

## Cytogenetic characterization of Punganur cattle

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### Abstract

Blood samples of a total of 30 Punganur cattle (15 male and 15 female) maintained at Livestock Research Station, Palamaner and 5 Non-Descript (ND) animals from the surrounding areas of Palamaner were utilized for short term lymphocyte culture technique. The diploid chromosome number was found to be 60, XY consisting of 29 pairs of acrocentric autosomes and a pair of allosomes. The sub- metacentric X- chromosome was the longest and acrocentric Y-chromosome was the shortest. The mean relative lengths of autosomes ranged from 5.34 to 1.87 percent in Punganur cattle and 5.10 to 1.97 percent in ND cattle. The effect of sex was non significant on relative length of all chromosomes except 29<sup>th</sup> pair of autosome in ND cattle. The NOR-banding revealed that NOR regions were located on chromosomes 2, 3, 4, 5 and 28.

**Keywords:** Chromosome number, Karyotype, Nondescript, Punganur Cattle, Relative length, Nucleolar organizing region

### Introduction

Punganur is a dwarf breed of Indian cattle with short and stumpy horns. The home tract of this breed is former Punganur tahsil in Chittoor district of Andhra Pradesh. Its breed characteristics have been recently documented by National Bureau of Animal Genetic Resources, Karnal (AGRI-IS, 1999). In spite of possessing good adaptability and disease resistance, it was grossly neglected over the past decades due to the low milk production resulting in the erosion of this valuable germplasm. This breed is on the verge of extinction and project on its conservation is in progress at Livestock Research Station, Palamaner, Chittoor district, Andhra Pradesh. Persual of available literature indicates that there are very few reports on cytogenetic studies on

this breed. Hence, the present study was conducted to unveil the chromosome architecture in this breed.

### Materials and methods

The peripheral blood samples (2 to 3 ml per animal) from a total of 30 Punganur Cattle maintained at Livestock Research Station, Palamaner and 5 non-descript Cattle from surrounding area of Palamaner, Chittoor district were collected aseptically by puncturing the external jugular vein and cultured by short term lymphocyte culture technique (Moorehead *et al.*, 1960). Each culture was set up in a complete medium containing 8 ml RPMI-1640 (GIBCO, USA), 2.5 ml foetal bovine serum (GIBCO, USA), 0.2 ml phytohaemagglutinin (GIBCO, USA), 0.1 ml antibiotic and antimycotic

(GIBCO, USA), 0.1 ml heparin and 0.8 ml blood sample and cultured for 72 hours at 37.5<sup>0</sup>C and 5% Carbon-dioxide level. About 200 µl colchicines (Qualikem) was added to each culture flask, 55minutes prior to harvesting to arrest the mitoses. Harvesting was done using 0.075M potassium chloride as hypotonic solution and the fixation was done in freshly prepared cool Carnoy's fluid (methanol and acetic acid 3:1). Slides were stained with Giemsa stain for 10 minutes and metaphase spreads captured into the digital camera attached to the microscope using 'Progres' software (Olympus, Mumbai), under 100X magnification with oil immersion. The best metaphases were karyotyped as per the International System for the Cytogenetic Nomenclature of Domestic Animals (ISCNDA, 1990). The length of chromosomes was measured using digital vernier calipers (Mitutoyo, Japan) and the relative length, arm ratio, centromeric index and morphological indices were computed as per Kumarasamy *et al.* (2008). The NOR banding was undertaken as per the procedure described by Goodpasture and Bloom (1975) and Barch *et al.* (1997). The slides were heated on a slide warmer up to 70<sup>0</sup>C and about two drops of colloidal developer and four drops of silver nitrate (50%) were placed on the pre-warmed cover slip, mixed well and placed the slide on the cover slip to enable chromosomes to take stain for about two minutes time or till the silver stain changed to golden yellow colour. The cover slip was then removed, excess stain rinsed with deionized water and counter stained with 2% Giemsa-stain for about 20 seconds. Slides were then rinsed, air dried and examined under the bright field. The silver stained NORs were visualized as black spherical bodies on yellow-brown chromosome arms. The data on percent relative length were transformed into angles using arc-sin

transformation (Snedecor and Cochran, 1989).

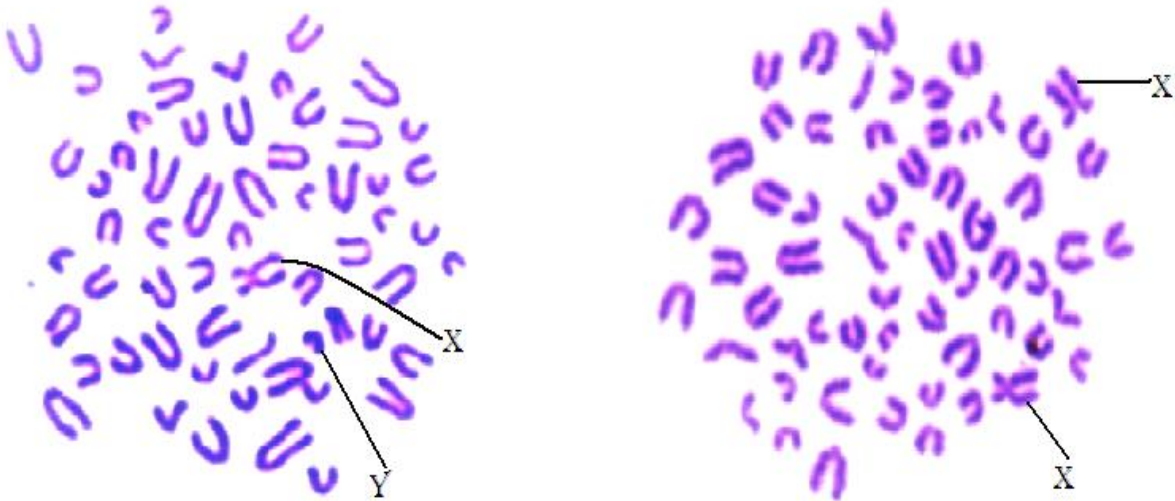
### Results and discussion

In the present study, a total of 1000 metaphases were examined for the chromosome count and morphology and 150 karyotypes of Punganur and 50 karyotypes of non-descript Cattle prepared for computing the morphometric measurements. All the metaphase spreads contained a diploid number (2n) of 60 chromosomes with 58 autosomes and two sex chromosomes with XY in males and XX complement in females (Figures:1 and 2), which was in agreement with the findings of Rao *et al.* (1996) and Kumarasamy *et al.* (2008) in *Bos indicus*, *Bos taurus* cattle and their crosses. All the 29 pairs of autosomes were found to be acrocentric, which corroborated with the reports of Halnan (1981), Rao (1995), Choudary *et al.* (1996), Kumarasamy *et al.* (2001, 2008), and in different *Bos indicus* and *Bos taurus* cattle. However, Arruga and Zarazaga (1985) reported the autosome pairs in Spanish bulls were telocentric in appearance. The X-chromosome of Punganur and non-descript Cattle in the present study was observed to be Sub metacentric which was similar to observations made by Halnan (1981), Chuanchai and Leusakui (1985), Turki *et al.* (1989), Sahoo *et al.* (1990), Rao *et al.* (1996) and Kumarasamy *et al.* (2008) in different Indian breeds of Cattle.

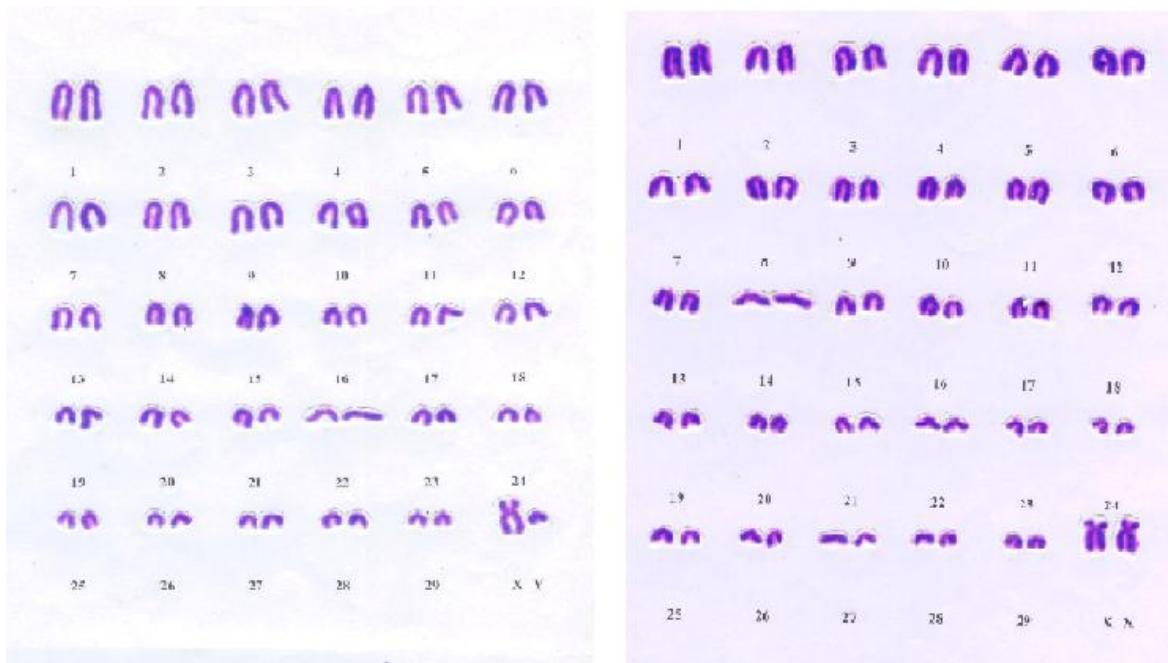
The Y-chromosome morphology of Punganur Cattle was found to be Acrocentric in appearance, which agreed well with reports of Gupta *et al.* (1974) in Red Sindhi and Sahiwal, Benjamin and Bhat (1977) in Hariana, Girija (1995) in dwarf Cattle of Kerala. In contrast, the Y-chromosome was reported to be metacentric in Africanders and their crosses with Brahman (Stranzinger *et al.*, 1987), Sub-

metacentric in crosses of taurine bulls (Kieffer and Cartwright, 1968), Holstein and Holstein x Hariana bulls (Benjamin and Bhat, 1977). Yu *et al.*, (1993) reported the Y- chromosome was to be metacentric,

acrocentric and sub-metacentric in humped and humpless cattle of china. There were different views on morphology of Y- chromosome among the breeds of *Bos indicus* and *Bos taurus* cattle.



**Fig. 1: Mitotic metaphase spreads of Punganur male (left) and female (right) cattle.**



**Fig. 2: Karyotypes of Punganur male (left) and female (right) cattle.**

### Relative Length

Results of the analysis of variance have revealed that there were significant differences among the genetic groups (Punganur and ND) with respect to the relative lengths of chromosomes 10, 26, 27, 28, 29 and Y- chromosome. The overall mean relative length of the autosomes varied from 1.92 (29th pair) to 5.21 (1st pair) percent, while the mean relative contribution of X- and Y-chromosomes to the total genome was 4.68 and 1.80 percent, respectively (Table 1), which are within the ranges reported in Assam local Cattle (Choudary *et al.*, 1996) and Punganur (Ravi kumar *et al.*, 2003). However, the relative length of the first chromosome obtained in the present study was slightly lower than that reported in Holstein- Friesian, Red Dane and Jersey (Desai *et al.*, 1984) and in Ongole (Rao 1995 and Kumarasamy *et al.*, 2001). Sex wise analysis of variance revealed that the Relative length of all the chromosomes except 9, 10, 11, 12, 13, 14, 28, 29 and X- chromosome showed significant differences among sexes in Punganur Cattle. Whereas, in ND cattle there was no significant difference among the sexes for all chromosomes except 29<sup>th</sup> pair of autosome. Autosomes of males contributed 1.87 to 5.34 percent, while those of the females contributed little lower ranging from 1.87 to 5.25 percent to the total genome in Punganur. Whereas, Autosomes of males contributed 1.97 to 5.10 percent, while those of females contributed ranging from 2.08 to 5.62 which is within the ranges found by Assam local Cattle (Choudary *et al.*, 1996) and Punganur (Ravi kumar *et al.*, 2003).

### Arm Ratio

Arm ratio of the submetacentric X-chromosome was found to be 1.55 and 1.56

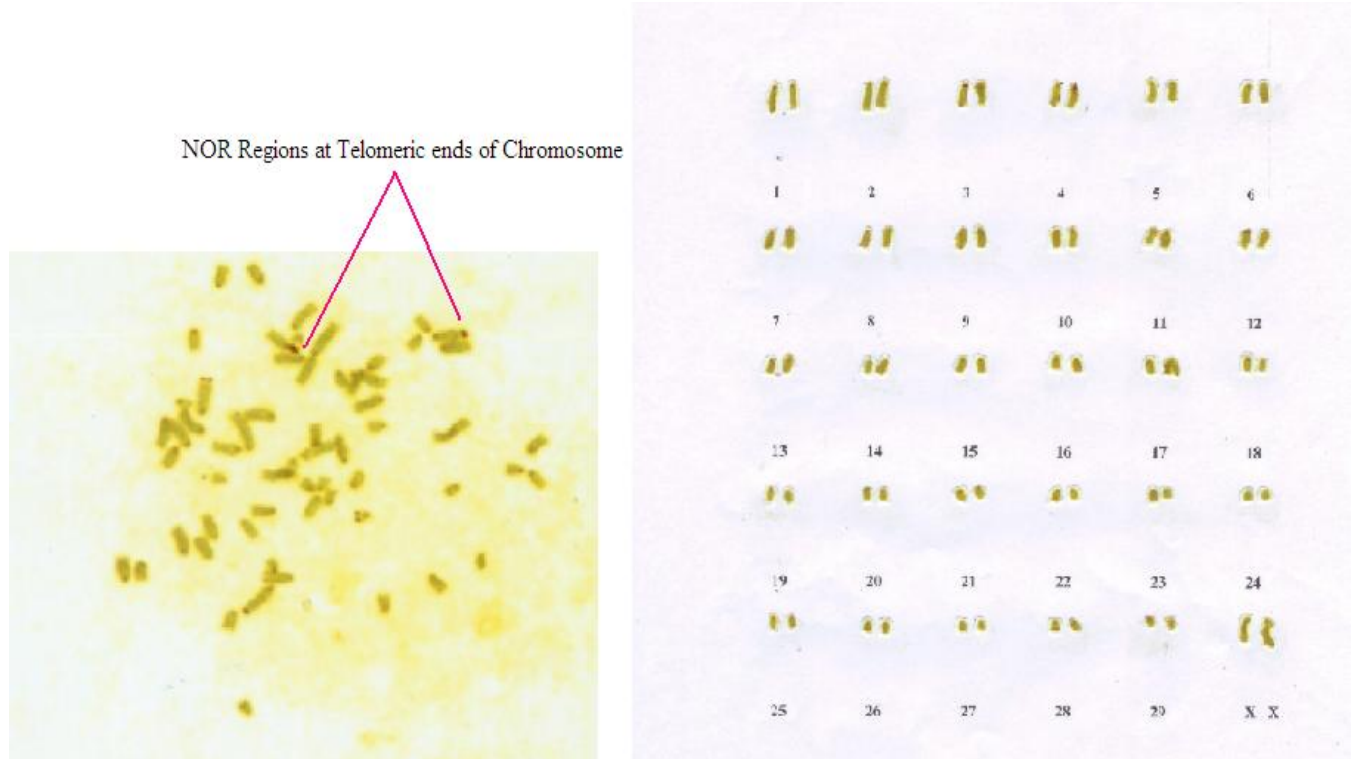
in Punganur and ND cattle. However, Kumarasamy *et al.*(2001) in Ongole Cattle reported 2.28, Ravikumar *et al.*(2003) in Punganur cattle reported 0.56 and Kumarasamy *et al.* (2008) in Umblichery Cattle reported 2.03.

**Table 1: Mean Relative Length (mm) of Chromosomes of Punganur and ND cattle.**

Chromosome No.	Punganur	ND	Over all
1	5.34	5.10	5.21
2	4.89	4.58	4.74
3	4.64	4.41	4.52
4	4.44	4.25	4.34
5	4.31	4.14	4.22
6	4.18	4.06	4.12
7	4.06	3.95	4.01
8	3.95	3.85	3.90
9	3.83	3.75	3.79
10	3.69 <sup>a</sup>	3.63 <sup>b</sup>	3.67
11	3.56	3.49	3.52
12	3.45	3.40	3.43
13	3.35	3.29	3.31
14	3.24	3.18	3.21
15	3.13	3.09	3.11
16	3.04	3.00	3.02
17	2.94	2.92	2.93
18	2.86	2.84	2.85
19	2.78	2.75	2.76
20	2.70	2.69	2.70
21	2.63	2.62	2.62
22	2.51	2.54	2.55
23	2.47	2.48	2.47
24	2.38	2.41	2.40
25	2.31	2.32	2.31
26	2.23 <sup>b</sup>	2.26 <sup>a</sup>	2.25
27	2.13 <sup>b</sup>	2.18 <sup>a</sup>	2.16
28	2.02 <sup>b</sup>	2.10 <sup>a</sup>	2.06
29	1.87 <sup>b</sup>	1.97 <sup>a</sup>	1.92
X	4.81	4.57	4.68
Y	1.69 <sup>b</sup>	1.93 <sup>a</sup>	1.80

\*Means with different superscript differ significant

**Fig. 3: NOR banded mitotic metaphase spread (left) and karyotype (right) of Punganur cattle.**



### Centromeric Index

Centromeric index of the X-chromosome was found to be 0.40 and 0.41 in Punganur and ND Cattle. However, Kumarasamy *et al.*(2001) in Ongole Cattle reported 0.30, Ravikumar *et al.*(2003) in Punganur cattle reported 0.36 and Kumarasamy *et al.* (2008) in Umblichery Cattle reported 0.31.

### Morphological Index

Morphological index of X-chromosome in the present study was 5.12 and 5.30 in Punganur and ND Cattle. These findings were lower than reported by Rao (1995) and higher than reported by Ravi kumar *et al* (2003).

### NOR Banding

In the present study the NOR bearing chromosomes were found to be 2, 3, 4, 5 and 28 which were located on telomeres and terminal ends of long arms in Punganur

cattle (Figure: 3) as reported by Henderson and Bruere (1979). Whereas Diberardino *et al.* (1980) reported 2, 3, 4, 11 and 28 chromosomes contained NORs in Cattle, which reflected the breed variation. The average number of NOR bearing chromosomes in Punganur Cattle were 5 in accordance with findings of Henderson and Bruere (1979, Diberardino *et al.* (1980).

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