Journal of Animal Research: v.10 n.2, p. 231-236. April 2020

DOI: 10.30954/2277-940X.02.2020.10

Karyomorphological Studies of Kangayam, Pulikulam, Crossbred Jersey and Crossbred Holstein Friesian Bulls

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Received: 06 Jan., 2020 **Revised:** 19 Jan., 2020 **Accepted:** 09 March, 2020

ABSTRACT

The present study was carried out in four genetic groups of cattle, *viz.* Kangayam, Pulikulam, crossbred Jersey and crossbred Holstein Friesian, to compare the karyomorphological pattern between *Bos indicus* and *Bos taurus* x *indicus* bull calves. Metaphase chromosomal spreads obtained by short term lymphocyte culture technique revealed chromosomal complement (2n) of 60, with 29 pairs of autosomes and one pair of sex chromosomes in four groups. All the autosomes were acrocentric, X-chromosome was sub-metacentric and Y-chromosome was acrocentric in *Bos indicus* and metacentric in crossbred bulls. There was no significant difference in relative length, arm ratio, centromeric index and morphological index of autosomes and X-chromosome between indicine and taurine groups; but Y-chromosome differed significantly ($P<0.01$) in relative length between *Bos indicus* and *Bos taurus* x *indicus* crosses. Y-chromosome polymorphism could help in the determination of breed origin and male lines used in the breeding programmes in order to prevent the possible interferences in the process of reproduction.

Keywords: Crossbreds, Kangayam, Pulikulam, relative length, Y-chromosome polymorphism

Among the breeding strategies, crossing of non-descript cows with exotic bulls has gained more importance so as to increase the milk production of non-descript cows in India. According to 20th Livestock Census (2019), the indigenous/non-descript cattle population in India is 142.11 millions, while the exotic/crossbred cattle population is 50.42 million accounting for 26.19 per cent of total cattle population. This has increased by 26.9% compared to previous census (39.73 millions). This is achieved by extensive coverage of crossbred and non-descript cows through artificial insemination network, utilizing the semen of elite sires.

The sires selected for frozen semen production are screened for chromosomal polymorphism, since chromosomal incompatibility results in fertility related problems. Though the chromosomal abnormalities are rare in mammalian species, Y-chromosome polymorphism (Stranzinger *et al*., 2007; Wurtser and Benirschke, 1960) had been reported among sex chromosomes, while C-band polymorphism observed in autosomes (Switonski *et al*., 1983; Switonski, 1984). Karyologically, the morphology of autosomes and X-chromosome is similar in *Bos taurus* and *Bos indicus*, whereas Y-chromosome exhibits differences (Parada *et al*., 2018). During meiosis, the synaptonemal complex is formed between short homologues of pseudo-autosomal regions (PAR) between X- and Y-chromosomes. This exchange between sex chromosomes will alter the length of the pseudo-autosomal region, thereby changing the arm ratio (Ohno and Weiler, 1962). In addition, it was also stated by Pearson and Borrow (Pearson and Borrow,

How to cite this article: Vemula, H., Periya, K., Doraiswamy, C. and Alagappan, M.G. (2020). Karyomorphological studies of kangayam, pulikulam, crossbred jersey and crossbred holstein friesian bulls. *J. Anim. Res.,* **10**(2): 231-236.

1970), if pairing of X and Y was not possible, then meiosis would be stopped and sperm production would not occur. Previously there were reports of reproductive failures in crossbreds of *Bos taurus* and *Bos indicus* (Rendel, 1980). Structural and numerical chromosomal abnormalities may not be the reasons for the fertility related problems; but, minor differences in the morphometric measurements such as relative length and arm ratio would cause reproductive failures (Stranzinger *et al*., 2007). With this backdrop, the current study was conducted to compare the morphometric measurements of Kangayam, Pulikulam, Jersey crossbred and Holstein Friesian crossbred, to find chromosomal differences among the *Bos indicus* and *Bos taurus* x *Bos indicus* cattle.

MATERIALS AND METHODS

A total of 122 bull calves (22 Kangayam, 13 Pulikulam, 48 crossbred Jersey and 39 crossbred Holstein Friesian) produced through field progeny testing programme were screened cytogenetically in the Department of Animal Genetics and Breeding, Madras Veterinary College, Chennai, India between October, 2018 to July 2019. Metaphase chromosomes of the peripheral blood lymphocytes were prepared through short term lymphocyte culture technique according to Bharti *et al*. (2017). Blood lymphocytes were cultured by adding 7 ml of complete chromosomal medium (Euroclone, Italy) to 0.5 ml of whole blood. The culture was incubated for 72 hours at 37ºC and added 8 µg of colchicine one and half hour prior to harvesting the culture. Later, the cells were treated with hypotonic solution (0.075M KCl) for 30 minutes at 37ºC and then fixation in carnoy's solution (3:1 ratio of Methanol : Glacial acetic acid). Cell suspension was dropped onto chilled wet slides from 2 to 3 feet height with pressure. Slides were air dried, stained with 5 per cent Giemsa stain for 30 minutes. Slides were screened under microscope and good metaphase spreads were photographed. Karyotyping was done with applied spectral imaging software Olympus, USA) as per the standard nomenclature (Di Berardino *et al*. 1989; Di Berardino *et al*., 2000). The length of the chromosomes was measured in millimeter with an accuracy of 0.05 mm, using a vernier calliper and morphometric measurements were recorded. Data on morphometric measurements were subjected to one-way ANOVA using IBM SPSS Statistics 20 to observe the significant differences between the genetic groups.

Formulas for the calculation of morphometric measurements were given below:

Relative length =

$$\frac{\text{Length of individual chromosome}}{\text{Total length of haploid genome including X-chromosome}} \times 100 \left(\begin{matrix}\text{Bhatia and}\\ \text{Shankar, 1991}\end{matrix}\right)$$

$$\text{Arm ratio} = \frac{\text{Length of long arm (q)}}{\text{Length of short arm (p)}}$$

Centromeric index =

$$\frac{\text{Length of short arm (p)}}{\text{Length of the chromosome (p+q)}} \times 100 \left(\begin{matrix}\text{Sahai and}\\ \text{Vijh, 1994}\end{matrix}\right)$$

$$\text{Morphological index} = \frac{\text{Total chromosome length}}{\text{Arm ratio}}$$

RESULTS AND DISCUSSION

Modal chromosome number and morphology

The bull calves of both *Bos indicus* and *Bos taurus* x *indicus* crosses analyzed in this study revealed a modal chromosome number (2n) of 60. The metaphase spreads and respective karyotypes of indigenous and crossbred bulls are presented in Fig. 1 and 2 respectively. All the autosomes were acrocentric, X -chromosome was sub-metacentric and Y -chromosome was acrocentric in *Bos indicus* and metacentric in crossbred bulls. These findings were in accordance with the earlier observations made in *Bos indicus* breeds of cattle *viz*. Khillari (Nakod, 2012), Sahiwal (Subramanyam, 2013), Tho-Tho (Choudhury *et al*., 2014), Punganur (Bharathi *et al*., 2015), Malnad Gida (Suresh *et al*., 2015), Ongole (Bharti *et al*., 2017), Alambadi (Parameswari *et al*., 2019), dwarf breeds of India (Rajesh Kumar *et al*., 2016) and Indonesian breeds (Ciptadi *et al*., 2017); *Bos taurus* breed, Holstein Friesian (Jamir *et al*., 2015) and *Bos indicus* x *Bos taurus* crosses *viz*. Jersey and Holstein Friesian crossbreds (Subramanyam, 2013; Nagpure *et al*., 2001; Cauveri and Sivaselvam, 2015).

There were two hypotheses given by the researchers for the differences in Y-chromosome morphology between

taurine and indicine bulls. First being translocation of chromosomal arms between autosomes and Y-chromosome (Rendel, 1980) which caused a balanced stable chromosomal polymorphism as evidenced from lower calving rates in F_2 generation of Brahman crosses with *Bos taurus*. Second hypothesis was centromere transposition or pericentric inversion which might be the reason for morphological differences in Y-chromosome (Goldammer *et al*., 1997; Di Meo *et al*., 2005). Comparative banding, *in situ* hybridization and southern blotting with specific DNA probes revealed pericentric inversion occurred in Y-chromosome between *Bos taurus* and *Bos indicus* cattle (Goldammer *et al*., 1997).

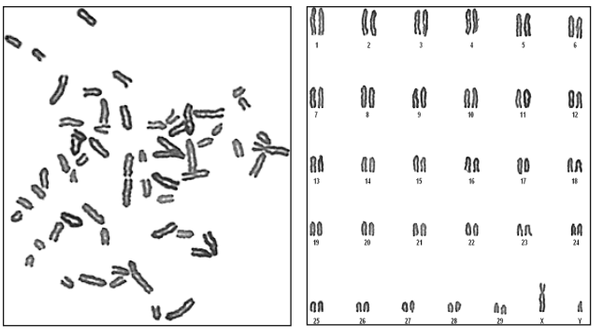

Fig. 1: Metaphase spread and karyotype of Kangayam bull calf

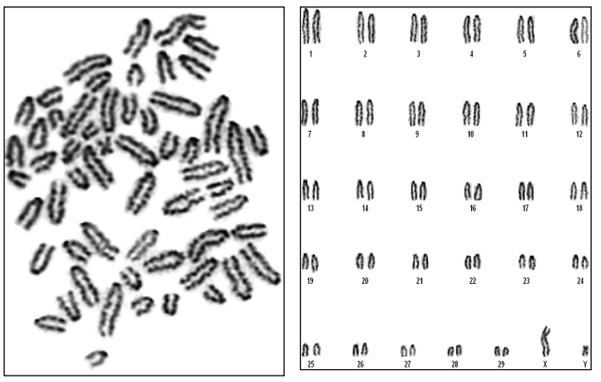

Fig. 2: Metaphase spread and karyotype of crossbred Jersey bull calf

Relative length

Analysis of variance revealed that there was no significant difference in relative lengths of autosomes and X-chromosome between the indigenous and crossbred bulls which concurred with the earlier studies in Sahiwal and Jersey crossbreds (Subramanyam, 2013), Punganur (Bharathi *et al*., 2015) and Malnad Gidda (Suresh *et al*., 2015). But, there was a significant difference ($P<0.01$) in relative length of Y-chromosome between *Bos indicus* and *Bos taurus* × *indicus* bulls (Stranzinger *et al*., 2007; Bharathi *et al*., 2015). The significant difference in the relative length of Y-chromosome was attributed to pericentric inversion causing loss of genetic material (Teale *et al*., 1995). The pericentric inversion should have resulted from splitting and dislocation of heterochromatin region and the influence of PAR region as opined by Stranzinger *et al*. (2007). In general, the relative length of a chromosome indicates the contribution of concerned chromosome to the haploid genome of the species under investigation, since it is important for classification of chromosomes, identification of deletions, duplications and translocations (Hansen, 1980). The relative lengths of X- and Y-chromosomes did not differ significantly between Kangayam and Pullikulam breeds.

The means of relative length of chromosomes in various genetic groups of cattle were given in Table 1. The relative lengths of autosomes were ranging from 5.30 ± 0.06 (1st chromosome) to 1.72 ± 0.04 per cent (29th chromosome); and 5.27 ± 0.07 (1st chromosome) to 1.70 ± 0.05 per cent (29th chromosome) in Kangayam and pulikulam bulls respectively. These values were similar to the earlier findings in indigenous breeds such as Red Kandhari (Katkade, 2005), Deoni (Balaji *et al*., 2006), Sahiwal (Subramanyam, 2013), Ongole (Bharti *et al*., 2017) and Alambadi (Parameswari *et al*., 2019).

The mean relative length of autosomes ranged from 5.36 ± 0.06 (1st chromosome) to 1.66 ± 0.04 (29th chromosome) in crossbred Jersey and 5.20 ± 1.73 (1st chromosome) to 1.73 ± 0.03 (29th chromosome) in crossbred Holstein Friesian bulls. The values for X- and Y-chromosomes were 5.83 ± 0.07 and 1.78 ± 0.06 in crossbred Jersey; and 5.50 ± 0.05 and 1.96 ± 0.06 in crossbred Holstein Friesian bulls respectively. These observations were slightly different from values reported in Holstein Friesian in Maharashtra (Nagpure *et al*., 2001), and Jersey crossbred cattle in Andhra Pradesh and Karnataka, India (Subramanyam *et al*., 2013; Suresh *et al*., 2015). Among the whole chromosomal complement, X-chromosome was the longest and Y was the smallest.

Table 1: Mean relative length of autosomes and allosomes in Kangayam, Pulikulam, crossbred Jersey and crossbred HF bull calves

Chromosome number	Genetic group - wise mean relative length (in percent)				F value
	Kangayam	Pulikulam	Crossbred Jersey	Crossbred HF	
1	5.30 ± 0.06	5.27 ± 0.07	5.36 ± 0.06	5.20 ± 0.04	1.28^{NS}
2	4.84 ± 0.03	4.75 ± 0.05	4.83 ± 0.05	4.79 ± 0.04	0.66 ^{NS}
3	4.59 ± 0.04	4.53 ± 0.04	4.52 ± 0.03	4.54 ± 0.03	0.69 ^{NS}
4	4.44 ± 0.03	4.36 ± 0.04	4.40 ± 0.03	4.39 ± 0.03	0.85 ^{NS}
5	4.28 ± 0.03	4.19 ± 0.03	4.28 ± 0.02	4.27 ± 0.03	1.23 ^{NS}
6	4.13 ± 0.03	4.07 ± 0.03	4.16 ± 0.02	4.16 ± 0.03	1.17 ^{NS}
7	4.03 ± 0.02	4.00 ± 0.03	4.033 ± 0.02	4.03 ± 0.03	0.14 ^{NS}
8	3.92 ± 0.02	3.87 ± 0.02	3.89 ± 0.02	3.89 ± 0.02	0.79 ^{NS}
9	3.79 ± 0.02	3.75 ± 0.02	3.79 ± 0.02	3.7 ± 0.02	0.48 ^{NS}
10	3.66 ± 0.01	3.63 ± 0.02	3.66 ± 0.02	3.67 ± 0.02	0.43 ^{NS}
11	3.53 ± 0.01	3.52 ± 0.01	3.54 ± 0.02	3.52 ± 0.02	0.29 ^{NS}
12	3.38 ± 0.01	3.41 ± 0.01	3.39 ± 0.02	3.39 ± 0.02	0.26 ^{NS}
13	3.27 ± 0.01	3.31 ± 0.01	3.28 ± 0.02	3.26 ± 0.02	0.92 ^{NS}
14	3.17 ± 0.01	3.20 ± 0.01	3.17 ± 0.02	3.16 ± 0.02	0.64 ^{NS}
15	3.07 ± 0.01	3.10 ± 0.02	3.07 ± 0.02	3.06 ± 0.02	0.67 ^{NS}
16	2.96 ± 0.01	2.99 ± 0.02	2.98 ± 0.02	2.97 ± 0.02	0.41 ^{NS}
17	2.86 ± 0.01	2.90 ± 0.02	2.87 ± 0.01	2.88 ± 0.02	0.58 ^{NS}
18	2.76 ± 0.02	2.81 ± 0.03	2.77 ± 0.02	2.80 ± 0.02	1.23 ^{NS}
19	2.67 ± 0.02	2.74 ± 0.03	2.68 ± 0.02	2.72 ± 0.01	2.00 ^{NS}
20	2.61 ± 0.02	2.65 ± 0.02	2.60 ± 0.02	2.63 ± 0.02	0.66 ^{NS}
21	2.54 ± 0.02	2.57 ± 0.03	2.54 ± 0.02	2.54 ± 0.02	0.27 ^{NS}
22	2.45 ± 0.02	2.49 ± 0.03	2.42 ± 0.02	2.45 ± 0.02	1.51 ^{NS}
23	2.34 ± 0.02	2.41 ± 0.02	2.32 ± 0.02	2.37 ± 0.03	2.45 ^{NS}
24	2.26 ± 0.02	2.32 ± 0.02	2.23 ± 0.03	2.29 ± 0.02	2.35 ^{NS}
25	2.16 ± 0.03	2.21 ± 0.03	2.15 ± 0.02	2.18 ± 0.02	0.92 ^{NS}
26	2.08 ± 0.03	2.13 ± 0.02	2.04 ± 0.03	2.11 ± 0.03	1.31 ^{NS}
27	2.00 ± 0.02	2.04 ± 0.08	1.94 ± 0.04	2.00 ± 0.03	1.38 ^{NS}
28	1.88 ± 0.03	1.92 ± 0.04	1.80 ± 0.05	1.88 ± 0.03	1.75 ^{NS}
29	1.72 ± 0.04	1.70 ± 0.05	1.66 ± 0.04	1.73 ± 0.03	1.42 ^{NS}
X	5.66 ± 0.06	5.60 ± 0.05	5.83 ± 0.07	5.50 ± 0.05	2.38 ^{NS}
Y	1.55 ± 0.04^{a}	1.48 ± 0.06^{a}	1.78 ± 0.06^{b}	1.96 ± 0.06^{b}	12.41**

** Significant (P<0.01); ^{NS}: Non - significant; Means bearing different superscripts differ significantly (P<0.01).

Arm ratio

The magnitude of the arm ratio indicates the nature of the chromosome, and alterations in the arm ratio denotes structural variations in the concerned chromosome pair (29). In the present study, the means for arm ratio for X -chromosome in Kangayam, Pulikulam, crossbred Jersey and crossbred Holstein Friesian bulls were 2.14 ± 0.08, 2.06 ± 0.10, 2.11 ± 0.04 and 2.16 ± 0.07, respectively. The mean arm ratios of Y-chromosome was 1.18 ± 0.03 in crossbred Jersey and 1.16 ± 0.03 in crossbred Holstein Friesian. The values found in the present study were in agreement with the earlier reports in Deoni (Balaji *et al*., 2006); Kangayam (Kumarasamy *et al*., 2008); Umblachery (Kumarasamy *et al*., 2006); Sahiwal and Jersey crossbreds (Subramanyam, 2013); Punganur (Bharathi *et al*., 2015); Malnad Gidda (Suresh *et al*., 2015); Ongole (Bharti *et al*., 2017) and Alambadi (Parameswari *et al*., 2019). However, all these findings confirmed the sub-metacentric nature of X-chromosome in indigenous and crossbred bulls; and metacentric nature of the Y-chromosome in crossbred Jersey and Holstein cattle.

Centromeric Index

Centromeric index is a morphological characteristic of the bi-armed chromosomes which determines the location

of the centromere. Chromosomes were identified in pairs based on the relative length and position of the centromere (Harshini, 2017). The mean centromeric indices of X-chromosome were 0.32 ± 0.007, 0.34 ± 0.011, 0.35 ± 0.004 and 0.32 ± 0.006 in Kangayam, Pulikulam, crossbred Jersey and crossbred Holstein bulls, respectively, while the means of Y-chromosome were 0.46 ± 0.007 and 0.46 ± 0.006 in crossbred Jersey and crossbred Holstein Friesian respectively. These values indicate the sub-metacentric nature of the X -chromosome in cattle, as the centromere was little away from the midpoint, and metacentric nature of the Y-chromosome in crossbred cattle due to midpoint positioning of the centromere. These values were in agreement with the findings of earlier researchers compared.

Morphological index

The morphological index is directly proportional to the total length of the chromosome. Hence, change in the length of the chromosome results in variation in morphologicl index. The means for morphological index of X-chromosome were 4.20 ± 0.18, 4.22 ± 0.31, 4.56 ± 0.27 and 4.38 ± 0.15 in Kangayam, Pulikulam, crossbred Jersey and crossbred Holstein bulls, respectively; while, the values for Y-chromosome were 3.48 ± 0.1 and 3.57 ± 0.2 in crossbred Jersey and Holstein bulls. These values concurred with the earlier studies on Deoni (Balaji *et al*., 2006), Sahiwal and Jersey crossbreds (Subramanyam, 2013), Punganur (Bharathi *et al*., 2015), Ongole (Bharti *et al*., 2017) and Alambadi (Parameswari *et al*., 2019) breeds of cattle.

CONCLUSION

Chromosomal number and morphology are the primary requirements for thorough understanding of the genetics of a species and any deviation from such as nombre fundamentale (NF) would pave the way for the determination of fertility related problems. In this study, the crosses have been made between taurine and indicine breeds which are classified as two different sub-species of cattle, and investigating the chromosomal structural variations becomes mandatory to make decisions on selection for semen production thereby preventing the possible interference in the process of reproduction. From the results obtained, it could be concluded that the sires chosen for frozen semen production are devoid of any chromosomal abnormality, suggestive of adoption of sound breeding strategies in selecting high genetic merit bulls.

ACKNOWLEDGEMENTS

The financial support research grant provided by Department of Animal Genetics and Breeding, Madras Veterinary College, Chennai is gratefully acknowledged.

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