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STANDARD KARYOTYPE OF SURTI BUFFALO FROM AN ORGANIZED FARM

Gaurav Pandya*, Nikhil Dangar[1], Mamta Janmeda, Yogendrasingh Gadhavi, Balkrushna Brahmkshtri[1] and Vishnu Kharadi[2]

Dept. of AGB, Veterinary College Navsari Agricultural University, Navsari
[1]Dept. of ILFC, Veterinary College Navsari Agricultural University, Navsari
[2]Livestock Research Station, Navsari Agricultural University, Navsari
E-mail: gmpandya@nau.in (**Corresponding Author*)

Abstract: Karyotypes, to study the basic chromosomal characteristics, of 6 male and 3 female Surti buffaloes maintained at Livestock Research Station, Navsari Agricultural University, Navsari were prepared. Short term lymphocyte culture technique was used to prepare the karyotypes. The karyotypes were studied for numerical abnormalities and relative length (%) as well as centromeric index of each chromosome. Diploid number of the chromosomes 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: Surti buffalo, Karyotype, Relative length, Centromeric index.

Introduction

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. 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.

Most of the Indian buffaloes are of Riverine type and are very important for the small and marginal farmers of the country. Surti is one of the well defined breeds of Indian buffaloes in the native tract of central and southern part of Gujarat. Surti buffalo is medium in body size as compared to heavy breeds, consumes less feed, thrives well both on limited and without green, and produce milk with higher fat and SNF content. It is maintained easily by

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landless, small and marginal farmers. These animals are preferred by city milk producers, due to its body size and regularity in calving.

The information pertaining to cytogenetic characteristics of Surti 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 Surti buffalo animals for any cytogenetic abnormalities through karyotyping.

MATERIALS AND METHODS

Blood samples from 6 male and 3 female buffalo calves were collected maintained at Livestock Research Station, Navsari Agricultural University. Short term lymphocyte culture technique for karyotyping and measurement of centromeric index and relative length of the chromosomes were followed as described by Bhattacharya et al. (2008) with minor modifications.

Describing briefly, approx. 5 ml of blood was collected aseptically in heparinized vacutainers from young male and female buffalo calves and was brought to the department laboratory under ice. One ml of the blood was mixed with 9.0 ml of RPMI 1640 media containing 10 % serum, 2.2 g sodium biocarbonate, 1 x antibiotic – antimycotic and Pokeweed @0.1 mg / 10 ml of media as mitogen, aseptically. The culture was allowed to incubate for 71 hrs at 37^0 c temperature and 5 % CO_2 in a humidified incubator. At the end of 71 hrs, colchicine @ 6.4 µg / 10 ml culture was added to the culture to arrest the cells in metaphase stage and the culture was again incubated for 1 hr. At the end of 1 hr, the culture was centrifuged at 1500 rpm for 15 minutes. The supernatant was discarded and the pellet was suspended in freshly prepared and pre-warmed 10 ml of hypotonic solution (0.075 M KCl) for 40 minutes. The hypotonic treatment was terminated by adding 1 ml of freshly prepared chilled fixative (3:1 Methanol: Glacial Acetic acid). The solution was mixed thoroughly and the content of the tubes were again centrifuged for 10 minutes and 1000 rpm. The supernatant was again discarded and cell pellet was resuspended in 5 ml chilled fixative. The process of washing the pellet with fixative was repeated thrice so as to get clear whitish pellet. After last wash, the supernatant was removed by serological pipettes leaving about 0.5 ml of the fixative over the pellet. The cells were suspended in the remaining fixative. The slides were prepared by air drying or flame drying method. Clean grease free slides were kept in chilled alcohol before about thirty minutes of the making of the slides. Three – four drops of the suspension were dropped on the slides by glass pipette from about two feet height. The slides were passed above the flames quickly for drying and subsequently were subjected to

staining. The slides were stained using Giemsa staining technique. The slides were kept in 4 % Giemsa staining solution for 30 minutes and then were rinsed in distilled water to remove the extra drops of the stains. The slides were allowed to dry for about two – three hrs at 37^0 c. The stained slides were evaluated under microscope under appropriate resolution. The good spreads from each slide were photographed. The chromosomes from the good quality photographs were cut and arranged according to their length to prepare the karyotype of each animal.

Relative length and Centromeric index

The length of each chromosome was measured from tip to tip on the karyotype using dial type vernier caliper with an accuracy of 0.02 mm. The length of the short and long arm were calculated from centromere of the chromosome. The average of homologous pair of the chromosome of each animal, separately for male and female, was considered to calculate the relative length and centromeric index.

Relative length of each chromosome was estimated by dividing the mean length of each chromosome with the length of total haploid genome (including mean of X chromosome in females and Y in males). The relative length of each chromosome was expressed as percentage. Centromeric index was calculated as the length of short arm of the chromosome, multiplied by 100 and then divided by length of whole chromosome.

RESULTS AND DISCUSSION

Metaphase spreads under 100x oil immersion and karyotypes for male and female buffalo calves are shown in figure 1 and 2, respectively. Chromosomal studies of Surti buffalo revealed diploid number of chromosomes as 50. Surti, being riverine buffalo, the presence of diploid number of 50 chromosomes is in agreement with the previous findings of other riverine buffaloes (Ahmad *et al.*, 2004, Dawood et al., 2014, Murali et al., 2009). Karyotype revealed 5 chromosome pairs of sub metacentric and 19 autosomal chromosome pair alongwith X and Y chromosomes as acrocentric. This finding is in agreement with that reported in Anatolian water buffalo (Yavasoglu et al., 2014). However, metacentric and telocentric alongwith the submetacentric chromosomes have been reported in riverine buffaloes (Murali et al., 2009, Kenthao et al., 2012).

Relative length (%) and Centromerix index of each chromosome are presented in table 1. Relative length of chromosome no. 1 was found highest as chromosome 1 being largest chromosome. X chromosome has been found to be largest acrocentric chromosome. Relative length of chromosomes from longest to shortest in Surti buffalo was reported 6.92 $\pm$

0.35 to 2.21 ± 0.24 by Kumar and Yadav, 1991, which is slightly higher to that of males while lower to that of females in the present study.

Cytogenetic studies of calves before using them for breeding should be made a routine practice, especially on organized farms and bull production centres. This study is a very basic step to carry out advanced cytogenetic techniques and thereby identifying the genetically abnormal animals.

ACKNOWLEDGEMENT

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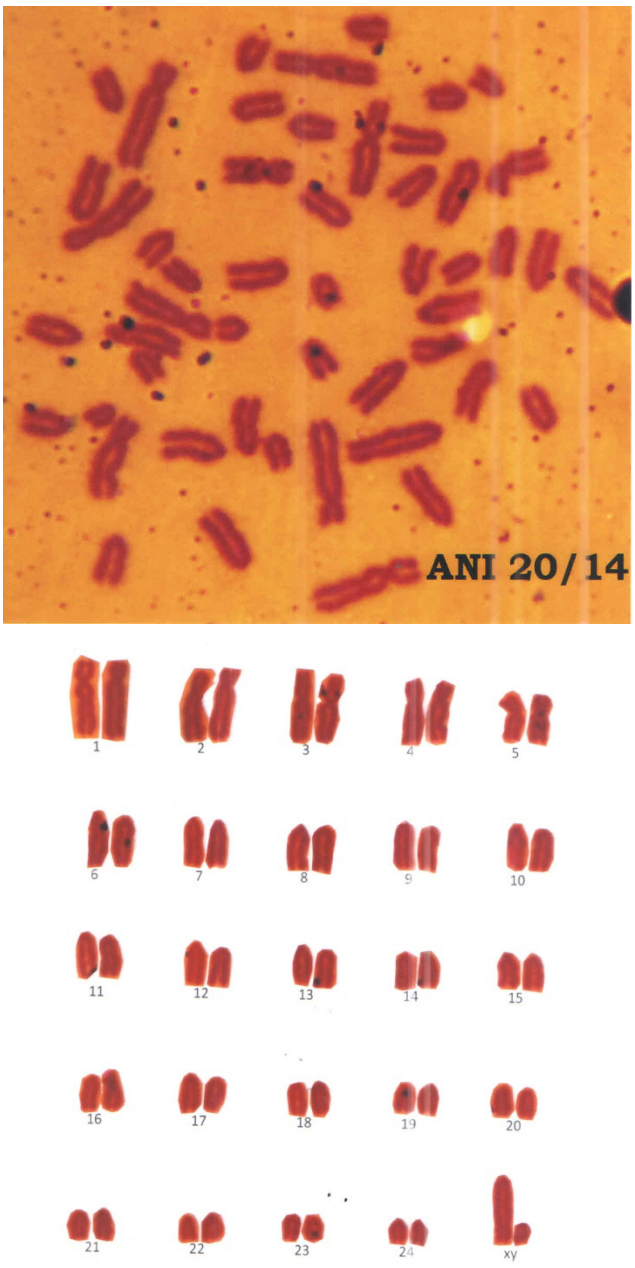


Photo 1: Metaphase spread and karyotype of male buffalo

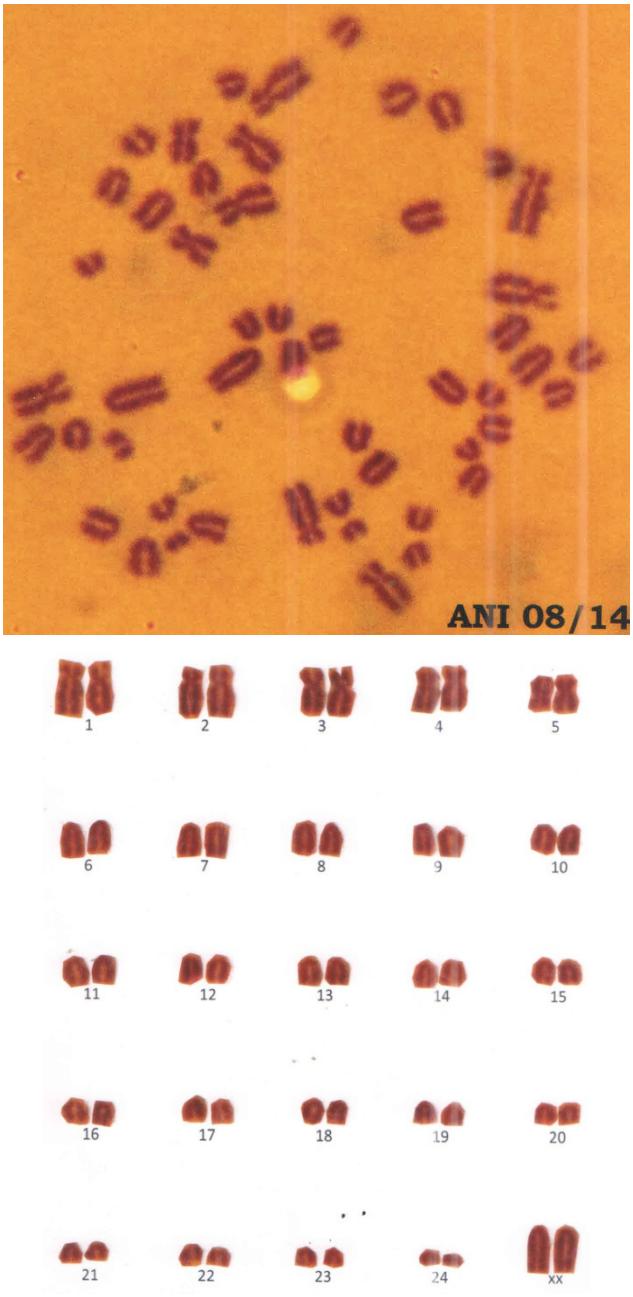
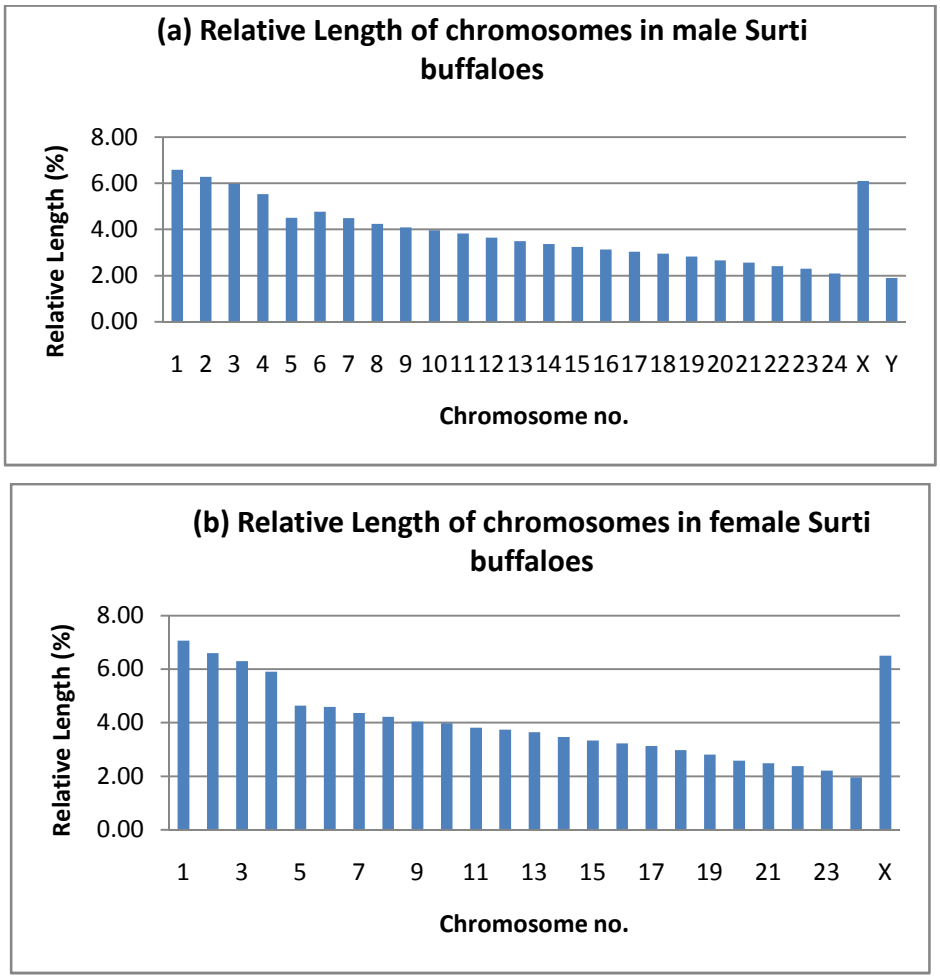


Photo 2: Metaphase spread and karyotype of female buffalo

Table 1: Centromeric index (CI) and Relative length (RL) (%) of chromosomes of Surti buffaloes

Chromosome no	Males (n = 6)		Females (n = 3)	
	CI ± SE	RL ± SE	CI ± SE	RL ± SE
1	27.67 ± 1.20	6.58 ± 0.09	25.63 ± 2.78	7.06 ± 0.13
2	29.89 ± 1.40	6.28 ± 0.08	34.36 ± 2.64	6.60 ± 0.05
3	35.96 ± 1.81	5.98 ± 0.04	36.94 ± 1.10	6.31 ± 0.05
4	32.35 ± 1.21	5.53 ± 0.07	30.00 ± 1.10	5.91 ± 0.11
5	35.63 ± 1.80	4.51 ± 0.22	37.01 ± 2.81	4.64 ± 0.06
6	0.00	4.77 ± 0.06	0.00	4.59 ± 0.03
7	0.00	4.50 ± 0.05	0.00	4.36 ± 0.02
8	0.00	4.24 ± 0.07	0.00	4.22 ± 0.08
9	0.00	4.09 ± 0.06	0.00	4.04 ± 0.05
10	0.00	3.97 ± 0.05	0.00	3.98 ± 0.03
11	0.00	3.82 ± 0.03	0.00	3.82 ± 0.04
12	0.00	3.65 ± 0.03	0.00	3.74 ± 0.05
13	0.00	3.50 ± 0.05	0.00	3.65 ± 0.06
14	0.00	3.37 ± 0.05	0.00	3.47 ± 0.04
15	0.00	3.24 ± 0.04	0.00	3.33 ± 0.05
16	0.00	3.14 ± 0.03	0.00	3.23 ± 0.08
17	0.00	3.03 ± 0.05	0.00	3.14 ± 0.10
18	0.00	2.95 ± 0.04	0.00	2.98 ± 0.12
19	0.00	2.83 ± 0.04	0.00	2.81 ± 0.07
20	0.00	2.67 ± 0.03	0.00	2.58 ± 0.05
21	0.00	2.56 ± 0.01	0.00	2.48 ± 0.02
22	0.00	2.41 ± 0.04	0.00	2.38 ± 0.03
23	0.00	2.30 ± 0.05	0.00	2.21 ± 0.01
24	0.00	2.09 ± 0.07	0.00	1.96 ± 0.09
X	0.00	6.10 ± 0.14	0.00	6.50 ± 0.16
Y	0.00	1.89 ± 0.04	-	-



Graph 1: Relative length (%) of chromosomes in (a) male and (b) female Surti buffalo

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