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Comparative study on cytogenetic profile of Ongole & Jersey x Sahiwal crossbred cattle

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Abstract
Thirty six Ongole and sixteen JerseyxSahiwal crossbred cattle were screened to study the chromosome profile by using peripheral short-term lymphocyte culture technique. The diploid chromosomal number was found to be 60 in both the breeds. All the 29 pairs of autosomes were found to be acrocentric, while X chromosome was sub-metacentric in both the cattle breeds. Y chromosome was found to be acrocentric in Ongole and submetacentric in JxS crossbred cattle. The mean relative length of autosomes ranged from 1.75±0.19 to 5.54±0.19 and 1.83±0.14 to 5.56±0.33 in Ongole and J x S crossbred cattle respectively. The X-chromosome was found to be largest sub metacentric in Ongole (5.42 ±0.31) and JxS crossbred (5.36 ±0.22), while the Y chromosome was the smallest acrocentric in Ongole (1.68 ± 0.05) and submetacentric in JerseyxSahiwal crossbred (2.55 ±0.31). Arm ratio and centromeric index of X chromosome in ongole cattle as well as and X & Y chromosomes in SahiwalxJersey crossbred cattle confirmed their sub metacentric nature.

Keywords: Ongole & Jersey x Sahiwal crossbred cattle, Mean relative lengths, morphometric measurements, karyotype

Introduction
Ongole is an excellent dual purpose cattle breed of India 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) [1]. Indiscriminate cross breeding in the breeding tract resulted in breed dilution and a gradual decline in their number which may result in complete genetic extinction. Consequently, the conservation of this versatile germplasm is need of the hour (Reddy *et al*., 2016) [2]. Sahiwal, considered as the best dairy cattle breed of Indian sub-continent well adapted to varied agro-climatic conditions of the country. Many synthetic breeds were developed as a result of upgradation or crossbreeding of indigenous breeds with exotic breeds for improving milk production in India.
Therefore, the present study was undertaken with an objective of cytogenetic characterization of Ongole and Jersey x Sahiwal crossbred cattle and to screen the young breeding bulls and cows from chromosomal abnormalities, so that it is useful to serve the farming community.

Materials & Methods
A total of thirty six Ongole cattle and sixteen JerseyxSahiwal crossbred cattle maintained at Livestock farm complex, College of Veterinary Science, Proddatur, Sri Venkateswara Veterinary University, Andhra Pradesh were utilized for the present study. About 2 ml of blood sample was collected from the external jugular vein into sterile heparinized vaccutiner tubes and used to set up cultures as per the short term lymphocyte culture method proposed by Moorehead *et al*. (1966) [3] with slight modifications. The cultures were set in a culture flasks by adding 8 ml of RPMI 1640 medium, 0.20 ml of PHA-M, 2.5 ml of Foetal Bovine Serum, 0.2 ml of antibiotic and antimycotic liquid. To this cocktail mixture, 0.5 ml of blood was inoculated under the laminar airflow and incubated at 37ºC, at 5% CO_2 for 72 hours. One hour prior to completion of incubation, 80 µl of colchicine was added to each culture flask. At the end of 72 hours, the culture was transferred into 15ml centrifuge tubes and were centrifuged at 1500 rpm for 10 minutes. The supernatant fluid was discarded from each tube and about 8 ml of hypotonic solution (0.075M KCl) was added slowly to cell pellet of each tube and the tubes were held in water bath at 37 ºC for 45 minutes and centrifuged at 1000 rpm for 10 minutes. The supernatant was removed and then, freshly prepared and pre-chilled (-20 ºC) Carnoy's fluid (8 ml) was added to the cell pellet, again 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 chilled glass slide held at 45º angle from a height of two feet and allowed to dry and then stained with 4% Giemsa stain for 30 minutes, then air dried and checked in microscope for metaphase spreads. All the good metaphase spreads were photographed and the best 5 were printed for karyotyping. The length of chromosomes were measured by using digital vernier callipers. All morphometric measurements like 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 spreads of male and female Ongole & JerseyxSahiwal crossbred cattle are presented in Fig. 1 and 2 respectively. The diploid chromosome number of both zebu and crossbred cattle was found to be 60, which is in agreement with the Cytogenetic characterization of Ongole cattle reported by Bharathi *et al.* (2015) [4] in punganur, Alok Bharti *et al* (2017) [5] in Ongole cattle. The karyotype of Ongole cattle (Fig. 3) showed that all 29 pairs of autosomes were found to be acrocentric and X-chromosome was sub-metacentric, while Y chromosome was acrocentric in morphology suggesting that the breed belongs to *Bos indicus*, Whereas the karyotype of JerseyxSahiwal crossbred cattle (Fig. 4) showed that all 29 pairs of autosomes were acrocentric, while the X and Y-chromosomes were sub-metacentric in nature, indicating the origin of Y-chromosome from *Bos taurus* cattle.

The mean relative length of chromosomes are presented in Table.1. The respective mean relative length of autosomes ranged from 1.75±0.19 to 5.54±0.19 and 1.83±0.14 to 5.56±0.33 in Ongole and JerseyxSahiwal crossbred cattle breeds. The X-chromosome was found to be the 2nd largest submetacentric chromosome in the karyotype both in the Ongole (5.42 ± 0.31) and crossbred (5.36 ± 0.22) cattle breeds. The Y chromosome was found to be the smallest acrocentric in Ongole cattle (1.68 ±0.05) smallest submetacentric in JerseyxSahiwal crossbred cattle (2.55 ±0.31). These findings are in accordance with reports of Nagpure *et al.* (2001) [6] in Hariana, Balaji *et al.* (2006) [7] in Deoni, Faske *et al.* (2009) [8] in Dangi, Kumarasamy *et al.* (2008) [9] in Umblachery, Subramanyam (2013) [10] in sahiwal and sahiwal cross, Bharathi *et al.,* (2015) [4] in Punganur cattle and Alok Bharti *et al.* (2017) [5] in Ongole cattle. In the present study, the Y-chromosome contributed to 30.32% and 45.86% of the relative length of first autosome in Ongole and JxS crossbred cattle breeds respectively. Desai (1987) [11] reported that Y-chromosome measured 35% of relative length of first autosome. The effect of sex was found to be non significant on relative lengths of all chromosomes in both the breeds. The idiogram is prepared based on percent relative length of chromosomes and presented in Fig. 5 & 6 which revealed that the rate of reduction of relative length of chromosomes was almost uniform from 1st to 29th chromosome in both breeds of cattle.

The mean arm ratio, centromeric index and morphological index of X-chromosome in Ongole cattle and X and Y chromosomes in J x S crossbred cattle are presented in Table 2 and 3 respectively.

The arm ratio, centromeric index and morphological index for the X-chromosome is 2.0±0.14, 0.37±0.18, 2.74±0.05 which is in accordance with the reports of Balaji *et al.* (2006) [7] in Deoni. Arm ratio of the chromosomes in the present study was higher than that reported by Bharathi *et al.,* 2015 [4] in Punganur, Alok Bharti *et al.,* 2017 [5] in Ongole cattle, whereas centromeric index and morphological index were found to be relatively lower.

The arm ratio, centromeric index and morphological index for the X-chromosome of JerseyxSahiwal crossbred cattle are 2.01±0.11, 0.37±0.23 and 2.71±0.08, respectively Whereas the corresponding values for the Y-chromosome are 1.67±0.06, 0.41±0.01 and 1.55±0.04, respectively. The present findings were on par with reports of subramanyam (2013) [10].

The t-test revealed non significant differences between the sexes for various morphometric measurements in both Ongole and JerseyxSahiwal crossbred cattle which is in agreement with the findings of Subramanyam, (2013) [10].

Table 1: Mean Relative Length of Chromosomes in Ongole and Jersey x Sahiwal crossbred Cattle

Ch. No	Ongole			Jersey x Sahiwal		
	Male	Female	Total	Male	Female	Total
1	5.62 ± 0.06	5.47 ± 0.32	5.54 ± 0.19	5.64 ± 0.53	5.41 ± 0.13	5.56 ±0.33
2	5.21 ± 0.51	5.13 ± 0.38	5.17 ±0.21	5.16 ± 0.53	5.03 ±0.38	5.06 ±0.43
3	4.93 ± 0.64	4.80 ± 0.34	4.86 ±0.31	4.75 ± 0.68	4.80 ±0.34	4.79 ±0.45
4	4.78 ± 0.45	4.59 ± 0.33	4.68 ±0.22	4.63 ± 0.51	4.61 ±0.31	4.62 ±0.36
5	4.62 ± 0.34	4.47 ± 0.21	4.51 ±0.25	4.32 ± 0.50	4.44 ±0.29	4.41 ±0.36
6	4.45 ± 0.24	4.29 ± 0.22	4.34 ±0.12	4.12 ± 0.58	4.29 ±0.28	4.25 ±0.38
7	4.04 ± 0.28	4.26 ± 0.26	4.18 ±0.14	3.94 ± 0.58	4.16 ±0.25	4.10 ±0.37
8	4.07 ± 0.19	4.03 ± 0.11	4.04 ±0.17	3.72 ± 0.59	4.03 ±0.24	3.95 ±0.38
9	3.85 ± 0.63	3.92 ± 0.20	3.89 ±0.09	3.56 ± 0.61	3.89 ±0.21	3.81 ±0.38
10	3.66 ± 0.62	3.86 ± 0.19	3.75 ±0.54	3.36 ± 0.62	3.76 ±0.19	3.66 ±0.39
11	3.52 ± 0.65	3.64 ± 0.27	3.60 ±0.43	3.22 ± 0.67	3.64 ±0.19	3.53 ±0.41
12	3.45 ± 0.18	3.56 ± 0.32	3.48 ±0.37	3.05 ± 0.80	3.51 ±0.21	3.49 ±0.48
13	3.32 ± 0.17	3.39 ± 0.33	3.38 ±0.23	3.79 ± 0.70	3.39 ±0.21	3.39 ±0.43
14	3.24 ± 0.19	3.28 ± 0.22	3.26 ±0.21	3.48 ± 0.49	3.28 ±0.22	3.33 ±0.32
15	3.14 ± 0.34	3.25 ± 0.24	3.16 ±0.54	3.24 ± 0.33	3.19 ±0.21	3.20 ±0.24
16	3.00 ± 0.29	3.07 ± 0.20	3.05 ±0.22	3.10 ± 0.28	3.07 ±0.20	3.08 ±0.22
17	2.89 ± 0.31	2.99 ± 0.24	2.96 ±0.44	2.77 ± 0.33	2.97 ±0.20	2.92 ±0.25
18	2.78 ± 0.45	2.91 ± 0.20	2.85 ±0.22	2.55 ± 0.41	2.88 ±0.20	2.80 ±0.30
19	2.71 ± 0.19	2.76 ± 0.13	2.75 ±0.22	2.71 ± 0.19	2.76 ±0.19	2.75 ±0.19
20	2.63 ± 0.27	2.68 ± 0.18	2.66 ±0.21	2.60 ± 0.22	2.67 ±0.18	2.65 ±0.19

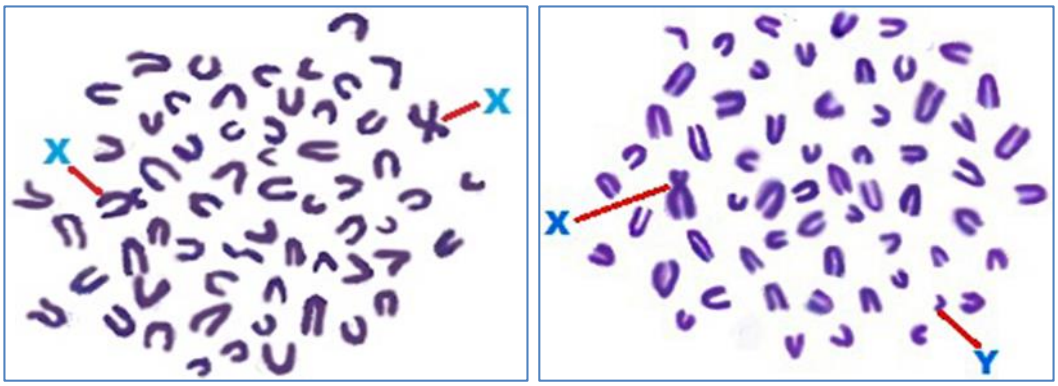
21	2.54 ± 0.25	2.63 ± 0.15	2.58 ±0.20	2.46 ± 0.24	2.59 ±0.17	2.55 ±0.19
22	2.43 ± 0.28	2.51 ± 0.16	2.47 ±0.12	2.30 ± 0.26	2.49 ±0.17	2.45 ±0.21
23	2.32 ± 0.31	2.40 ± 0.22	2.38 ±0.11	2.15 ± 0.32	2.40 ±0.17	2.40 ±0.24
24	2.25 ± 0.11	2.30 ± 0.26	2.28 ±0.13	2.68 ± 1.21	2.30 ±0.17	2.34 ±0.63
25	2.12 ± 0.21	2.18 ± 0.16	2.17 ±0.27	2.62 ± 1.04	2.18 ±0.18	2.29 ±0.57
26	2.02 ± 0.16	2.11 ± 0.18	2.05 ±0.25	2.42 ± 0.86	2.11 ±0.18	2.19 ±0.47
27	1.95 ± 0.15	1.99 ± 0.34	1.96 ±0.21	2.25 ± 0.73	1.99 ±0.15	2.06 ±0.40
28	1.85 ± 0.31	1.88 ± 0.43	1.87 ±0.14	1.85 ± 0.41	1.96 ±0.48	1.94 ±0.46
29	1.72 ± 0.29	1.78 ± 0.38	1.75 ±0.19	1.70 ± 0.31	1.88 ±0.09	1.83 ±0.14
X	5.51 ± 0.27	5.35 ± 0.34	5.42 ±0.31	5.53 ± 0.17	5.26 ±0.27	5.36 ±0.22
Y	1.68 ± 0.05	-	1.68 ±0.05	2.55 ± 0.31	-	2.55 ±0.31

Table 2: Mean values of arm ratios, centromeric index, and mophological index of allosomes in Ongole cattle

Chromosome number	Arm ratio	Centromeric index	Morphological index
Female	2.04±0.23	0.37±0.22	2.65±0.11
Male	1.96±0.03	0.38±0.11	2.83±0.01
Overall	2.0±0.14	0.37±0.18	2.74±0.05

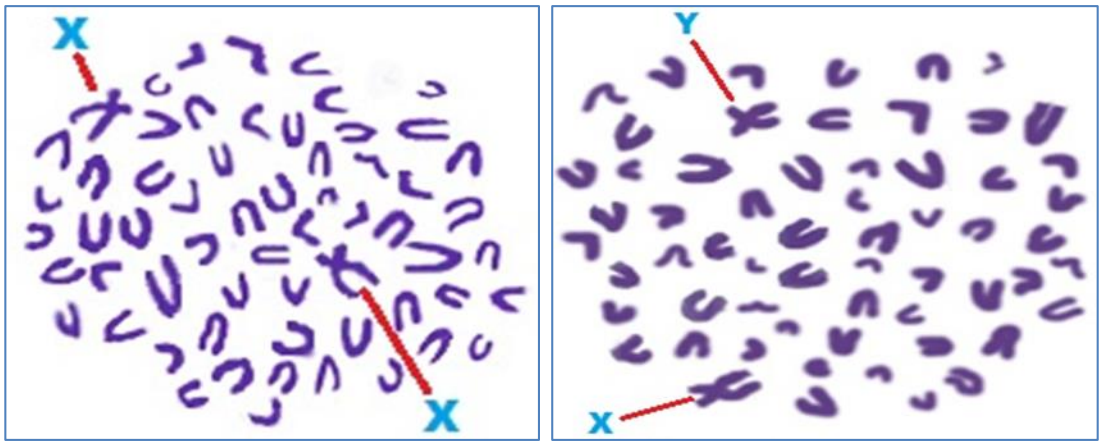
Table 3: Mean values of arm ratios, centromeric index, and mophological index of allosomes in Jersey x Sahiwal cattle

Chromosome number	Arm ratio	Centromeric index	Morphological index
X-chromosome	2.01±0.11	0.37±0.23	2.71±0.08
Y- chromosome	1.67±0.06	0.41±0.01	1.55±0.04
Overall	1.84±0.09	0.39±0.11	2.13±0.06



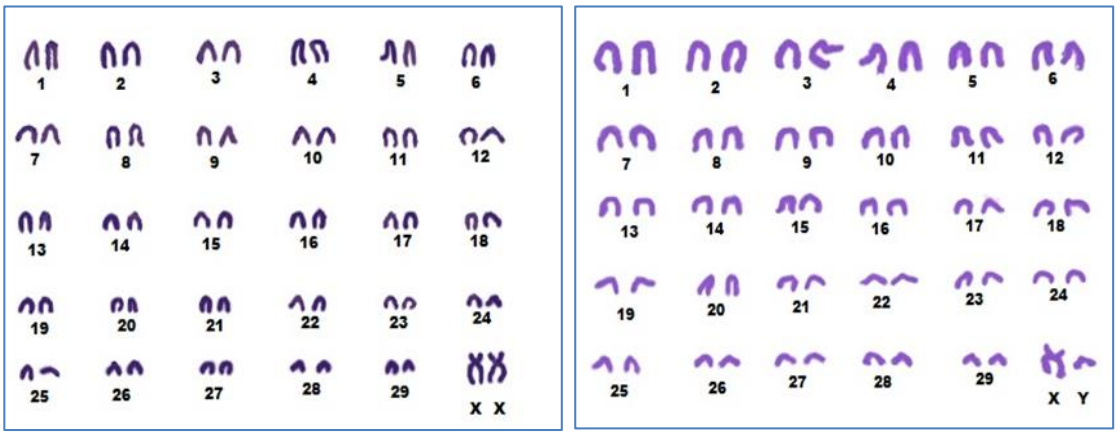
Female Male

Fig 1: Metaphase spread of Ongole cattle



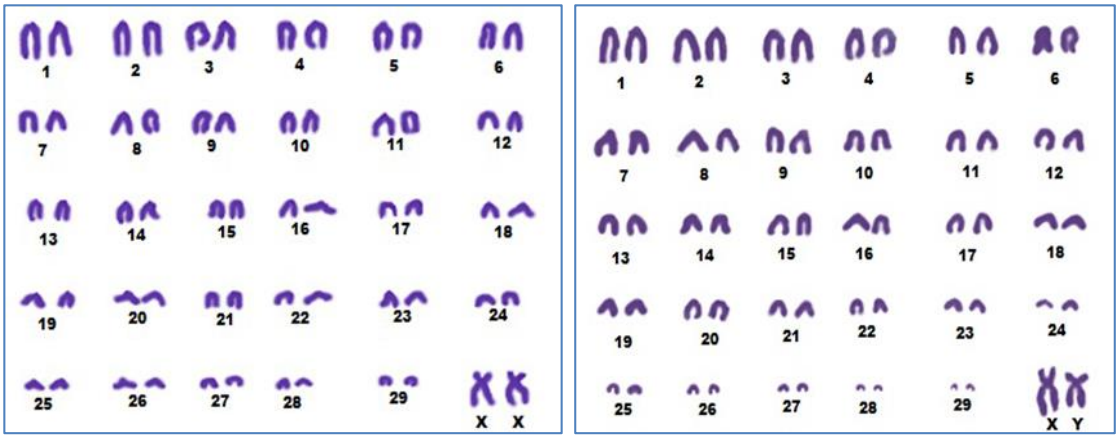
Female Male

Fig 2: Metaphase spread of Jersey x Sahiwal crossbred cattle



Female Male

Fig 3: Karyotype of Ongole cattle



Female Male

Fig. 4: Karyotype of Jersey x Sahiwal crossbred cattle

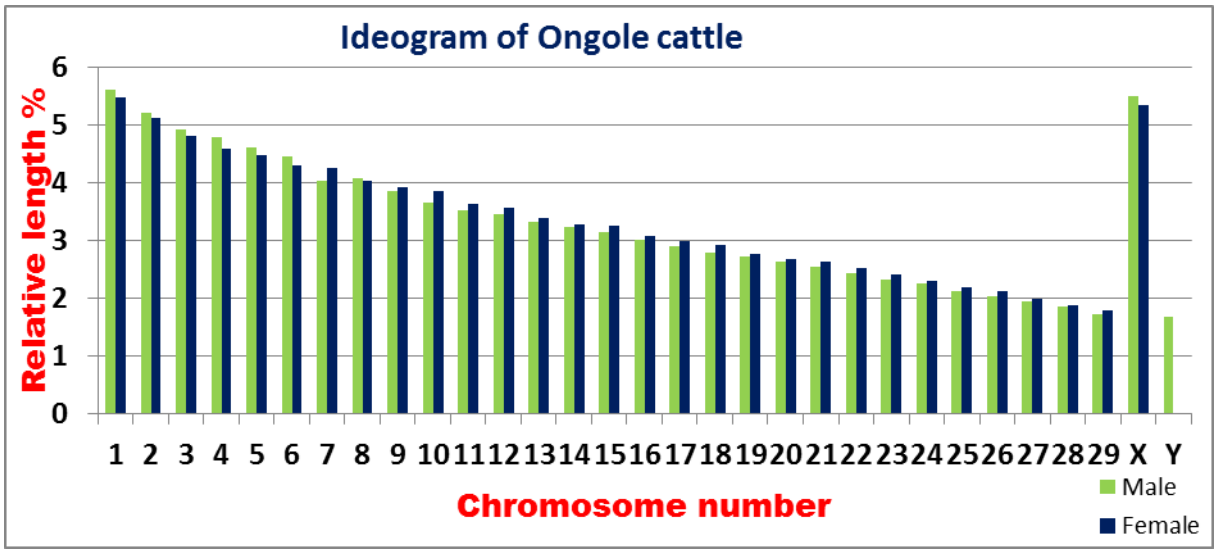


Fig 5: Ideogram of Ongole cattle

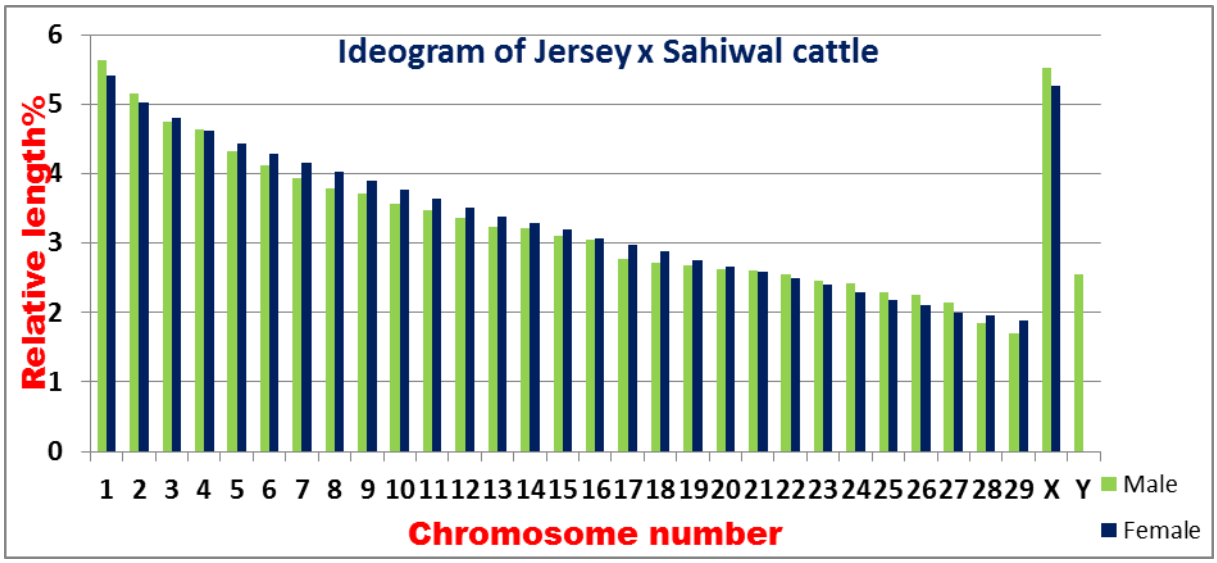


Fig 6: Ideogram of Jersey x Sahiwal crossbred cattle

Conclusion

It was concluded that, the modal chromosome number in ongole & JxS crossbred cattle was found to be 60, which constituted 29 pairs of acrocentric autosomes and sub-metacentric X & Y chromosomes in JxS crossbred cattle. The Y chromosome in Ongole cattle was found to be acrocentric. Various morphometric measurements, suggested that the chromosome architecture of Ongole cattle is similar to that of different recognized breeds of *Bos indicus* cattle, where for JerseyxSahiwal crossbred cattle it was found to be similar as in Bos taurus in cattle.

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