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Sex determination through Barr Bodies of Neutrophils in cattle and buffalo

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Short Communication

ABSTRACT

The present study was designed to assess the presence/absence and frequency of Barr Bodies on Polymorph Nuclear Neutrophils (PMNs) as a confirmatory guide towards sex determination in cattle and buffalo. Blood samples of adult cattle (n=26) and buffaloes (n=26) grouped as males and females (n=13 each), were collected from the jugular vein through appropriate measures. Thin blood smears were stained using Field stain 'A' and 'B'. The PMNs were counted and observed for Barr Bodies attached to them. Only the neutrophils with terminable lobes were examined and counted. The mean value ±SE of the Barr Bodies was calculated and the difference between males and females of both species was analyzed through independent T-test. The mean ±SE values for Barr Bodies were significantly higher (P≤0.05) in female cattle (9.61±1.11) as compared to their male counterparts (0.92±0.28). Similar results were revealed for buffaloes being 6.23±1.15 and 0.92±0.28 for females and males, respectively. In a nutshell, it is concluded that female cattle and buffaloes have a higher occurrence of Barr Bodies on their PMNs as compared to males. Furthermore, it can also be deduced that sex determination in cattle and buffalo through this cyto-diagnostic technique is quiet certain and precise.

Keywords: Barr bodies, Polymorph neutrophils, Sex determination, Cholistani cattle

INTRODUCTION

For almost a decade, the presence / absence and frequency of occurrence of Barr Bodies (also known as 'nucleolar satellites' or 'sex chromatin' or 'Davidson's Bodies' or 'Drumsticks') on the Polymorph Nuclear Neutrophils (PMNs) have provided a clinical tool for assessment of gender both in humans and in animals (Miller, 2006; Brahimi *et al.,* 2013; Ajuogu *et al.,* 2014).The "drumsticks" of the PMNs were first identified by Barr &Bertram (1949) while working on effects of stress on nervous system. Later on, Davidson and Smith (1954) related these nuclear appendages to sex chromatin. In their original observation, Davidson and Smith classified five types of nuclear appendages in mature neutrophils: drumsticks, sessile, nodules, small clubs, minor lobes and racket formation. They considered only 'drumsticks' to be related to the sex chromatin. It is now established that the sex chromatin seen as darkly staining mass at the nucleus of all non-dividing cells of genotypical females, represents the heterochromatin of the inactivated X-chromosome.

Cytogenetic studies have long been an integral part of a better understanding towards pathophysiology of numerous problems in livestock. One of the cytogenetic procedures which is gaining strong footing is the assessment of X-chromatin (Pandey & Mann, 2000; Ajuogu *et al.,* 2014; Verma & Adinarayan, 2017). These assessments undermine the fact that they represent the sexual status of the animal. The X-chromatin status and assessment of gender through presence/absence and frequency of occurrence of Barr Bodies on the PMNs has been reported for various breeds of goats (Okonkwo *et al.,* 2010), cattle (Ajuogu *et al.,* 2014) and sheep (Barjatiya *et al.,* 2016). Similar studies have also been conducted for various laboratory animals (Cadar, 2009). Recently, our group reported similar technique for humans and dogs (Lashari *et al.,* 2018). However, to the best of our knowledge, no such work has yet been reported on cattle and buffalo from Pakistan. The present work, is thus, aimed towards the assessment of the presence/absence and frequency of occurrence of

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Barr bodies as a confirmatory guide towards sex determination in cattle and buffalo.

MATERIALS AND METHODS

Study design and animals

The study was approved by the Directorate of Research, Innovation and Commercialization of the Islamia University of Bahawalpur (IUB), Pakistan through the Department of Life Sciences and the University College of Veterinary and Animal Sciences (UCV&AS).

The cattle (n=26) and buffalo (n=26) being harbored at the UCV&AS, IUB Farm were selected to be incorporated in the study. The adult animals (from 3 to 7 years old) were grouped as males and females having n=13 in each group. The cattle incorporated in presented study belonged to Cholistani breed which is a humped indigenous cattle breed of Pakistan and has lately acclaimed both national and international fame through our previous detailed work (Farooq *et al.,* 2010; 2012; 2013; 2015; 2017; Mahmood *et al.,* 2014), whereas the buffalo belonged to Nili-Ravi breed (*Bubalusbubalis*).The mean live weight of cattle and buffaloes incorporated in the study was 300±32.4 and 519±46.2kg, respectively. Their feeding regimen consisted (g/kg DM) of fresh green forage of sorghum (844), lucern hay (88), cottonseed cake (30) and a commercial concentrate mixture (37) with a roughage to concentrate ratio of 80:20 on a Dry Matter (DM) basis. The chemical composition (g/kg DM) of the ration was: DM (302), crude protein (CP; 58), ether extract (EE; 18), neutral detergent fibre (aNDF, 580) non-fibre carbohydrates (NFC; 230) and ash (113). All animals were tied up, individually fed and given access to fresh clean water as per requirements.

Blood collection

Blood was collected from the jugular vein of the animal after proper restraint in3mL vacutainers containing EDTA. The same technique and timing of blood collection (10:00AM), same day of collection, and same personnel were assigned for sampling in order to minimize the stress to the animal. Samples were brought to the Physiology laboratory of UCV&AS, IUB for further analyses.

Slide preparation and staining

A drop of whole blood was used for making a thin smear which was air dried, fixed in absolute alcohol and stained using Field's Stain (SDL, Pakistan) (Chunge *et al.,* 1989). Field's stain consists of two parts - Field's stain 'A' is methylene blue and Azure 1 dissolved in phosphate buffer solution; Field's stain 'B' is Eosin Y in buffer solution.

Observations

Dried and stained slides were observed under the microscopic magnification of 100X (oil immersion lens). Total neutrophils were counted throughout the slide and those having Barr Bodies attached to them were counted separately. Only neutrophils with identifiable terminal lobes were examined. Terminal lobes correspond to the two end lobes in the linear array of lobes that form the neutrophil nucleus (Karni *et al.,* 2001).

Statistical analysis

Statistical analysis was conducted through Statistical Package for Social Science (SPSS for Windows version 12, SPSS Inc., Chicago, IL, USA). Descriptive analysis was implied and the difference between male and female cattle and buffaloes was analyzed through independent T-test (Verma & Adinarayan, 2017).

RESULTS AND DISCUSSION

The present study is the first of its kind being reported from Pakistan with an aim to assess gender in cattle and buffalo through presence/absence and frequency of Barr Bodies on PMNs. Review of literature revealed very scanty details on similar work for cattle/buffalo. Hence, the results of present study have been compared with previous work on other livestock species.

The result of Barr Bodies on the PMNs of male and female cattle is presented in Table 1 whereas that for buffaloes is given in Table 2. The mean ±SE values for Barr Bodies were significantly higher ($P \leq 0.05$) in female cattle (9.61±1.11) as compared to their male counterparts (0.92±0.28). Similar results were revealed for buffaloes being 6.23±1.15 and 0.92±0.28 for females and males, respectively. The percentage for occurrence ranged from 2 to 13 in all the females, and from 0 to 2 in all males.

Table 1: Comparison of Barr Bodies of neutrophils in male and female cattle

Groups	Neutrophils Counted	Barr Bodies						
		No. of Barr Bodies	% of Barr Bodies	Mean ± SE	Range	Min. value	Max. value	P value
Males (n=13)	1300	12	0.92	0.92 ± 0.28	0-3	0	3	0.00
Females (n=13)	1180	125	10.59	9.61 ± 1.11	2-16	2	16	

Significant at P≤0.05

Table 2: Comparison of Barr Bodies of neutrophils in male and female buffaloes

Groups	Neutrophils counted	Barr Bodies						
		No. of Barr bodies	% of Barr bodies	Mean ± SE	Range	Min. value	Max. value	P value
Males (n=13)	940	12	1.28	0.92 ± 0.28	0-3	0	3	0.00
Females (n=13)	1140	81	7.10	6.23 ± 1.15	0-11	0	11	

Significant at P≤0.05

In the present study, number and percentage incidence of Barr Bodies being higher in females (2% to 13%) for both species (cattle and buffalo) as compared to that in males (0%-2%) is in accordance with the work of Ajuogu *et al*., (2014) who reported a reference range of 0% to 2% drumsticks on PMNs of male bulls while working on Nigerian indigenous cattle breeds. Lower percentages though have been reported for other livestock breeds. Okonkwo *et al.,* (2010) while working on Nigerian indigenous goat breeds reported a mean percentage of 0.15 Barr Bodies for males and 3.55 for females. They concluded that the drumstick incidence may be determined from either the buccal cavity or the polymorph nuclear cells (PMNS) of goats with equal efficacy and accuracy. A 1% to 9% occurrence has been reported for female llamas by Azwai *et al*., (2007), whereas a prevalence of 0.3% to 1% Barr Bodies has been reported for male sheep by Barjatiya *et al.,* (2016). A higher percentage for females in our study could be a species specific characteristic. Furthermore, breed disposition can also not be overruled.

Comparing our results with other species, remarkable differences were noted. A complete absence of Barr Bodies on PMNs of males has been reported by Pothiwong *et al.,* (2006) in wild cats from five species of family Felidae. Cadar (2009) reported work on some laboratory animal species including white mouse, rat, guinea pig, rabbit and dog. It was concluded that sexual dimorphism reflected through Barr Bodies on the PMNs in reliable at highest certainty only for dogs. Frequency of Barr Bodies in human PMNs has been reported as 2.4% to 5.1% and 0.4% to 1% for females and males, respectively (Barjatiya *et al*., 2016). It can plausibly be submitted that the accuracy of sex determination through this cytogenetic technique in various species will depend on specie itself (Pothiwong *et al*., 2006; Cadar, 2009; Barjatiya *et al*., 2016). Furthermore, though it was stated in the literature that drumsticks are never seen in males, our study and several other studies in literature suggest that true drumsticks are also present in males though their percentage incidence is less.

Though the present study incorporated healthy and normal animals, however, the detection of various nuclear appendages on PMNs can be used for identification of various genetic anomalies and fertility related issues of the livestock as reported earlier (Mayr *et al.,* 1983; Nicholas, 2009; Beckelmann *et al*., 2012).

CONCLUSION

The present study is a preliminary study and provides a baseline data on sex determinism in cattle and buffalo. Results indicate that female cattle and buffaloes have a higher occurrence of Barr Bodies on their PMNs as compared to males. Furthermore, it can also be deduced that determination of sex in cattle and buffalo through this cytogenetic technique is quiet certain and precise. Future horizons include better cytogenetic analyses for livestock with a higher population, and in various species. These studies need to be elaborated in correlation to various genetic anomalies as well.

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