The Pharma Innovation Journal 2020; 9(1): 139-142

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(1): 139-142
© 2020 TPI
www.thepharmajournal.com
Received: 22-11-2019
Accepted: 24-12-2019

S Vani
Department of Animal Genetics and Breeding, College of Veterinary Science, Proddatur, Andhra Pradesh, India

K Sakunthala Devi
Department of Animal Genetics and Breeding, College of Veterinary Science, Proddatur, Andhra Pradesh, India

M Kalyan Chakravarthy
Livestock Farm Complex, College of Veterinary Science, Proddatur, Andhra Pradesh, India

V Manasa
Department of Veterinary Biochemistry, College of Veterinary Science, Proddatur, Andhra Pradesh, India

Corresponding Author:
S Vani
Department of Animal Genetics and Breeding, College of Veterinary Science, Proddatur, Andhra Pradesh, India

Karyological study on Murrah buffalo

S Vani, K Sakunthala Devi, M Kalyan Chakravarthy and V Manasa

Abstract
Eleven Murrah buffaloes were utilized to study the basic chromosomal characteristics using Short term lymphocyte culture technique. The karyotypes were studied to find out numerical chromosomal abnormalities by measuring relative length (%) as well as centromeric index of each chromosome. Diploid chromosomal number were found to be 50 including XY in males and XX chromosomes in females. Each karyotype consisted of 5 pairs of submetacentric and 19 pairs of acrocentric chromosomes. X chromosome was found to be the largest acrocentric chromosome. The relative length of chromosome no. 1 was found to be highest in both males and females, while that of Y chromosome was found to be least in males.

Keywords: Murrah buffalo, karyotype, relative length, centromeric index, arm ratio

Introduction
Most of the Indian buffaloes are of Riverine type and are very important for the small and marginal farmers of the country. Murrah is one of the well defined breeds of Indian buffaloes in the native tract of Punjab and Haryana. It is large in size, Jet black in colour with tightly curled horns. Murrah buffalo is known for its highest milch production of the country. The science of cytogenetics, has got important role to play in farm animal breeding since last few decades. The study pertaining to full set of metaphase spread of chromosomes (karyotype) is necessary to know the basic cytogenetic characteristics of a species or breed. Karyotype of an apparently normal animal helps in identifying cytogenetically abnormal animal, which otherwise, may propagate the abnormalities in the subsequent generations (Gaurav *et al*., 2016) [1]. Embryonic and fetal abnormalities can be reduced by about 20 – 30 % in farm animals by chromosomal screening (Roberts, 1971). So it is important to screen the breeding animals for cytogenetic abnormalities. The information pertaining to cytogenetic characteristics of Murrah buffalo is very scanty and there are many scopes to carry out the basic and advanced cytogenetic studies of the animals. So to start with, the present study was carried out to screen the Murrah buffalo animals for any cytogenetic abnormalities through karyotyping.

Meterials and Methods
Blood samples were collected under aseptic conditions using sterile heparinised vacuitainers from eleven murrah buffaloes (3M and 8F) maintained at Livestock farm complex, College of Veterinary Science, Proddatur, Andhra Pradesh. Cold chain was maintained to transfer the samples to the laboratory. The lymphocytes were multiplied using the peripheral lymphocyte culture technique proposed by Moorehead *et al*., 1960 [3]. For preparing cocktail media, 8 ml of RPMI 1640 taken into a cell culture flask and 0.2ml phytohemagglutinin-M (a potent mitogen), 2.5ml of FBS, antibiotic and antimycotics were added. 0.5 ml of blood sample was dropped into the culture flask and mixed well. The culture bottles were incubated at 37 ^{o}C, and 5% CO_2 for 72 hours. One hour prior to completion of incubation, colchicine was added and contents were mixed well, followed by further incubation upto 72 hours. Then the culture media was transferred to 15ml centrifuge tubes and centrifuged at 1500 rpm for 10minutes, the supernatant was discarded and the pellet was suspended in freshly prepared and pre-warmed 8 ml of hypotonic solution (0.075 M KCl) for 45 minutes at 37 ^{o}C. Supernatant was discarded after centrifugation at 1,000 rpm for 10 minutes. Cells were fixed in fresh cool fixative (3 methanol: 1 glacial acetic acid) to make up volume up to 8 ml then centrifuged at 1,500 rpm for 10 minutes, and the supernatant was discarded. The fixative washings were repeated until the supernatant was clear and the pellet turns into white colour. The cell suspension was dropped onto a clean and chilled glass slide by using micropipette, and allowed for air-drying.

The slide was conventionally stained with 4% Giemsa solution for 30 minutes.

Metaphase spreads and karyotyping

Chromosome counting was performed on mitotic metaphase cells using phase contrast microscope. Ten well spread metaphases from each male and female were selected and photographed. The length of short arm and long arm of the chromosomes were measured and total length of the chromosome was calculated. Relative length (RL), centromeric index (CI) and morphological index (MI) of the chromosomes were estimated. These measurements were used for preparation of karyotypes.

Results and Discussion

The present cytogenetic study using peripheral lymphocyte culture technique revealed that, the diploid chromosome number of the Murrah buffalo is 50. Similar results were reported for river buffaloes by Chauhan *et al*. 2009 and Murali *et al*., 2009, Kenthao *et al.* 2012, Gaurav *et al.*, 2016 [4, 5, 6, 1]. The first 5 pairs of autosomes were found to be sub metacentric, and the remaining 19 pairs were acrocentric. The X and Y chromosome of Murrah buffalo were found to be largest and smallest acrocentric chromosomes respectively. These features are similar to the reports of Bondoc *et al*., 2002, Murali *et al*. 2009, Kenthao *et al*., 2012 and Gaurav *et al.*, 2016) [1]. Metaphase spreads and karyotypes of the Murrah buffalo are presented in Fig. 1 and 2.

The relative length of chromosomes in Murrah buffaloes are presented in Table.1. The idiogram is prepared based on percent relative length of chromosomes and are presented in Fig.3. The mean relative length of autosomes in Murrah buffaloes ranged from 1.96±0.22 to 7.18±0.28. Based on the mean relative lengths, it is confirmed that X-chromosome had highest relative length (6.10±0.53) among the acrocentric chromosomes and is considered as largest acrocentric chromosome whereas Y chromosome is considered as smallest acrocentric chromosome (1.86±0.11). The findings of the present study are in accordance with the reported values by Gaurav *et al*. 2016 [1].

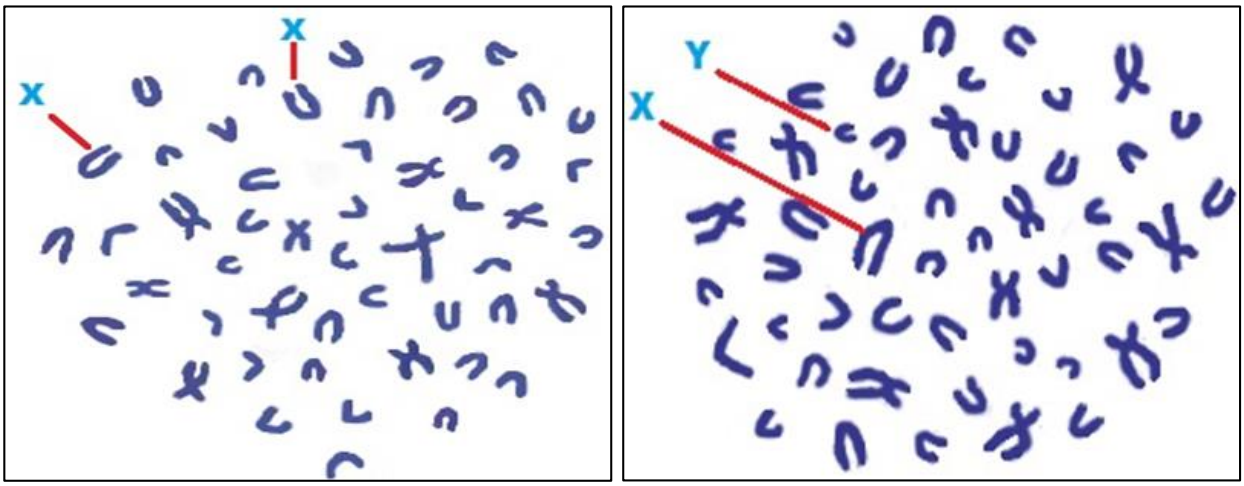
Mean values of arm ratio, centromeric index, and morphological index of first 5 pairs of sub metacentric autosomes are presented in Table.2. The overall mean arm ratio, centromeric index, and morphological index of first 5 pairs of autosomes ranged from 1.17±0.18 to 1.67±0.16, 0.33±0.07 to 0.38±0.07 and 3.56±0.07 to 4.28±0.08. The arm ratio and centromeric index values of the above autosomes confound to their sub metacentric nature.

Table 1: Mean relative lengths of murrah buffaloes

Chromosome	Male	Female	Total
1	7.13 ±0.38	7.20±0.24	7.18±0.28
2	5.64±0.30	5.47±0.24	5.51±0.64
3	5.46±0.31	5.15±0.26	5.24±0.57
4	5.07±0.30	5.11±0.26	5.07±0.45
5	4.97±0.46	4.85±0.29	4.91±0.44
6	4.91±0.29	4.73±0.22	4.67±0.41
7	4.52±0.46	4.58±0.27	4.67±0.39
8	4.23±0.22	4.44±0.25	4.37±0.39
9	4.19±0.48	4.25±0.30	4.24±0.37
10	4.04±0.24	4.19±0.22	4.12±0.34
11	3.93±0.47	4.02±0.22	4.03±0.33
12	3.64±0.40	4.01±0.34	3.91±0.33
13	3.64±0.28	3.85±0.34	3.76±0.32
14	3.52±0.37	3.70±0.20	3.68±0.32
15	3.48±0.31	3.56±0.25	3.47±0.32
16	3.21±0.27	3.35±0.22	3.38±0.32
17	3.19±0.42	3.31±0.38	3.22±0.31
18	2.99±0.43	3.19±0.71	3.19±0.30
19	2.97±0.32	3.05±0.45	2.96±0.28
20	2.71±0.32	2.94±0.61	2.95±0.27
21	2.47±0.14	2.72±0.49	2.65±0.25
22	2.23±0.12	2.45±0.46	2.39±0.23
23	2.09±0.11	2.23±0.39	2.19±0.23
24	2.00±0.07	1.95±0.27	1.96±0.22
X	5.91±0.39	6.17±0.56	6.10±0.53
Y	1.86±0.11	-	1.86±0.11

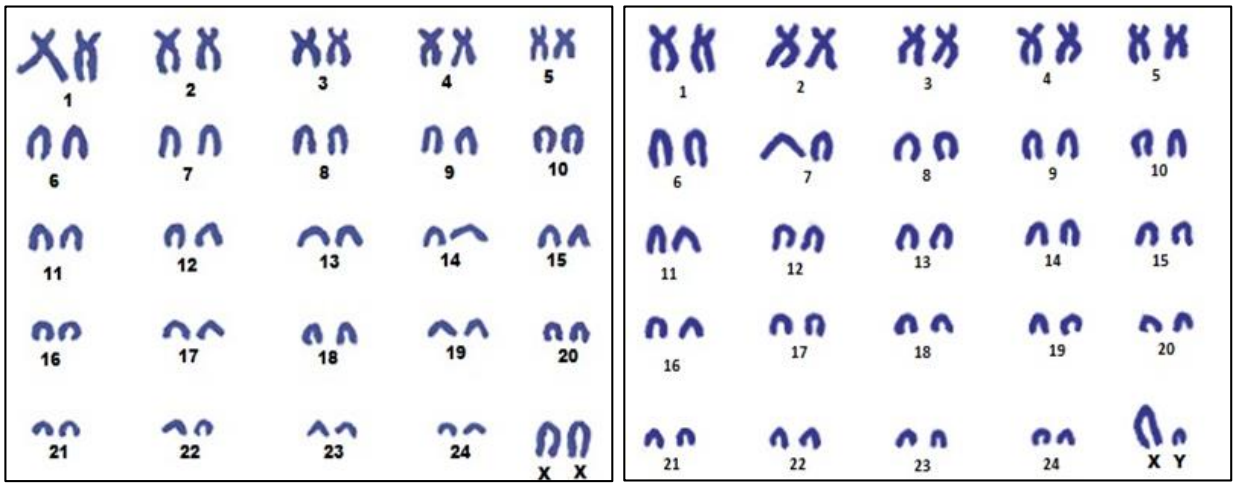
Table 2: Mean values of arm ratio, centromeric index, and mophological index of first 5 pairs of sub metacentric autosomes in Murrah buffaloes

Female			Male			Overall		
AR	CI	MI	AR	CI	MI	AR	CI	MI
1.72±0.11	0.33±0.12	4.19±0.05	1.62±0.21	0.32±0.02	4.36±0.12	1.67±0.16	0.33±0.07	4.28±0.08
1.58±0.02	0.31±0.03	3.52±0.11	1.56±0.04	0.38±0.01	3.60±0.17	1.57±0.03	0.35±0.02	3.56±0.07
1.51±0.21	0.37±0.07	3.63±0.14	1.54±0.10	0.37±0.04	3.58±0.18	1.53±0.16	0.37±006	3.60±0.10
1.46±0.18	0.37±0.12	3.55±0.22	1.39±0.17	0.39±0.02	3.66±0.12	1.43±0.18	0.38±0.07	3.61±0.17
1.14±0.14	0.33±0.18	4.17±0.26	1.24±0.23	0.36±0.06	4.01±0.10	1.17±0.18	0.35±0.12	4.09±0.18



Female Male

Fig 1: Metahase spread of Murrah Buffalo



Female Male

Fig 2: Karyotype of Murrah buffalo

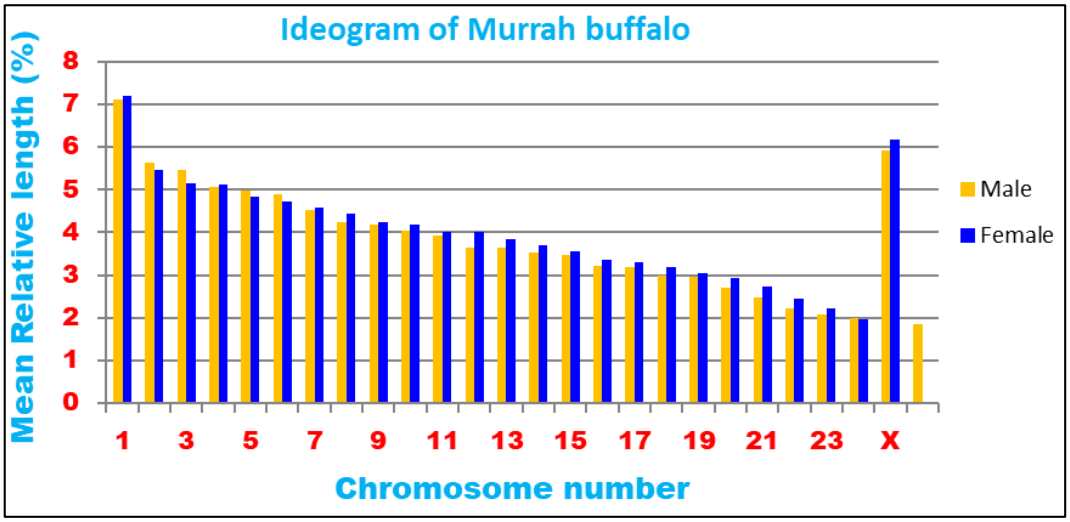


Fig 3: Ideogram showing mean relative length of chromosomes in Murrah buffalo

Conclusion

The modal chromosome number of Murrah buffalo is found to be 50, which constituted 5 pairs of sub metacentric and 19 pairs of acrocentric autosomes and X & Y chromosomes. Various morphometric measurements, suggested that the chromosome architecture of Murrah buffalo is similar to that of different recognized breeds of *Bubalus bubalus.* All the breedable animals of Livestock farm complex, CVSc., Proddatur are found to be free of chromosomal abnormalities.

Acknowledgement
We thank sincerely to Sri Venkateshwara Veterinary University for sanctioning project entitled "Cytogenetic screening of reproductive disorders for cattle and buffaloes" and providing necessary fund to carry out the present study.

References

1. Gaurav Pandya, Nikhil Dangar, Mamta Janmeda, Yogendrasingh Gadhavi, Balkrushna Brahmkshtri, Vishnu Kharadi *et al*. Standard karyotype of surti buffalo from an Organized farm. International Journal of Science, Environment and Technology. 2016; 5(3):1108-1115.
2. Roberts SJ. Veterinary Obstetrics and Genital Diseases (Theriogenology). 2nd End., Edward Bros. Ann. Arbor, Michigon, USA. c.f. Ahmad *et al*, 1971.
3. Moorehead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leucocytes cultured from human peripheral blood. Exp. Cell Res. 1960; 20:613-616.
4. Chauhan JB, Patel RK, Singh KM, Soni KJ. Impact of a novel cytogenetic finding (unusual X; X translocation) on fertility of a buffalo bull (*Bubalus bubalis*). Buffalo Bull. 2009; 28(3):151-153.
5. Murali N, Devendran P, Panneerselvan S. Cytogenetic studies on the chromosomes of Toda buffaloes. Buffalo Bulletin. 2009; 28(2):95-100.
6. Kenthao A, Tanomtong A, Supanuam P, Pinyoteppratan C, Muangprom P, Buranarom K *et al*. Standardized karyotype and idiogram of mehsani buffaloes, *Bubalus Bubalis* by conventional staining, GTG-banding, CBG-banding and AG-NOR Banding techniques. Buffalo Bulletin. 2012; 31(1):24-39.
7. Bondoc OL, Flor MCGT, Rebollos SDN, Albarace AG. Variations in Karyotypic Characteristics of Different Breed Groups of Water Buffaloes (*Bubalus bubalis*). Asian-Aust. J Anim. Sci. 2002; 15(3):321-325.