

# Morphological Features and Influence of Age and Breed on the Morphometry of Red Blood Cells of Female Cattle

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**Abstract:** *The study was carried out on three female cattle groups namely; local (indigenous), Red Sindhi and cross breed Jersey in order to study the influence of age (10 months-2 years, i.e., group 1, 2-6 years, i.e., group 2 and 6-10 years, i.e., group 3) and breed on length and breadth of erythrocytes. Since the morphometric (length and breadth) data on these particular breeds and ages are inconsistent and inadequate and to prevent any possible confusion of anemic syndrome on the basis of size this study is undertaken. Poikilocytosis were seen. For each animal blood samples were collected by jugular venipuncture; smears were prepared on slides immediately after the blood collection and stained with Giemsa stain. Among the three breeds, there was significant increase ( $p < 0.01$ ) in length of red blood cell size in 2-6 years local cattle and significant decrease ( $p < 0.01$ ) in 2-6 years Red Sindhi cattle. Breadth of erythrocyte was significantly larger ( $p < 0.01$ ) in 6-10 years cross breed Jersey cattle and smaller in 10 months-2 years Red Sindhi cattle. Therefore, age and breed have profound effect on the morphometry of red blood cells. Careful attention must be observed in studying and interpretation of anemic syndromes on the basis of size.*

**Keywords:** Local, Red Sindhi, Cross breed Jersey, Erythrocyte

## 1. Introduction

Erythrocytes or red blood cells (RBCs) provide vital functions of oxygen transport, carbon dioxide transport and buffering of hydrogen ions [1], [2]. The matured red blood cell of the adult bovine is biconcave in shape [3]-[5], has a width of 5-6  $\mu\text{m}$ , and has minimal central pallor and relatively lifespan of approximately 130 days [6]. RBCs lack nuclei and organelles and thereby no ability to synthesize proteins. The full complement of functional proteins must be present by the time of reticulocyte maturation [7]. Variation in erythrocyte size is termed as anisocytosis [8]. Anisocytosis is mild to moderate in bovine. Polychromatophils are generally absent from the blood of normal adult cattle [9], [6]. Poikilocytosis is a general term for variation in shape of RBC [4], [2]; it can occur in a variety of conditions, so poikilocytosis is non-specific [2]. Morphometry is a quantitative description of geometrical structures in all dimensions [10], [11]. It provides a numerical objectification of the most subtle modifications unavailable to visual estimation and as such less clinical and research applications that are becoming more numerous, especially in cytology and histopathology [12]-[14]. Since studies on morphometrical parameters, i.e., length and breadth of red blood cells of local (indigenous), Red Sindhi and cross breed Jersey breed female cattle based on age groups (10 months-2 years, 2-6 years, and 6-10 years) in a reinforced manner are inadequate the study was conducted to know the influence of both age and breed on the morphometry of blood cells as well as to prevent any possible confusion of anemic syndrome on the basis of size.

## 2. Materials and Methods

### 2.1 Blood samples collection and preparation of smears

Three breeds of female cattle namely local (indigenous), Red Sindhi and cross breed Jersey, each having three different age groups namely, 10 months-2 years, 2-6 years and 6-8 years were taken for this study. After disinfecting the sampling area, blood samples were taken from the jugular vein [15]-[17] of each animal. Dry and sterilized needles [Dispo Van Single Use Needle, Hindustan Syringes & Medical Devices Ltd., Faridabad, India] and dry syringes [Dispo Van Single Use Syringe, Hindustan Syringes & Medical Devices Ltd., Faridabad, India] were used for collection of blood samples [15]. Smears were prepared on microscopic slides (BLUE STAR, PIC 2, Polar Industrial Corporation, Mumbai, India) just after venipuncture without anticoagulants which may interfere and induce some cytoplasmic and morphometric cell changes and on extreme provoke degranulation of some blood cells [18], [4], [19]. Slides were precisely identified according to their respective breed and age.

### 2.2 Blood smears staining and morphometric study

In the laboratory, smears were stained with Giemsa stain prepared from Giemsa powder (Qualigens CAS NO.51811-82-6 Product NO. 39382, scientific India Pvt. Ltd., Mumbai, Maharashtra, India) as protocol cited by Lillie [20]. For several and even until the last years, morphometric studies of red blood cells are essentially based on linear measures of erythrocyte size. Using an ocular micrometer and an objective micrometer is the only valid and recognized method to measure the size of erythrocytes [21]. The entire data (20 observations) per age group of each breed were subjected for morphometrical analysis by using an ocular micrometer that was standardized against a stage micrometer (ERMA TOKYO, Japan made) using a standard light

microscope (LABOSCOPE MICROSCOPES Research microscope M.No. BD-08 B, S. No. 21320 Mfg. by B.D. INSTRUMENTATION, Ambala Cantt, India) under 40X objective.

### 2.3 Photomicrography

Photomicrography of blood cells were done by CC130-1.3 mega pixel microscopic camera (Mfg. by Catalyst Biotech, Maharashtra, India) connected to microscope (LABOSCOPE MICROSCOPES Research microscope M. No. BD-08 B, S. No. 21320 Mfg. By B.D. INSTRUMENTATION, Ambala Cantt, India) under 40X objective. Identifications of erythrocytes were done according to Harvey [4] and Barger [5].

### 2.4 Statistical analyses

Each parameter is expressed as mean±SE for all the breeds and Microsoft Office Excel 2007 was used for statistical analyses. Data analyses for comparison were done with the help of Paleontological Statistics (PAST) version 2.17 [Natural History Museum, University of Oslo] for One-Way Analysis of Variance (ANOVA) followed by Turkey's pair wise comparison tests. Differences were classified as significant at  $p < 0.05$  and highly significant at  $p < 0.01$ .

## 3. Results and Discussion

### 3.1 Results

#### 3.1.1 Morphology of red blood cells

The erythrocytes were observed to occur in various forms. They were either biconcave in shape with central pallor (Figure 1) or in different shapes. Ten months-2 years is considered as group 1, 2-6 years is considered as group 2 and 6-10 years is considered as group 3 for all the breeds of cattle. Some irregular forms such as match stick RBCs (Figure 2) were observed in group 2 and group 3 of local (indigenous) breed, group 1 and group 3 of Red Sindhi and all the age groups of cross breed Jersey cattle. Crenated RBCs or echinocytes (Figure 3) having relatively evenly spaced spicules were observed in group 1 of local breed, group 1 and group 3 of Red Sindhi and in all the age groups of cross breed Jersey cattle. Spindle shaped RBC (Figure 3) having both side tapered end was observed in group 1 and group 2 local breed and in all the age groups of cross breed Jersey cattle. Acanthocytes or spur cells (Figure 4) or erythrocytes with irregularly spaced variable sized spicules were observed in group 1 local and all the age groups of cross breed Jersey cattle. Comma shaped RBCs (Figure 5) were observed only in group 1 cross breed Jersey cattle. Dacryocyte or tear drop shaped RBCs (Figure 6) were observed in all the age groups of all the breeds except group 3 of Red Sindhi cattle. Schistocyte or erythrocyte fragment (Figure 7) was observed in group 1 of both local and cross breed Jersey cattle. Leptocyte (Figure 8) was observed in all the age groups of all the breeds except group 2 Red Sindhi cattle. Stomatocytes or cup shaped erythrocytes with oval or elongated areas of central pallor were (Figure 9) observed in group 2 of local breed. Erythrocyte with two central pallors (Figure 10) was observed in group 3 local, group 1 Red Sindhi and in group 3 cross breed Jersey cattle. Two dividing

erythrocyte (Figure 11) was observed in 6 years of cross breed Jersey cattle. Aggregated erythrocytes or rouleau formation (Figure 12) was observed in all the age groups of all breeds.

#### 3.1.2 Influence of age

The influences of age groups on the three breeds of female cattle are observed (Table 1). Ten months-2 years is considered as group 1, 2-6 years is considered as group 2 and 6-10 years is considered as group 3 for all the breeds. Among the local breeds, the length of erythrocyte is greater in group 2 than group 1 cattle followed by group 3 and highly significant difference ( $p=0.008$ ) is found between group 2 and group 3 cattle. Breadth of erythrocyte is greater in group 2 than group 1 followed by group 3 among local breeds. Among the Red Sindhi cattle, length of erythrocyte is greater in group 1 than group 3 followed by group 2 and highly significant difference ( $p=0.002$ ) is found between group 1 and group 2. Breadth of erythrocyte is largest in group 3 than group 1 followed by group 2 among the Red Sindhi cattle. Among the cross breed Jersey cattle length of erythrocyte is largest in group 1 than group 3 followed by group 2 and differences are insignificant among them. For breadth of erythrocyte group 3 has largest breadth than group 2 followed by group 1 among the cross breed Jersey cattle and no significant differences are found among the groups.

#### 3.1.3 Influence of breed

The influences of breeds on three different age groups of female cattle are observed (Table 2). Among the group 1, the length of erythrocyte is largest in local breed cattle than Red Sindhi cattle followed by cross breed Jersey but no significant differences were found among them. Among the group 1, local breed has largest erythrocyte breadth than cross breed followed by Red Sindhi but no significant differences are found among them. Among the group 2, erythrocyte length is largest in local breed than cross breed Jersey followed by Red Sindhi cattle and highly significant differences are found among them. Highly significant difference is found between group 2 local breed and group 2 Red Sindhi cattle ( $p=0.0001$ ) and highly significant difference is also found between group 2 local breed and group 2 cross breed Jersey cattle ( $p=0.005$ ). Among the group 3, cross breed Jersey has both largest erythrocyte length and breadth than Red Sindhi cattle followed by local breed cattle.

According to our results, it seems that both age and breed can affect the length and breadth of erythrocytes in female cattle.

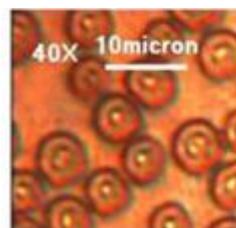


Figure 1: Biconcave erythrocytes.

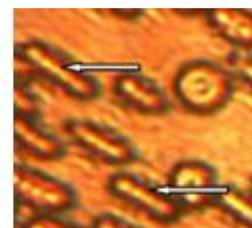


Figure 2: Matchstick RBCs.



Figure 3: Crenated RBCs (echinocytes) are shown by (↔) and spindle shaped RBC by (←).

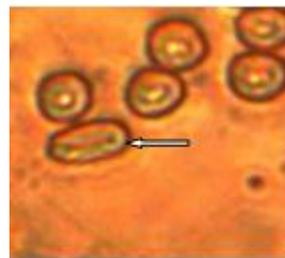


Figure 8: Leptocyte.

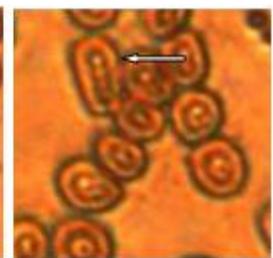


Figure 9: Stomatocyte.

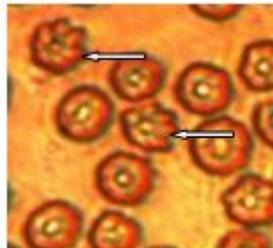


Figure 4: Acanthocytes.

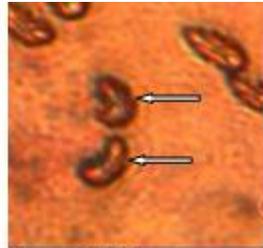


Figure 5: Comma shaped RBCs.

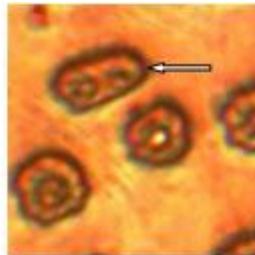


Figure 10: RBC with two central pallors.

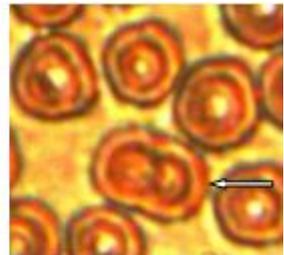


Figure 11: Dividing erythrocyte.



Figure 6: Dacryocyte (tear drop shaped RBC).

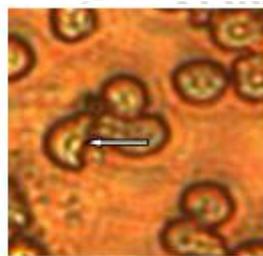


Figure 7: Schistocyte.

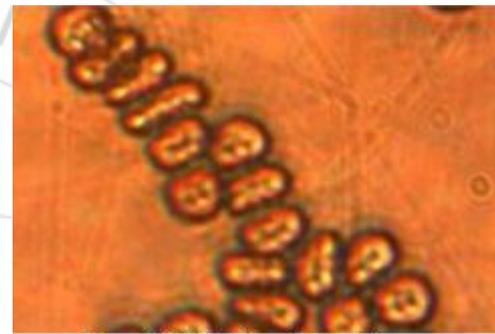


Figure 12: Rouleaux formation.

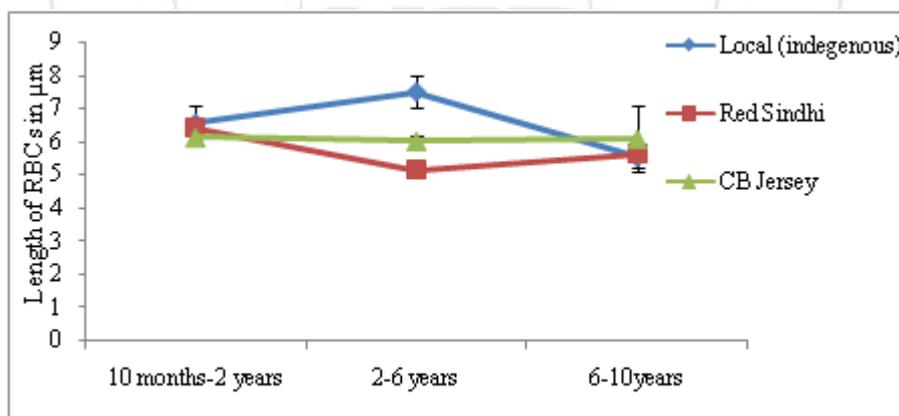


Figure 13: Influences of ages with respect to breeds on the length of RBCs. (CB Jersey, i.e., cross breed Jersey).

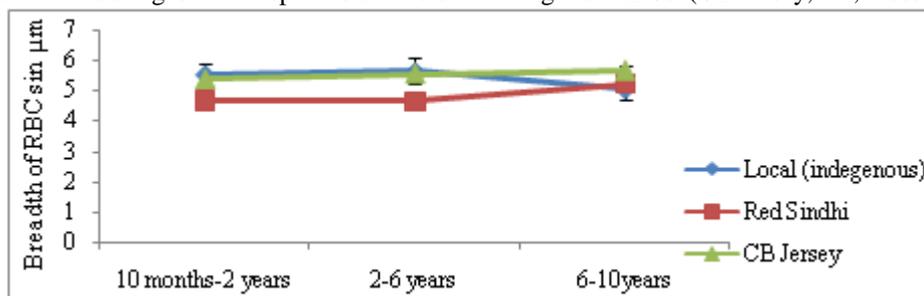


Figure 14: Influences of ages with respect to breeds on the breadth of RBCs. (CB Jersey, i.e., cross breed Jersey).

**Table 1:** Influences of age groups on the size of erythrocytes of three breeds of female cattle (Mean ± SE expressed in μm)

Types of breed	Parameter	Age groups			F value
		10months-2 years or Group1 (n=20)	2-6years or Group 2 (n=20)	6-10 years or Group 3 (n=20)	
Local (indigenous)	Length	6.56±0.50	7.51±0.48 <sup>b</sup>	5.54±0.34 <sup>b</sup>	4.75*
	Breadth	5.55±0.38	5.65±0.43	5.07±0.27	0.69 <sup>NS</sup>
Red Sindhi	Length	6.40±0.31 <sup>a</sup>	5.15±0.21 <sup>a</sup>	5.62±0.19	6.47**
	Breadth	4.68±0.30	4.66±0.23	5.23±0.21	1.61 <sup>NS</sup>
Cross breed Jersey	Length	6.13±0.14	6.01±0.17	6.09±0.09	0.20 <sup>NS</sup>
	Breadth	5.37±0.18	5.55±0.24	5.66±0.15	0.69 <sup>NS</sup>

<sup>1</sup>Mean±SE with similar superscripts in the same row differ significantly at p<0.05 and p<0.01

<sup>2</sup>\* means Significant at p<0.05, \*\* means significant at p<0.01 and NS means not significant

<sup>3</sup>Figures in parentheses represent the number of observations in each case

SE: Standard error, F value: Fischer's value

**Table 2:** Influences of breeds on the size of erythrocytes of different age groups of female cattle (Mean ± SE expressed in μm)

Age groups	Parameters	Breed			F value
		Local (indigenous)	Red Sindhi	Cross breed Jersey	
10 months-2 years (Group 1) (n=20)	Length	6.56±0.50	6.40±0.31	6.13±0.14	0.36 <sup>NS</sup>
	Breadth	5.55±0.38	4.68±0.30	5.37±0.18	2.31 <sup>NS</sup>
2-6 years (Group 2) (n=20)	Length	7.51±0.48 <sup>a</sup>	5.15±0.21 <sup>a</sup>	6.01±0.17 <sup>a</sup>	13.59**
	Breadth	5.65±0.43	4.66±0.23	5.55±0.24	2.89 <sup>NS</sup>
6-10 years (Group 3) (n=20)	Length	5.54±0.34	5.62±0.19	6.09±0.09	1.56 <sup>NS</sup>
	Breadth	5.07±0.27	5.23±0.21	5.66±0.15	1.95 <sup>NS</sup>

<sup>1</sup>Mean±SE with similar superscripts in the same row differ significantly at p<0.01

<sup>2</sup>\*\* means significant at p<0.01 and NS means not significant

<sup>3</sup>Figures in parentheses represent the number of observations in each case

SE: Standard error, F value: Fischer's value

### 3.2 Discussion

#### 3.2.1 Morphology

Poikilocytosis is a general term used to describe the presence of erythrocytes having abnormal shape [4]. Poikilocytosis may be seen in clinically normal young cattle [22]. Echinocytes are spiculated erythrocytes having relatively evenly spaced and similar sized spicules [23]. When the surface area of the outer lipid monolayer increases relative to the inner monolayer echinocytosis form [4]. Acanthocytes or spur cells are erythrocytes with irregularly spaced, variably sized spicules [24]. When erythrocyte membranes contain excess cholesterol compared to phospholipids acanthocytes form [4]. Marked acanthocytosis is reported in young goats and some young cattle [22], [25]. Erythrocyte fragments with pointed extremities are called schistocytes. Erythrocyte

fragmentation may appear when erythrocyte are forced to flow through altered vascular channels or exposed to turbulent blood flow [4]. Some leptocytes appear folded (Figure 8) [4]. Leptocytes may be seen in iron deficiency anemia [26]. Polychromatophilic erythrocytes may sometimes appear as leptocytes [4]. Dacryocytes are teardrop shaped erythrocytes with single pointed or elongated extremities (Figure 6) [4]. In iron-deficient ruminants, dacryocytes are common erythrocyte shape abnormalities [27]. Due to thick blood film preparations stomatocytes most often occur as artifacts [4].

#### 3.2.2 Influence of age

Anisocytosis are seen in different age groups. According to some authors [28]-[30] age can be considered when establishing the references values in domestic animal. According to Schlam and Carlson [31], Harvey et al [28], Meinkoth and Clinkenbeard [32] and Harvey [1], the fetal erythrocytes are larger than those of adults. During gestation and at birth, the erythron compartment increase, at birth 9% of the red blood cells are reticulocytes [33]. Fetal calf red blood cells are less fragile and larger than adult bovine red blood cells [28]. The increasing of erythrocyte diameter with increase in age in group 1 cattle except local breed, observed in our study could be interpreted by the persistence of red blood cells after parturition formed during embryonic life and decreasing of the diameter or length by the stem cell adaptation to new conditions of life after parturition [34].

#### 3.2.3 Influence of breed

Anisocytosis are seen in different breeds. Sex [35], breed [36], exercise [37], pregnancy and lactation [38]-[40], emotional states [15] are variables to be considered when establishing references values in domestic animal. Breed difference for both length and breadth is observed in our study which can be interpreted with some workers [15, 36] who had considered the breed as one of the factors for reference values. There is overlap between length of RBCs of local and Red Sindhi cattle among 10 months-2 years age (Figure 13). There is also found overlap between length of RBCs of 6-10 years of local and Red Sindhi cattle. There is slight overlap between breadth of 2-6 years of local and cross breed Jersey and between 6-10 years of local and Red Sindhi cattle (Figure 14).

### 4. Conclusion

Age and breed have effect on the morphometry of local (indigenous), Red Sindhi and cross breed Jersey female cattle and possible confusion of anemic syndromes can be avoided by this type of study. These results could serve as a base line for the diagnostic interpretation of anemic syndromes in veterinary medicine especially concerning normocytic, microcytic and macrocytic anemia. Extended studies to other breeds are highly recommended.

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