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CYTOGENETIC CHARACTERIZATION OF ONGOLE CATTLE

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ABSTRACT
Thirty eight Ongole cattle from its breeding tract were utilized to study the chromosome profile through short-term lymphocyte culture method. The diploid chromosome number was 60. All the 29 pairs of autosome were acrocentric, while X- was sub-metacentric and Y was acrocentric. The mean relative length of autosomes ranged from 1.92 ± 0.01 to 5.24 ± 0.02. The X-chromosome was the largest in the karyotype (5.42 ± 0.03), while the Y was the smallest (1.79 ± 0.02). The arm ratio, centromeric index and morphological index of X-chromosome were 1.87, 0.35 and 5.25, respectively. The present study revealed that the chromosome architecture of Ongole cattle was similar to that of the other breeds of Zebu cattle.

KEY WORDS: Ongole cattle, Chromosomes, Arm ratio, Autosomes, karyotype.

INTRODUCTION

Ongole is an excellent dual purpose cattle breed of India, and well noted for its body size, strength and sturdiness. By virtue of its adaptability traits and superior productive capacity under harsh tropical conditions, they are beneficial in tropical cattle production (FAO, 1953). Although native to coastal districts of Andhra Pradesh state, the breeds' well renowned adaptability to the adverse environmental conditions extends their spread throughout most of the America, Africa, the far-east and Australia. Indiscriminate cross breeding in the breeding tract resulted in breed dilution and a gradual decline in their number which may results in complete genetic extinction. Consequently, the conservation of this versatile germplasm is need of the hour (Reddy *et al*., 2016). Consequently, the conservation of this versatile germplasm is need of the hour. Cytogenetic studies are highly useful in genetic characterization and for effective conservation of the specie seriously at risk of extinction (Benirschke and Kumamoto, 1991). Research strategies involving cytogenetics hold the promise of yielding insight into the mechanisms underlying chromosome instability in embryos and the impact of the *in vitro* environment on the chromosome make-up of embryos (King, 2008). In addition, cytogenetic characterization of animals will help to identify the animals with congenital and acquired chromosome abnormalities whose elimination will help to maintain the herds cytogenetically clean. Therefore, the present study was undertaken with an objective of cytogenetic characterization of ongole breed, which is a fundamental step for the breeds' conservation.

MATERIALS & METHODS

A total of thirty eight Ongole cattle maintained at Livestock Research Station, Lam, Andhra Pradesh were utilized for the present study. About 5 ml of blood from the external jugular vein drawn into sterile heparinated vaccutiner tubes was used to set up cultures as per the short term lymphocyte culture method of Moorehead *et al*. (1966) with slight modifications. The cultures were set in a culture tube by adding 8 ml of RPMI 1640 medium, 0.20 ml of PHA-M, 2.5 ml of Fetal Bovine Serum, 0.2 ml of antibiotic antimycotic liquid and 0.80 ml of blood aseptically in the laminar airflow and incubated at 37.5ºC at 5% CO_2 concentration for 72 hours.

Colchicine (0.2 ml) was added to each culture tube, 45 minutes prior to harvesting. At the end of 72 hours, the culture vials were centrifuged at 1500 rpm for ten minutes. The supernatant fluid was discarded from each tube and about 7 ml of hypotonic solution (0.075M KCl) was added to cell pellet of each tube, held at 37.5^o C for 20 minutes, centrifuged at 1500 rpm for 10 minutes. The supernatant was removed and, freshly prepared and pre-chilled (-20^o C) Carnoy's fluid (8 ml) was added to the cell pellet, centrifuged and the supernatant was discarded. The washings with Carnoy's fluid were repeated several times till the pellet appears white. About 20ul of cel1 suspension was dropped on the slide held at 45^o angle from a height of two feet, stained with 2% or 4% Giemsa stain for 10 minutes, air dried and checked for metaphase spreads. All the good metaphase spreads were photographed and the best 10 were printed for karyotyping. Vernier callipers was used for measuring the arms and total length of chromosomes Relative length, Arm ratio, Centromeric index and Morphological index were estimated The relative lengths of each chromosome was measured as the percentage of it to the total haploid genome length (excluding Y-chromosome).

RESULTS & DISCUSSION

The mitotic metaphase chromosome spread of male and female Ongole cattle is presented in Fig. 1 and 3, respectively. The diploid chromosome number of Ongole cattle was found to be 60, which is in agreement with the

reports of Kumarasamy *et al.* (2006) in ongole, Kumar *et al.* (2003) in punganur, Balaji *et al.* (2006) in Deoni, Kumarasamy *et al.* (2008) in Umblachery and Faske *et al.* (2009) in Dangi cattle. The karyotype (Fig. 2 and 4), showed that the all 29 pairs of autosomes were acrocentric. The X-chromosome was sub-metacentric while Y was acrocentric in morphology suggesting that the breed belongs to *Bos indicus.* These findings are in accordance with reports of Kumarasamy *et al.* (2006) in Ongole, Balaji et *al.* (2006) in Deoni, Kumarasamy *et al.* (2008) in Umblachery and Faske *et al.* (2009) in Dangi.

The idiogram (Fig. 5) revealed that the rate of reduction of relative length (RL) of chromosomes was not uniform for first to second (17.2%) and second to third pair (6.9 percent), thereafter it was gradual. Furthermore, a steep decline was noticed between 28th and 29th chromosome (11.1%). The respective mean RL of autosomes was 1.99 ± 0.01 to 5.15 ± 0.02 and 1.85 ± 0.01 to 5.30 ± 0.03% for males and females with an overall range from 1.92 ± 0.01 to 5.24 ±0.02. The RL of the first autosome was 5.24 ± 0.02%, which is in agreement with reports of Balaji *et al*. (2006) in Deoni.

The mean relative lengths of chromosomes are presented in table. 1. The X-chromosome was the largest, which was corroborating with the reports of Balaji *et al.* (2006) in Deoni and Kumarasamy *et al.* (2008) in Umblachery cattle. The Overall mean RL of X chromosome was 5.42 ± 0.03%. It was 5.35 ± 0.05 and 5.35 ± 0.04% for males and females, respectively, which is in agreement with the findings of Kumarasamy *et al.* (2006) in Ongole. The Y was the smallest and contributed 1.79 ± 0.02% to the genome. Several scientists ((Kumar *et al.* (2003) in Punganur, Rao (1995) in Ongole, Nagpure *et al.* (2001) in Hariana and Kumar *et al.* (2003) in Punganur cattle) supported these findings. In the present study, the Y-chromosome contributed to 34.16% of the RL of first autosome. Likewise, Desai (1987) reported that Y-chromosome measured 35 % of RL of first autosome.

TABLE 1: Mean Relative Lengths of Chromosomes based on transformed data

Chromosome No.	Male	Female	Overall
1	13.10 ± 0.03^{q}	13.34 ± 0.04 ^{p}	13.22 ± 0.02^{a}
2	12.47 ± 0.02	12.50 ± 0.02	12.49 ± 0.02^{b}
3	12.15 ± 0.02	12.17 ± 0.02	12.16 ± 0.01^{c}
4	11.93 ± 0.02	11.98 ± 0.02	11.95 ± 0.01^{c}
5	11.77 ± 0.02 ^{q}	11.82 ± 0.02 ^{p}	11.79 ± 0.01 ^{c}
6	11.63 ± 0.02 ^{q}	11.67 ± 0.02 ^{p}	11.65 ± 0.01 ^{c}
7	11.49 ± 0.01	11.52 ± 0.02	11.50 ± 0.01 ^{c}
8	11.36 ± 0.01	11.37 ± 0.01	11.36 ± 0.01 ^{c}
9	11.22 ± 0.01	11.20 ± 0.01	11.21 ± 0.01 ^{c}
10	11.06 ± 0.01	11.03 ± 0.01	11.05 ± 0.01 ^{c}
11	10.86 ± 0.01	10.85 ± 0.01	10.85 ± 0.01 ^{c}
12	10.68 ± 0.01	10.67 ± 0.01	10.67 ± 0.01 ^{c}
13	10.51 ± 0.01	10.51 ± 0.01	10.51 ± 0.01 ^{c}
14	10.38 ± 0.01	10.35 ± 0.01	10.36 ± 0.01 ^{c}
15	10.24 ± 0.01 ^{p}	10.20 ± 0.01 ^{q}	10.22 ± 0.01 ^{c}
16	10.11 ± 0.01 ^{p}	10.08 ± 0.01 ^{q}	10.09 ± 0.01 ^{c}
17	9.97 ± 0.01 ^{p}	9.94 ± 0.01 ^{q}	9.96 ± 0.01 ^{c}
18	9.83 ± 0.01 ^{p}	9.80 ± 0.01 ^{q}	9.81 ± 0.01 ^{c}
19	9.70 ± 0.01	9.67 ± 0.01	9.68 ± 0.01 ^{c}
20	9.57 ± 0.01	9.55 ± 0.01	9.56 ± 0.01 ^{c}
21	9.43 ± 0.01	9.42 ± 0.01	9.42 ± 0.01 ^{c}
22	9.29 ± 0.02	9.26 ± 0.02	9.27 ± 0.01 ^{c}
23	9.15 ± 0.02 ^{p}	9.10 ± 0.02 ^{q}	9.13 ± 0.01 ^{c}
24	9.03 ± 0.02 ^{p}	8.96 ± 0.02 ^{q}	8.99 ± 0.01 ^{c}
25	8.89 ± 0.02 ^{p}	8.81 ± 0.02 ^{q}	8.85 ± 0.01 ^{c}
26	8.74 ± 0.02 ^{p}	8.66 ± 0.02 ^{q}	8.70 ± 0.01 ^{c}
27	8.58 ± 0.02 ^{p}	8.50 ± 0.02 ^{q}	8.54 ± 0.02 ^{c}
28	8.39 ± 0.02 ^{p}	8.26 ± 0.03 ^{q}	8.33 ± 0.02 ^{c}
29	8.12 ± 0.03 ^{p}	7.82 ± 0.03 ^{q}	7.96 ± 0.02^{d}
X	13.29 ± 0.05 ^{q}	13.58 ± 0.06 ^{p}	13.44 ± 0.04^{a}
Y	7.68 ± 0.04		7.68 ± 0.04^{e}

*Means with similar superscripts do not differ significantly (P≤ 0.05)

**a, b & c – Between different chromosomes; p & q – Between sexes.

TABLE 2: Mean Arm ratio, Centromeric index and Morphological index of X-chromosome

Sex	Arm Ratio	Centromeric Index	Morphological Index
Male	1.88 ± 0.023	0.35 ± 0.003	5.44 ± 0.11^{a}
Female	1.86 ± 0.02	0.35 ± 0.002	5.07 ± 0.10^{b}
Overall	1.87 ± 0.015	0.35 ± 0.002	5.25 ± 0.075

*Means with different superscripts in a column differ significantly

The mean arm ratio, centromeric index and morphological index of X-chromosome are presented in table 2. Arm ratio was 1.87 which is slightly lower than the reports of Balaji *et al.* (2006) in Deoni, Kumarasamy *et al.* (2006) in Ongole and Kumarasamy *et al.* (2008) in Umblachery, whereas, lower values 0.54 and 0.56 were reported by

Rao (1995) in Ongole and by Kumar *et al.* (2003) in Pungannur respectively. Further, the centromeric index of X- was 0.35, which confirmed its sub-metacentric nature and this was similar to reports of Balaji *et al.* (2006) in Deoni. The morphological index of X-was 5.25, which was higher than the reports of Balaji *et al.* (2006) in Deoni, and Kumar *et al.* (2003) in Punganur. However, centromeric index and morphological index were in agreement with the reports of Rao (1995) and Kumarasamy *et al.* (2011) in Ongole.

It was concluded that, the modal chromosome number in ongole cattle was (2n=60), which constituted 29 pairs of acrocentric autosomes and sub-metacentric X and acrocentric Y and various morphometric measurnments, suggested that the chromosome architecture of Ongole cattle is similar to that of different recognized breeds of *Bos indicus* cattle.

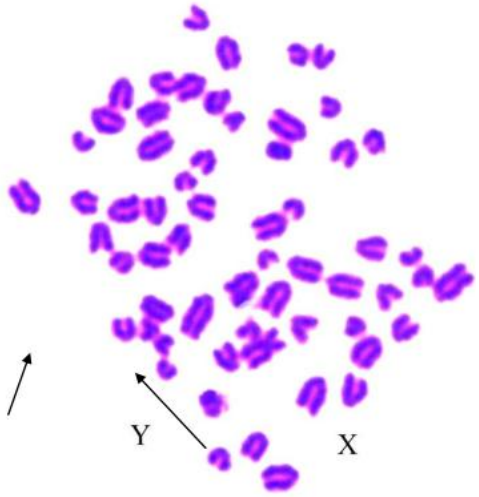

FIGURE 1: Metaphase spread on Ongole Bull

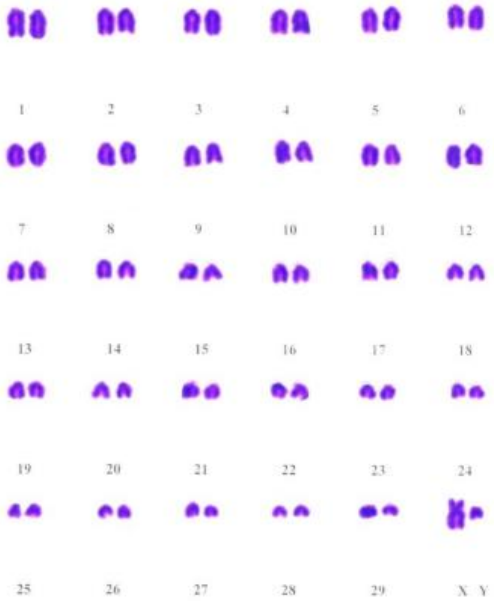

FIGURE 2: Karyotype on Ongole Cow

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