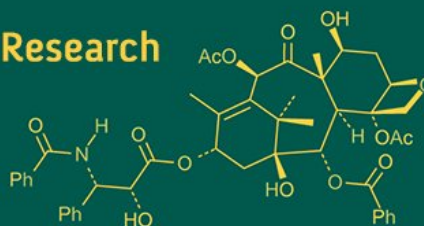


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Milk differential leukocyte counts in relation to somatic cell count in Alpine × Beetal and Saanen × Beetal crossbred does

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Abstract

Somatic cell count (SCC) is the main screening tool for udder health in dairy ruminants but is difficult to interpret in goats, whose milk carries a high somatic cell load from apocrine secretion and in which neutrophils, not macrophages, dominate even in health. No reference data describe the caprine leukocyte profile across the normal SCC range in Indian crossbred goats. The milk differential leukocyte count was measured across six SCC groups in 72 clinically healthy crossbred does with no mastitis history (36 Alpine × Beetal, AB; 36 Saanen × Beetal, SB; n = 6 per group). Neutrophil percentage rose steadily with SCC in both crosses (AB, $52.00 \pm 0.82\%$ to $72.50 \pm 0.76\%$; SB, $54.33 \pm 0.49\%$ to $75.33 \pm 0.49\%$; linear trend $P < 0.001$), while macrophages fell reciprocally. The neutrophil-to-macrophage (N:M) ratio rose by 155.7% (AB) and 188.0% (SB), the largest change observed. Lymphocytes declined in both crosses ($P \leq 0.003$). Two-way analysis of variance showed main effects of cross and SCC group, but no cross × SCC interaction for neutrophils, N:M or lymphocytes ($P \geq 0.055$); the crosses differ in baseline composition but respond to rising SCC in parallel. No bacteriology was performed, and the SCC range studied (<400 to $>1200 \times 10^3$ cells/mL) lies within that reported for healthy goats. These are physiological reference data, not evidence of subclinical mastitis. They provide baseline values for two Indian crossbred populations and show the N:M ratio varies systematically within the normal range.

Keywords: goat milk; somatic cell count; differential leukocyte count; neutrophil-to-macrophage ratio; caprine physiology; reference values

1. Introduction

Somatic cell count (SCC) is the standard indirect measure of udder health in dairy ruminants. It underpins milk payment schemes and udder health programmes worldwide (Contreras *et al.*, 2007; Podhorecka *et al.*, 2021) [4, 17]. Moving it from cattle to goats is not simple, and the reason is physiological rather than numerical. Goats secrete milk by an apocrine process. This sheds cytoplasmic particles and cell fragments that contain DNA, and automated counters read these as somatic cells. Apparent SCC therefore rises without any inflammation (Paape and Capuco, 1997; Kaskous *et al.*, 2023) [9, 14]. As a result, normal SCC in goat milk averages several hundred thousand cells per millilitre and can reach 1.5×10^6 cells/mL in clearly healthy animals. Most of the variation comes from lactation stage, parity, oestrus and season rather than from infection (Haenlein, 2002; Luengo *et al.*, 2004; Jimenez-Granado *et al.*, 2014; Smistad *et al.*, 2021) [7, 8, 11, 21]. The SCC threshold for detecting intramammary infection in goats lies near 1.5×10^6 cells/mL (Koop *et al.*, 2011) [10], about ten times the usual cattle figure. The cells themselves also differ. In a healthy cow quarter, macrophages are the main cell type, and neutrophils rise sharply only when infection occurs. In the goat, neutrophils are already the main cell type in milk from healthy, uninfected udders. They make up 40–80% of the somatic cell population, and they stay dominant in infected udders as well (Sierra *et al.*, 1999; Paape *et al.*, 2001; Bagnicka *et al.*, 2011; Kaskous *et al.*, 2023) [2, 9, 19]. The cattle rule macrophages mean health, neutrophils mean disease therefore does not hold in goats. Any diagnostic use of differential cytology in this species must start from goat baselines.

Differential leukocyte counting (DLC) has been proposed as a way to get more from the goat somatic cell population than the total count gives (Souza *et al.*, 2012; Alhussien and Dang,

2018; Kaskous *et al.*, 2023) ^[1, 9]. It divides the cell pool instead of only measuring its size, so it should be less affected by the particle problem. The approach has precedent in small ruminants: Cuccuru *et al.* (1997) ^[5] related differential cell counts to total counts in Sardinian ewes. Its interpretation, however, requires knowledge of how the leukocyte profile behaves across the normal SCC range in the population concerned. Such reference goatsarce for goats in general, and for Indian crossbred dairy goats they do not appear to exist at all. Alpine $\times$ Beetal and Saanen $\times$ Beetal are the main exotic $\times$ indigenous crosses bred for milk production in India, and no published work describes their milk leukocyte composition.

The milk DLC was therefore measured across six SCC groups in clinically healthy does of both crosses under uniform management. The objectives were threefold: to characterise how the leukocyte profile changes across the normal SCC range; to determine whether the neutrophil-to-macrophage (N:M) ratio tracks that change more clearly than any single cell type; and to test whether the two crosses differ in their baseline composition, in their response to rising SCC, or in both.

2. Materials and methods

2.1. Ethics statement

All procedures were approved by the Institutional Animal Ethics Committee of ICAR–National Dairy Research Institute, Karnal, under the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India (approval no. 1705/GO/Re/L/13/CPCSEA, dated 23 July 2021).

2.2. Study site

The study was conducted at the Livestock Research Centre, ICAR–National Dairy Research Institute, Karnal, Haryana, India (29°43'14.4" N, 76°58'55.2" E; 250 m above mean sea level). The region experiences summer maxima up to 45 °C and winter minima of 4–5 °C, with a diurnal range of 15–20 °C and mean annual rainfall of approximately 700 mm, falling mainly between early July and mid-September.

2.3. Experimental animals and management

Seventy-two crossbred does were enrolled: 36 Alpine $\times$ Beetal (AB) and 36 Saanen $\times$ Beetal (SB). All were in early lactation, between the third and sixth parity, and carried a body condition score of 3–4. Only clinically healthy does with no history of clinical mastitis were included; animals showing any abnormality of the udder or milk on physical examination were excluded at enrolment.

The herd was housed in a covered pen with an attached open area and was stall-fed with ad libitum green fodder (maize) and a concentrate mixture (18% crude protein, 70% total digestible nutrients) formulated to NRC (2001) recommendations, with free access to water. Does were hand-milked twice daily at 04:00 and 16:00 h in a dedicated milking shed. Animals were groomed and washed before milking; udders were washed with clean water (lukewarm during winter months) and milkers washed their hands in 1:1000 chlorhexidine–cetrimide solution before milking. All does were managed identically throughout.

2.4. Milk sampling

Milk was collected at the evening milking. Udders were wiped with single-use aseptic wipes and the first jets

discarded. Approximately 150 mL of pooled milk was collected from each doe by manual full-hand milking into sterile 50 mL tubes following National Mastitis Council guidelines and transported to the laboratory in insulated ice-pack boxes at approximately 4 °C for immediate processing. Each doe was sampled on three consecutive days, and every determination reported here somatic cell count and differential leukocyte count alike was performed on each of the three samples. The three values were then averaged to give a single figure per doe. The doe, not the sample, was therefore the experimental unit throughout, and all sample sizes given below refer to numbers of does.

2.5. Somatic cell counts and group allocation

Somatic cell count was determined by two independent methods on every sample. Automated counts were obtained on a Lactoscan SCC (Milkotronic Ltd., Stara Zagora, Bulgaria), which uses fluorescent image cytometry. Fresh milk (100 μ L) was added to an Eppendorf tube pre-coated with lyophilised Sofia Green DNA-binding dye and vortexed 10–15 times. The tube was held for 2 min and vortexed again. A volume of 8.5 μ L of the stained sample was then loaded onto a Lactochip cassette and counted. Direct microscopic counts were made in parallel on the Pyronin Y–methyl green stained smears described in Section 2.6, which also provided the differential count. Automated counters of this type have been evaluated against direct microscopic counts as indicators of intramammary infection in dairy goats (Persson and Olofsson, 2011) ^[16].

Group allocation used the mean of the three daily automated determinations. Does within each cross were assigned to six groups: <4, 4–6, 6–8, 8–10, 10–12 and >12 $\times 10^5$ cells/mL ($n = 6$ per group per cross). The two counting methods differ in what they enumerate. The automated method registers the DNA-bearing cytoplasmic particles characteristic of apocrine secretion; the microscopic count is restricted to nucleated cells (Section 2.6). The automated value is therefore the more conservative basis for allocation, and is the one reported throughout.

2.6. Differential leukocyte count

Differential counts were performed microscopically on stained milk smears, independently of the automated SCC. One smear was prepared from each of the three daily samples, giving three smears per doe; all three were read and the resulting differential counts averaged to a single value per animal. A thin smear was prepared by spreading 5–10 μ L of fresh milk over 1 cm² of a clean glass slide and drying at 30–40 °C. Fat globules were removed by immersion in xylene for 20 min; slides were fixed in methanol for 1 min, flooded with distilled water for 1 min, and dried. Smears were stained for 5 min under subdued light with freshly prepared Pyronin Y–methyl green. The stain was prepared by dissolving 1 g Pyronin Y and 0.56 g methyl green in 196 mL distilled water, filtering through 10–12 μ m filter paper and storing in an actinic bottle; it is light-sensitive and was prepared fresh. Excess stain was rinsed off with tap water.

Slides were examined under oil immersion on an Olympus IX51 microscope (Olympus, Tokyo, Japan). Neutrophils were identified by their multilobed, bridged nucleus; lymphocytes by a densely stained, often eccentric nucleus with scant cytoplasm; and macrophages by their large size and abundant cytoplasm. Epithelial cells, identified by their

smaller size and spherical nucleus, were excluded from the differential. Only round, nucleated cells with nuclei stained dark red by Pyronin Y–methyl green were enumerated. Cells smaller than 4 μm were disregarded; fragments were counted only when more than half of the nuclear material was visible; cell clusters were scored as a single unit unless nuclei were clearly separate; and doubtful objects were not counted. Counts are expressed as the percentage of each cell type within the total leukocyte population.

2.7. Neutrophil-to-macrophage ratio

The N:M ratio was computed for each doe individually as the quotient of her neutrophil and macrophage percentages, and these individual ratios were then averaged within group. Ratios were not derived from group mean percentages, since the mean of a ratio is not the ratio of means.

2.8. Statistical analysis

Analyses were performed in SPSS v22.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean $\pm$ standard error. Each response variable was examined by two-way analysis of variance with cross (2 levels), SCC group (6 levels) and their interaction as fixed effects. The cross $\times$ SCC interaction was the formal test of whether the two crosses differed in their response to rising SCC; main effects

were interpreted only in the light of that term. Effect sizes are reported as partial η^2 .

The six SCC groups are ordered rather than nominal. The dose–response question was therefore addressed by a linear polynomial contrast within each cross (coefficients $-5, -3, -1, +1, +3, +5$). Pairwise post hoc comparison was not used for this purpose: it is underpowered for monotone trends and does not test the hypothesis of interest. Pairwise differences among groups were additionally examined by Tukey's honest significant difference test and are indicated by superscript letters in Tables 1 and 2; where the pairwise and trend analyses diverge, both are reported. Significance was declared at $P < 0.05$.

Neutrophil, macrophage and lymphocyte percentages are compositional: they are constrained to sum to 100 and therefore cannot vary independently. A decline in one fraction is in part the arithmetic complement of a rise in another. This constraint is acknowledged throughout the interpretation, and the reciprocal neutrophil–macrophage relationship is not treated as two independent findings.

3. Results

Tables 1 and 2 give the differential leukocyte counts for both crosses across the six SCC groups. Table 4 summarises the percentage changes relative to the lowest group, and Table 3 gives the two-way analysis of variance.

Table 1: Milk differential leukocyte count (mean $\pm$ SE) across somatic cell count groups in clinically healthy Alpine $\times$ Beetal crossbred does (n = 6 per group).

SCC group ($\times 10^5$ cells/mL)	Neutrophil (%)	Macrophage (%)	N:M ratio	Lymphocyte (%)
<4	52.00 ^d $\pm$ 0.82	33.00 ^a $\pm$ 0.93	1.58 ^d $\pm$ 0.02	15.00 ^a $\pm$ 1.73
4-6	55.00 ^d $\pm$ 0.82	29.30 ^{ab} $\pm$ 0.77	1.88 ^{cd} $\pm$ 0.02	15.70 ^a $\pm$ 1.57
6-8	60.00 ^c $\pm$ 0.86	27.83 ^b $\pm$ 0.83	2.17 ^c $\pm$ 0.08	12.17 ^a $\pm$ 1.01
8-10	63.33 ^{bc} $\pm$ 0.99	23.33 ^c $\pm$ 0.92	2.74 ^b $\pm$ 0.12	13.33 ^a $\pm$ 1.17
10-12	67.00 ^b $\pm$ 0.93	22.00 ^c $\pm$ 0.93	3.06 ^b $\pm$ 0.09	11.00 ^a $\pm$ 1.86
>12	72.50 ^a $\pm$ 0.76	18.17 ^d $\pm$ 0.95	4.04 ^a $\pm$ 0.20	9.33 ^a $\pm$ 1.52

Means within a column bearing different superscript letters differ significantly (Tukey's HSD, $P < 0.05$). N:M, neutrophil-to-macrophage ratio, computed per doe and then averaged. Linear trend across groups: neutrophil, macrophage and N:M, all $P < 0.001$; lymphocyte, $P = 0.003$.

Table 2: Milk differential leukocyte count (mean $\pm$ SE) across somatic cell count groups in clinically healthy Saanen $\times$ Beetal crossbred does (n = 6 per group).

SCC group ($\times 10^5$ cells/mL)	Neutrophil (%)	Macrophage (%)	N:M ratio	Lymphocyte (%)
<4	54.33 ^f $\pm$ 0.49	36.33 ^a $\pm$ 0.49	1.50 ^f $\pm$ 0.03	10.17 ^a $\pm$ 0.21
4-6	58.33 ^e $\pm$ 0.42	31.50 ^b $\pm$ 0.43	1.85 ^e $\pm$ 0.04	9.33 ^{ab} $\pm$ 0.17
6-8	63.00 ^d $\pm$ 0.37	29.00 ^c $\pm$ 0.37	2.17 ^d $\pm$ 0.04	8.67 ^{bc} $\pm$ 0.12
8-10	66.50 ^c $\pm$ 0.43	24.83 ^d $\pm$ 0.48	2.68 ^c $\pm$ 0.07	8.00 ^{cd} $\pm$ 0.21
10-12	72.17 ^b $\pm$ 0.31	21.00 ^e $\pm$ 0.37	3.44 ^b $\pm$ 0.07	7.17 ^{de} $\pm$ 0.31
>12	75.33 ^a $\pm$ 0.49	17.50 ^f $\pm$ 0.43	4.32 ^a $\pm$ 0.13	6.83 ^e $\pm$ 0.17

Means within a column bearing different superscript letters differ significantly (Tukey's HSD, $P < 0.05$). N:M, neutrophil-to-macrophage ratio, computed per doe and then averaged. Linear trend across groups: all variables $P < 0.001$.

3.1. Neutrophils

Neutrophil percentage rose steadily across the SCC gradient in both crosses (Tables 1 and 2; Fig. 1A). In AB does the mean rose from 52.00 $\pm$ 0.82% in the $<4 \times 10^5$ cells/mL group to 72.50 $\pm$ 0.76% in the $>12 \times 10^5$ group, an increase of 39.4%. In SB does it rose from 54.33 $\pm$ 0.49% to 75.33 $\pm$ 0.49%, an increase of 38.7% (Table 4). The linear trend was highly significant in both crosses (AB, $t(30) = 19.56$, $P <$

0.001; SB, $t(30) = 42.37$, $P < 0.001$). SCC group explained most of the variance (partial $\eta^2 = 0.956$; Table 3). From 6–8 $\times 10^5$ cells/mL upward, every group differed from the lowest by Tukey's HSD.

Even the lowest-SCC does were neutrophil-dominant. At $<4 \times 10^5$ cells/mL, more than half of all milk leukocytes were neutrophils in both crosses.

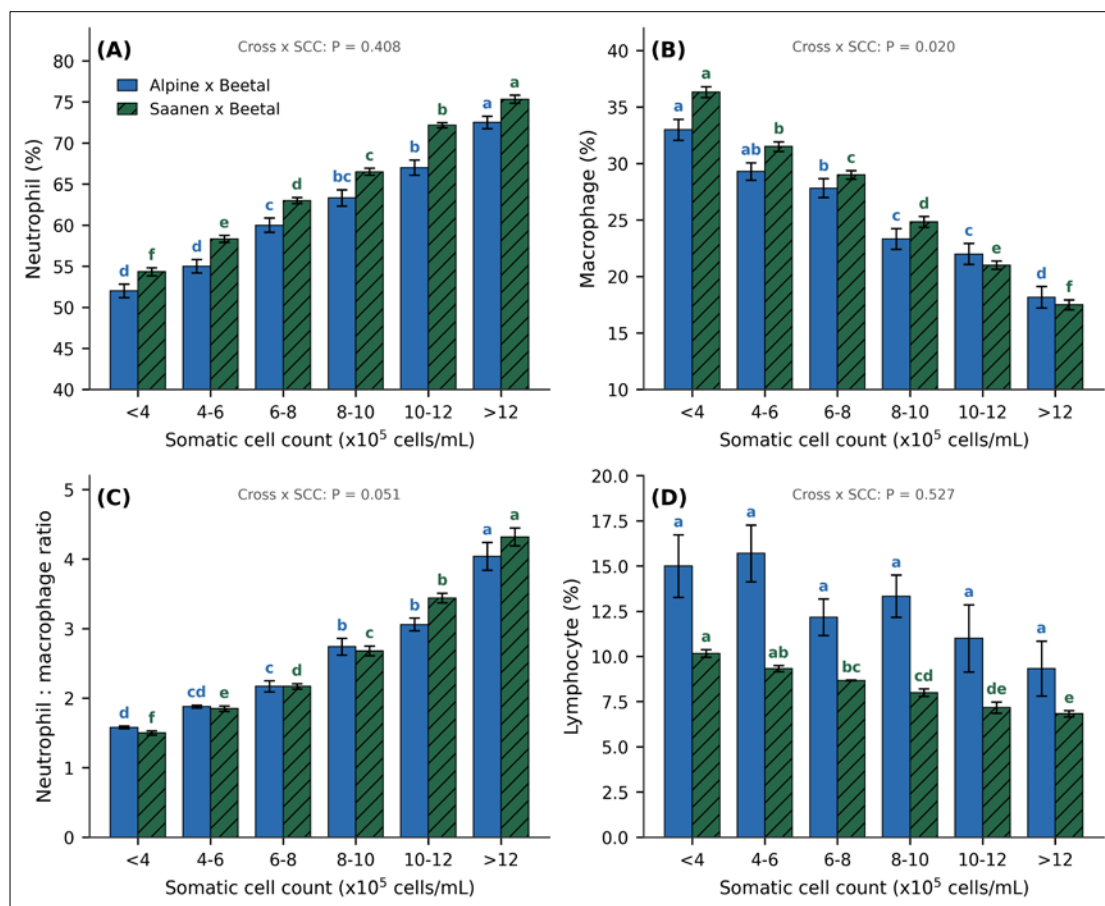


Fig 1: Milk differential leukocyte indices across six somatic cell count groups in Alpine $\times$ Beetal (blue) and Saanen $\times$ Beetal (green) crossbred does. (A) neutrophil percentage; (B) macrophage percentage; (C) neutrophil-to-macrophage ratio; (D) lymphocyte percentage. Bars are means $\pm$ SE ($n = 6$). Letters denote Tukey's HSD groupings within cross. The cross $\times$ SCC interaction P-value from two-way ANOVA is shown in each panel.

3.2. Macrophages

Macrophage percentage fell in the opposite direction: from $33.00 \pm 0.93\%$ to $18.17 \pm 0.95\%$ in AB (-44.9%) and from $36.33 \pm 0.49\%$ to $17.50 \pm 0.43\%$ in SB (-51.8%). Both linear trends were significant ($P < 0.001$; Fig. 1B; Table 4).

In SB, all six groups separated cleanly and carried distinct superscripts. Fig. 2 shows how the leukocyte pool redistributes across the gradient. As Section 2.8 notes, this fall is partly the arithmetic mirror of the neutrophil rise and is not independent of it.

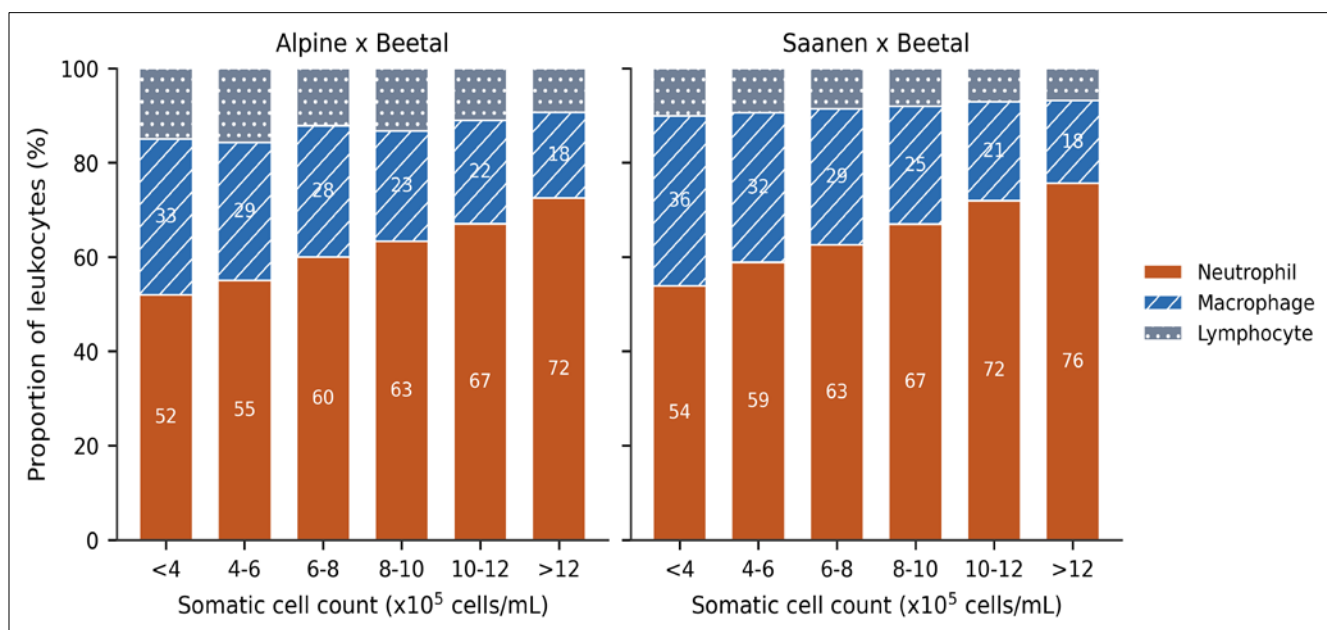


Fig 2: Composition of the milk leukocyte population across somatic cell count groups. Percentages are normalised to sum to 100 within each group, illustrating the compositional constraint discussed in the text: the reciprocal neutrophil-macrophage movement is partly arithmetic, and the three fractions cannot vary independently

3.3. Neutrophil-to-macrophage ratio

The N:M ratio rose from 1.58 ± 0.02 to 4.04 ± 0.20 in AB (+155.7%) and from 1.50 ± 0.03 to 4.32 ± 0.13 in SB (+188.0%). Both linear trends were highly significant ($P < 0.001$; Fig. 1C). An exponential function of SCC fitted the

data closely in both crosses (AB, $N:M = 1.18e^{0.091 \cdot SCC}$, $R^2 = 0.984$; SB, $N:M = 1.07e^{0.105 \cdot SCC}$, $R^2 = 0.995$; Fig. 3). Of all the measures examined, the ratio changed the most in proportional terms, and at low SCC its standard errors were the smallest relative to the mean.

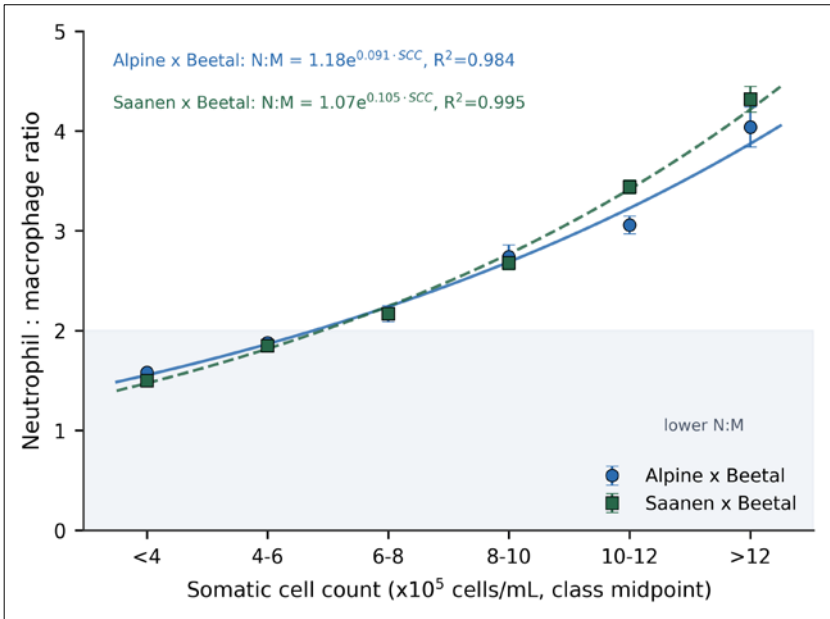


Fig 3: Neutrophil-to-macrophage ratio as a function of somatic cell count class midpoint, with fitted exponential curves. Points are means $\pm$ SE (n = 6). The ratio more than doubles across a somatic cell count range that lies within the reported physiological range for healthy goats, which is the principal caution against using it as a standalone diagnostic index.

3.4. Lymphocytes

Lymphocyte percentage fell across the gradient in both crosses: from $15.00 \pm 1.73\%$ to $9.33 \pm 1.52\%$ in AB (−37.8%) and from $10.17 \pm 0.21\%$ to $6.83 \pm 0.17\%$ in SB (−32.8%). Pairwise post hoc testing found no significant differences among the AB groups, all six-sharing superscript a (Table 1). The SB groups separated clearly (Table 2). This difference comes from the precision of the two datasets, not from the underlying response. The mean standard error was 1.48 percentage points for AB lymphocytes against 0.18 for SB about eight times larger. A linear contrast uses the ordered structure of the groups, and on this test the decline was significant in both crosses (AB, $t(30) = -3.28$, $P = 0.003$; SB, $t(30) = -14.18$, $P < 0.001$). The proportional decline was in fact slightly larger in AB than in SB (Table 4). The apparent stability of AB lymphocytes under pairwise testing is therefore an artefact of low precision, not evidence

that the response is absent (Fig. 1D).

3.5. Comparison between crosses

Two-way analysis of variance (Table 3) showed significant main effects of cross for neutrophils ($F(1,60) = 70.45$, $P < 0.001$), macrophages ($F(1,60) = 7.26$, $P = 0.009$) and lymphocytes ($F(1,60) = 50.13$, $P < 0.001$). Across all groups, SB does carried on average 3.3 percentage points more neutrophils and 4.4 percentage points fewer lymphocytes than AB does. The N:M ratio did not differ between the crosses overall ($P = 0.127$). The cross $\times$ SCC interaction was not significant for neutrophils ($F(5,60) = 1.02$, $P = 0.411$), N:M ($F(5,60) = 2.31$, $P = 0.055$) or lymphocytes ($F(5,60) = 0.84$, $P = 0.528$). For macrophages it was only marginally significant ($F(5,60) = 2.84$, $P = 0.023$). The two crosses therefore differ in their baseline leukocyte composition, but they respond to rising SCC in much the same way.

Table 3: Two-way analysis of variance for the effects of cross, somatic cell count group, and their interaction on milk differential leukocyte indices (error df = 60).

Source	df	F	P	Partial η^2
Neutrophil (%)				
Cross	1	70.45	<0.001	0.540
SCC group	5	262.10	<0.001	0.956
Cross $\times$ SCC	5	1.02	0.411	0.079
Macrophage (%)				
Cross	1	7.26	0.009	0.108
SCC group	5	155.85	<0.001	0.929
Cross $\times$ SCC	5	2.84	0.023	0.191
N:M ratio				
Cross	1	2.39	0.127	0.038
SCC group	5	228.18	<0.001	0.950
Cross $\times$ SCC	5	2.31	0.055	0.162

Lymphocyte (%)				
Cross	1	50.13	<0.001	0.455
SCC group	5	5.64	<0.001	0.320
Cross × SCC	5	0.84	0.528	0.065

The cross × SCC interaction tests whether the two crosses differ in their response to rising somatic cell count. Its non-significance for neutrophils, N:M ratio and lymphocytes indicate parallel responses on different baselines.

Table 4: Percentage change in milk differential leukocyte indices relative to the lowest somatic cell count group ($<4 \times 10^5$ cells/mL).

Variable	<4	4-6	6-8	8-10	10-12	>12
Alpine × Beetal						
Neutrophil	—	+5.77	+15.38	+21.79	+28.85	+39.42
Macrophage	—	-11.21	-15.67	-29.30	-33.33	-44.94
N:M ratio	—	+18.99	+37.34	+73.42	+93.67	+155.70
Lymphocyte	—	+4.67	-18.87	-11.13	-26.67	-37.80
Saanen × Beetal						
Neutrophil	—	+7.36	+15.96	+22.40	+32.84	+38.65
Macrophage	—	-13.29	-20.18	-31.65	-42.20	-51.83
N:M ratio	—	+23.33	+44.67	+78.67	+129.33	+188.00
Lymphocyte	—	-8.26	-14.75	-21.34	-29.50	-32.84

Values are percentage change from the reference group. Column headings are somatic cell count groups ($\times 10^5$ cells/mL). Direction and magnitude are descriptive; formal inference is by linear contrast (Tables 1 and 2) and two-way ANOVA (Table 3).

4. Discussion

The milk leukocyte profile of clinically healthy crossbred does change sharply and steadily across the SCC range normally regarded as healthy for goats. Neutrophils rose by about two-fifths, macrophages fell by almost half, lymphocytes declined, and the N:M ratio more than doubled. All of this happened within a gradient of less than 400 to more than 1200×10^3 cells/mL. Where that gradient sits is what governs how it should be read. Published work puts normal goat milk SCC at a mean of about 764×10^3 cells/mL, ranging from 200 to 1500×10^3 , and reports uninfected goats between 270 and 2000×10^3 cells/mL (Kaskous *et al.*, 2023) [9]. Latent class analysis suggests an infection cutoff near 1500×10^3 cells/mL (Koop *et al.*, 2011) [10], and the median of more than a million Norwegian goat milk records was 440×10^3 cells/mL (Smistad *et al.*, 2021). The range studied therefore lies almost entirely within what a healthy, uninfected goat udder can produce. Finite mixture modelling of goat somatic cell scores points the same way, suggesting that the caprine SCC distribution contains more than one underlying population rather than a single graded one (Boettcher *et al.*, 2005) [3].

It would be tempting to read the rising neutrophil fraction as subclinical mastitis, but that reading does not hold. No bacteriological culture was performed, the animals were clinically sound, and the SCC range never reaches the level at which infection becomes likely in this species. Goat udders with high SCC have been examined histologically and found free of mastitis (Kaskous *et al.*, 2023) [9]. More than 90% of the variation in goat SCC has been traced to physiological rather than bacteriological causes (Luengo *et al.*, 2004; Jimenez-Granado *et al.*, 2014) [11]. Min *et al.* (2007) [8, 12] found that SCC in goat milk does not correlate strongly with intramammary infection at all, and stage of lactation interacts with subclinical mastitis in shaping goat milk quality (Silanikove *et al.*, 2014) [20]. The shift described here is better read as the cellular signature of graded cell mobilisation in a healthy gland.

The baseline values reported here support this. In the lowest group, neutrophils already made up 52.0% (AB) and 54.3% (SB) of milk leukocytes. That sits squarely inside the 40–80% reported for healthy uninfected goats (Sierra *et al.*, 1999; Paape *et al.*, 2001; Kaskous *et al.*, 2023) [9, 19], and it

is nothing like the macrophage-dominant profile of a healthy cow quarter. Any framework that treats neutrophil dominance in goat milk as inherently abnormal would misclassify every healthy doe in this study. These data also refine the findings of Bagnicka *et al.* (2011) [2], who reported that mastitis raises neutrophil content in ewe milk but not in goat milk, where neutrophils dominate regardless. Dominance did persist here, but its degree was far from fixed: it ranged from roughly 3:2 to better than 4:1 across the gradient.

The N:M ratio responded most steeply and most consistently, following SCC as a near-exponential curve (Fig. 3). Its size comes from combining a rising numerator with a falling denominator. That is both its strength and its weakness, because the underlying percentages are constrained to sum to 100, so part of its sensitivity is arithmetic rather than biological. It is still useful as a summary measure, because both numbers come from the same slide. This makes it immune to the particle problem that inflates automated SCC in goats. Fluorescent cytometry counts the roughly 150×10^3 DNA-bearing particles per mL of goat milk as if they were cells (Paape and Capuco, 1997; Kaskous *et al.*, 2023) [9, 14]. A microscopic count limited to nucleated cells above 8 μm does not. The practical message is a warning rather than a recommendation. Because N:M more than doubles across the normal range in healthy animals, any threshold built on it must be judged against that gradient. A doe with an N:M of 4.0 is not therefore inflamed; healthy does here reached that value routinely.

The two crosses differed in their baselines SB does were consistently richer in neutrophils and poorer in lymphocytes but showed no significant cross × SCC interaction for neutrophils, N:M or lymphocytes. The response to rising cell load therefore looks the same in both, with the crosses separated by starting point rather than slope. This agrees with reports that SCC does not differ between goat breeds once management is held constant (Kaskous *et al.*, 2023) [9]. None of this means the trait is uninformative: divergent selection on milk SCC in goats improves udder health and milk quality (Rupp *et al.*, 2019) [18], so SCC clearly carries real signal despite its physiological noise. This comparison should not be read more strongly. Treating a significant result in one cross and a non-significant result in the other as

a real difference between them is a known error: the difference between significant and not significant is not itself statistically significant (Gelman and Stern, 2006) [6]. The lymphocyte data show this clearly. Pairwise testing suggested a difference between the crosses, but the interaction term ($P = 0.528$) rejects it and the effect sizes contradict it, since the AB decline was actually the larger of the two. The eight-fold gap in lymphocyte standard errors between crosses most likely reflects differences between observers or reading sessions rather than biology. Three constraints limit these conclusions, and all three point to the same next experiment. No bacteriology was performed, so udder health rests on clinical examination alone. The cell fractions sum to 100, so the neutrophil rise and macrophage fall are not independent. And the does came from one herd, in early lactation, at parities 3–6, chosen to span a wide gradient. That design controls the largest non-infectious sources of goat SCC variation (Luengo *et al.*, 2004; Smistad *et al.*, 2021) [11], but it yields no information on prevalence and limits how far the results can be extended. Pairing differential cytology with bacteriological culture would turn this baseline into a diagnostic threshold. Expressing the counts as absolute concentrations ($\text{SCC} \times \text{percentage}$) would free the fractions from the sum-to-100 constraint and test whether macrophages fall in absolute number rather than only in share. Together, these two steps would show whether the N:M ratio can separate infection from normal variation in a species where the total somatic cell count clearly cannot.

5. Conclusions

In clinically healthy Alpine $\times$ Beetal and Saanen $\times$ Beetal crossbred does, the milk differential leukocyte profile changes steadily and predictably across the normal somatic cell count range. Neutrophils rise, macrophages fall in step, lymphocytes decline, and the neutrophil-to-macrophage ratio more than doubles. These does were neutrophil-dominant even at the lowest cell counts, which confirms that goat milk cytology follows its own pattern rather than the cow's. The two crosses start from different baselines but respond in the same way, and there is no evidence that either reacts more strongly than the other. No bacteriology was performed, and the somatic cell range studied lies within the range reported for uninfected goats. These results are therefore reference data on normal physiology for two Indian crossbred dairy goat populations that had none before not a diagnostic test for subclinical mastitis. That is exactly where their value lies. They show how much the goat milk leukocyte profile varies in health, and therefore how much variation any future diagnostic threshold must be able to see past. Setting that threshold will need differential cytology paired with bacteriological culture and absolute cell counts across a wider somatic cell range.

Declarations

Ethical approval: The approval of all the experiments carried out in this research work was obtained from the Animal Ethics Committee of the National Dairy Research Institute, Karnal, according to the Committee for Control and Supervision of Experiments on Animals rules laid down by the Government of India (No. 1705/GO/Re/L/13/CPCSEA, dated 23/07/2021).

Conflict of interest: The authors declare no conflict of interest.

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CRedit authorship contribution statement: Animesh Patel: Investigation, Formal analysis, Data curation, Writing – original draft. Ajay Kumar Dang: Conceptualization, Supervision, Resources, Project administration, Funding acquisition, Writing – review & editing. Both authors have read and approved the final manuscript.

Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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