total Leukocyte Count (TLC) in lambs from primiparous ewes suggest a potentially heightened innate immune response and adaptability to the challenges of the extrauterine environment. This finding underscores the intricate interplay between maternal factors, such as colostrum quality, and the development of the lamb's immune system.

The Total erythrocytic counts and hemoglobin levels did not show any variation along the time or between the groups but the values in both the groups were in accordance with Saddiqi *et al.* (2011) [41]. Azab and Abdel-Maksoud (1999) [42] reported significant differences in Hb level and a decrease in the TEC during the early neonatal period which is completely

in contrast to our study.

The results presented in this study contribute to the broader field of maternal-fetal interactions in livestock and highlight the importance of considering ewe parity as a determinant of offspring hematological profiles. As sheep farming practices evolve, integrating this knowledge into management strategies can optimize lamb health outcomes and contribute to the overall success of the flock. Further research is encouraged to delve deeper into the mechanisms shaping these hematological variations and to explore potential interventions that may enhance the resilience and well-being of lambs born to ewes with varying reproductive histories.

Table 1: Blood hematology at different stages after lambing in lambs born to primiparous and multiparous ewes

Parameter	Time	Primiparous group lambs	Multiparous group lambs	T	G	TXG
Absolute lymphocyte count (10 ³ cells/μL)	DAY 0	2.60±0.46 ^A	1.49±0.36 ^a	0.01	0.01	0.003
	DAY 7	5.70±1.01 ^B	3.37±0.69 ^{ab}			
	DAY 14	3.78±1.27 ^{AB}	3.75±0.64 ^{bc}			
	DAY 28	5.85±0.51 ^B	4.48±0.75 ^{bc}			
	DAY 60	5.36±0.28 ^{B*}	2.77±0.22 ^{ab}			
	DAY 90	5.77±0.32 ^B	4.89±0.43 ^c			
Absolute eosinophil count (10 ³ cells/μL)	DAY 0	.39±0.08 ^{AB*}	.05±0.03 ^a	0.028	0.013	0.01
	DAY 7	.74±0.27 ^{AB}	.35±0.09 ^{bc}			
	DAY 14	.55±.10 ^{AB}	.28±.10 ^{abc}			
	DAY 28	.61±.18 ^{AB}	.30±.09 ^{abc}			
	DAY 60	.39±.03 ^{AB*}	.07±.00 ^{abc}			
	DAY 90	.22±.02 ^B	.20±.00 ^c			
Absolute neutrophil count (10 ³ cells/μL)	DAY 0	2.40±0.38 ^{A*}	1.16±0.71 ^a	0.00	0.002	0.00
	DAY 7	4.51±0.95 ^{ABC}	5.03±1.50 ^b			
	DAY 14	4.56±0.75 ^{BC*}	1.47±0.19 ^a			
	DAY 28	5.43±0.43 ^{C*}	1.52±0.23 ^a			
	DAY 60	3.08±0.24 ^{AB*}	1.85±0.32 ^a			
	DAY 90	2.61±0.20 ^A	2.12±0.40 ^a			
Absolute monocyte count (10 ³ cells/μL)	DAY 0	.12±0.03 ^A	.05±0.03 ^a	0.06	0.123	0.283
	DAY 7	.41±0.19 ^A	0.02±0.02 ^a			
	DAY 14	0.22±0.06 ^A	0.08±0.07 ^a			
	DAY 28	0.26±0.11 ^A	0.19±0.06 ^a			
	DAY 60	0.06±0.05 ^A	0.02±0.02 ^a			
	DAY 90	0.06±0.03 ^A	0.08±0.03 ^a			
Total leukocyte count (TLC) (* 10 ³ /μl)	DAY 0	5.43±0.79 ^{A*}	2.82±0.56 ^a	0.008	0.001	0.040
	DAY 7	11.18±1.49 ^{BC}	8.20±1.95 ^{ab}			
	DAY 14	8.86±1.42 ^{AB}	6.00±.95 ^{ab}			
	DAY 28	12.86±1.25 ^{CD*}	6.20±.51 ^b			
	DAY 60	8.74±.74 ^{C*}	4.16±.30 ^{ab}			
	DAY 90	8.56±.50 ^{BD}	7.20±.74 ^b			
Total erythrocyte count (*10 ⁶ /μl)	DAY 0	10.50±0.38 ^A	10.00±0.18 ^a	0.847	0.082	0.304
	DAY 7	10.47±0.59 ^A	9.35±0.36 ^a			
	DAY 14	9.73±0.30 ^A	9.26±0.46 ^a			
	DAY 28	11.42±0.43 ^A	10.57±0.38 ^a			
	DAY 60	10.54±0.95 ^A	9.75±2.06 ^a			
	DAY 90	10.24±0.16 ^A	10.25±0.63 ^a			
Haemoglobin (g/dl)	DAY 0	12.78±0.51 ^A	12.77±0.30 ^a	0.460	0.960	0.695
	DAY 7	12.32±0.79 ^A	11.35±0.40 ^a			
	DAY 14	10.70±0.42 ^A	10.57±0.25 ^a			
	DAY 28	10.46±0.49 ^A	11.73±0.48 ^a			
	DAY 60	10.34±0.44 ^A	10.00±1.25 ^a			
	DAY 90	10.36±0.52 ^A	10.72±0.89 ^a			

Values with at least one common superscript (PP: A,B; MP: a,b) do not differ significantly (P>0.05) between the different stages within both groups.

* Significant (P<0.05) differences between lambs of Primiparous and Multiparous group.

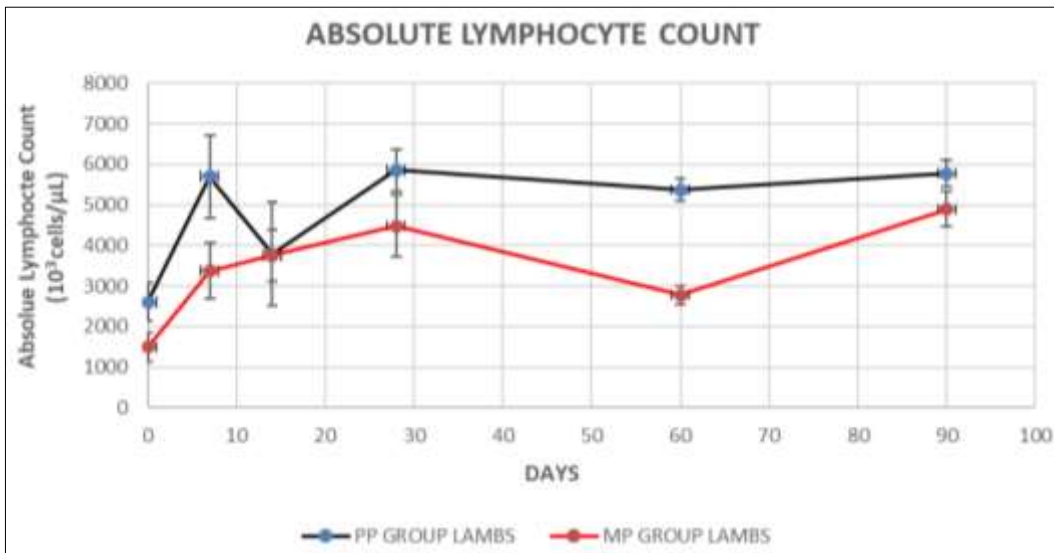


Fig 1: Mean (±SE) Absolute Lymphocyte Count (ALC) in lambs born to primiparous and multiparous ewes

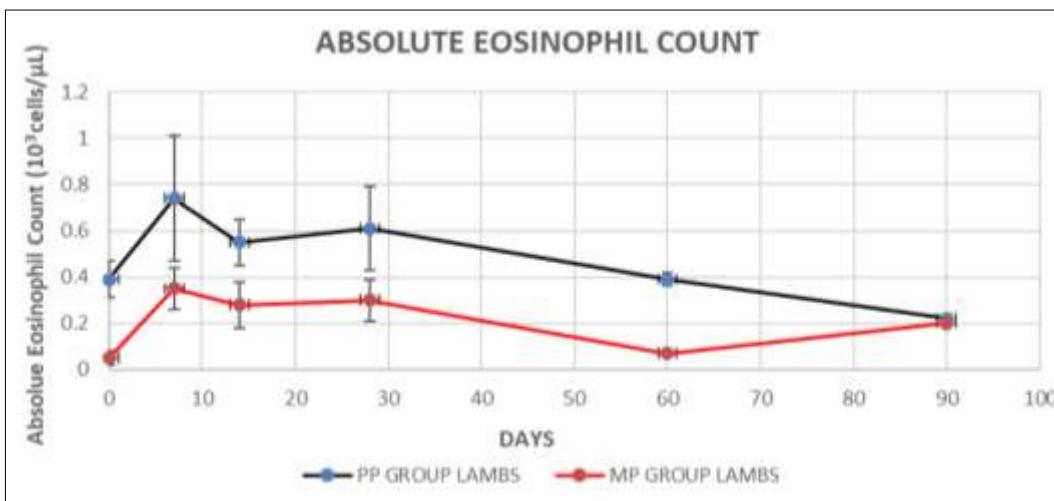


Fig 2: Mean (±SE) Absolute Eosinophil Count (AEC) in lambs born to primiparous and multiparous ewes

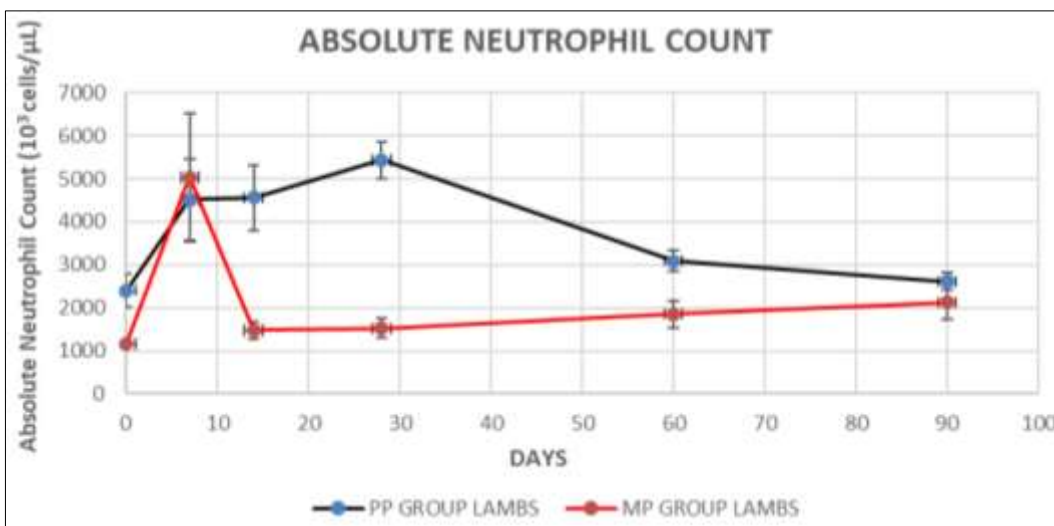


Fig 3: Mean (±SE) Absolute Neutrophil Count (ANC) in lambs born to primiparous and multiparous ewes

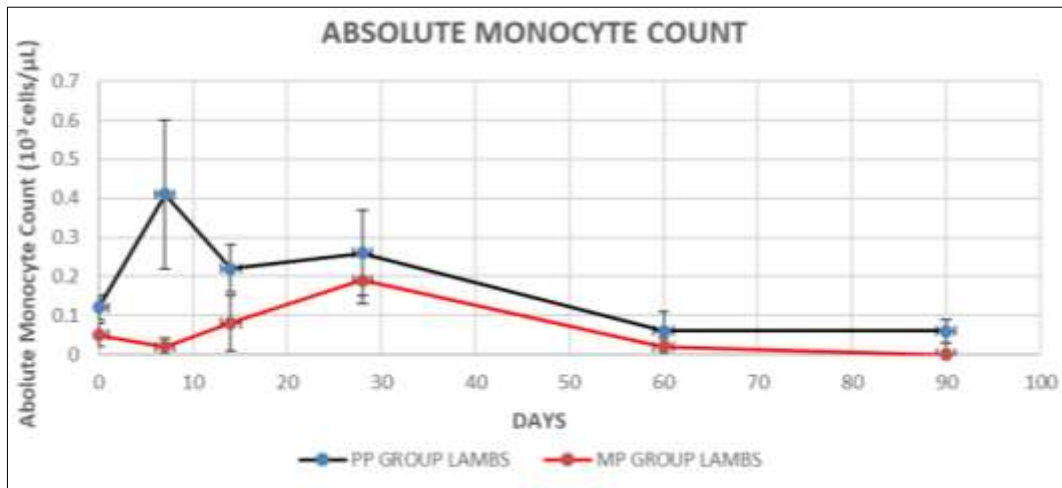


Fig 4: Mean (\pm SE) Absolute Monocyte Count (AMC) in lambs born to primiparous and multiparous ewes

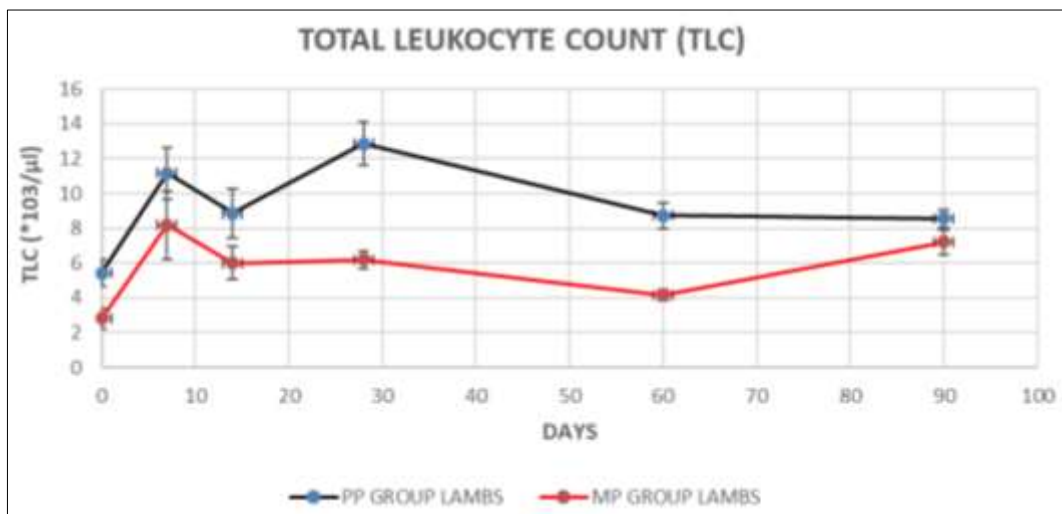


Fig 5: Mean (\pm SE) Total Leukocyte Count (TLC) in lambs born to primiparous and multiparous ewes

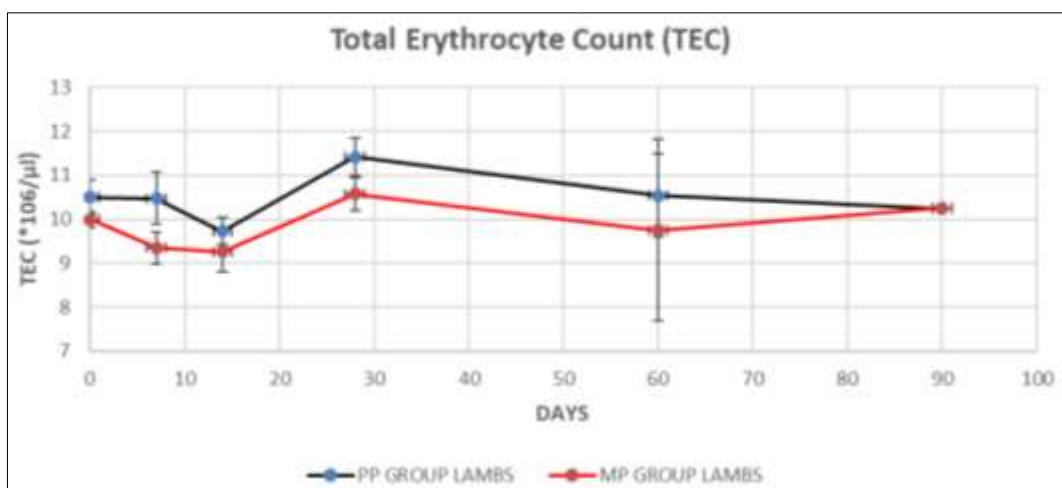


Fig 6: Mean (\pm SE) Total Erythrocyte Count (TLC) in lambs born to primiparous and multiparous ewes

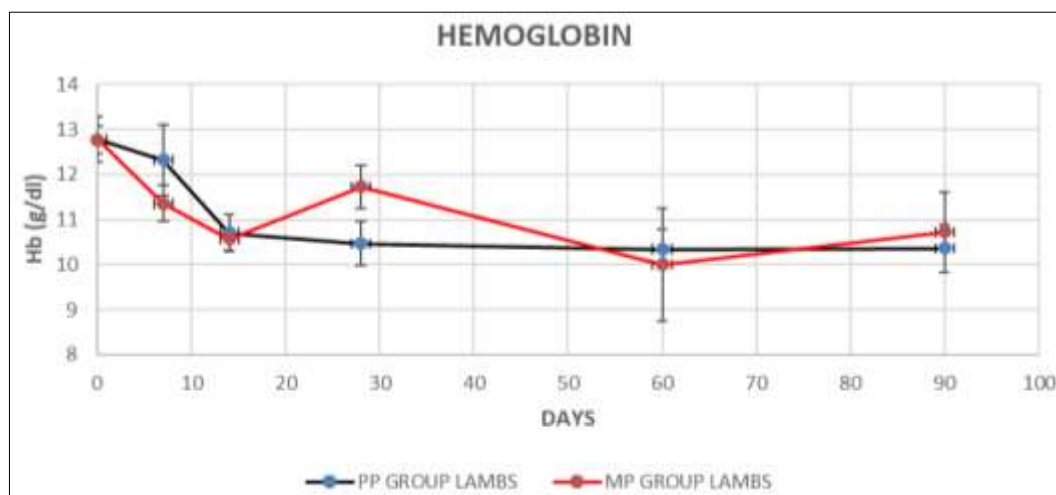


Fig 7: Mean (\pm SE) Hemoglobin (Hb) in lambs born to primiparous and multiparous ewes

Conclusion

Ewe parity emerges as a significant factor influencing the hematological profile of lambs. These findings underscore the importance of considering maternal reproductive history when assessing the health and immune status of newborn lambs. Further research is warranted to delve deeper into the underlying mechanisms and long-term implications of ewe parity on lamb hematological parameters. This study thus contributes to our understanding of how maternal factors shape the hematological landscape of offspring, providing valuable insights for optimizing lamb health and management practices in sheep farming.

Conflict of Interest

Authors declare that there is no conflict of interest.

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