

Nucleolar organizer region (NOR) banding in Punganur cattle

G. Bharathi*, M. Gnanaprakash, B. Punya Kumari

College of Veterinary Science, Rajendranagar, Hyderabad-500 030, India.

Correspondence Address: *G. Bharathi, College of Veterinary Science, Rajendranagar, Hyderabad-500 030, India.

Abstract

Nucleolus organizer regions (NORs) on the Chromosomes of Punganur Cattle were studied by Short – Term Lymphocytic culture technique. The Ammonical Silver staining procedure was followed with minor modifications. The Chromosomes were stained with Silver- Giemsa staining technique using gelatine colloidal developer. The frequency distribution of number of metaphases containing the Ag- NOR bands were tested with Chisquare test. A Total of 373 NORs was detected in 98 metaphases. The mean number of NORS per metaphase was 3.806, with a range from 3.2 to 4.07, while the number of NORs per metaphase ranged from 1 to 8. An inverse relationship was found between Frequency of metaphases and Number of NORs per metaphase. There exists a significant departure was noticed between the observed and expected frequencies ($P < 0.01$). The highest frequency of metaphases (25.51%) examined had 3 NORs, while the lowest frequency (4.08%) had 8 NORs. The Chromosome morphology, number and morphometric measurements and NORs in Punganur Cattle were similar to other Zebu Cattle in India.

Keywords: Nucleolar Organizing Regions, Banding, Cattle

Introduction

Nucleolar Organizing Regions (NORs) are elements of genomes involving heterochromatin and late replicating regions. These are associated with secondary constrictions, terminal and centromeric regions of defined autosomes (Halnan, 1989). The location and distribution of Ag-NORs on different chromosomes are the characteristic features of given Species of Livestock (Barchet.al c1997). But such work is yet to report on NORs in Punganur Cattle. Hence, this study is attempted to localize the NORs in these Cattle.

Materials and methods

The Short term Lymphocyte culture method, as described by Moorhead et.al (1960) with slight modifications was followed to get good quality metaphase spreads. The Ammonical silver staining procedure as described by Bloom and Good Pasture (1976) and Barch et al (1997) was followed with minor modifications. The unstained slides were screened under phase contrast microscope and those having good quality metaphases were subject to NOR banding. The surface of the slide with cover slip is exposed to warmer duly covered with a silver foil.

The slides were heated on a slide warmer up to 70°C. A 24X50 mm cover slip was placed on the slide. About four drops of colloidal developer and 8 drops of Silver Nitrate (50%) were placed on cover slip and then inverted on the slide. The Chromosomes to take stain for about 2 minutes (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. The slides were rinsed with water, air dried and examined under the bright field. The Silver stained NORs (Ag-NORs) were visualized as black spherical bodies on Yellow- Brown chromosome arms. The Good metaphases showing the NORs per metaphase were counted and subject to the analysis. The frequency distribution of number of metaphases containing the Ag-NOR bands were tested for significance of Chisquare test (Snedecor and Cochran 1989).

Results

The number of metaphases examined, number of NORs detected and mean number of NORs per metaphase for the five Punganur Cattle are detailed in Table 1. And the Frequency distribution of NORs per metaphase is given in Table 2. The metaphase spread showing the location of NORs on the Chromosomes and NOR banded Karyotype of Punganur Cattle is represented in fig1. In present study, a total of 373 NORs were detected among 98 good metaphases examined. The mean number of NORs per metaphase was 3.806, which is ranged from 3.2 (in P6, Female) to 4.07 (in P65 Female) The number of NORs per metaphase ranged from 1 to 8. In general, An Inverse relationship was noticed between frequency of metaphases and the number of NORs per metaphase. Further it was observed there exists significant departure of observed frequencies from the expected frequencies ($P < 0.01$). The highest frequency of metaphases (25.51%) examined had 3 NORs, while the lowest frequency (4.08%) had 8 NORs.

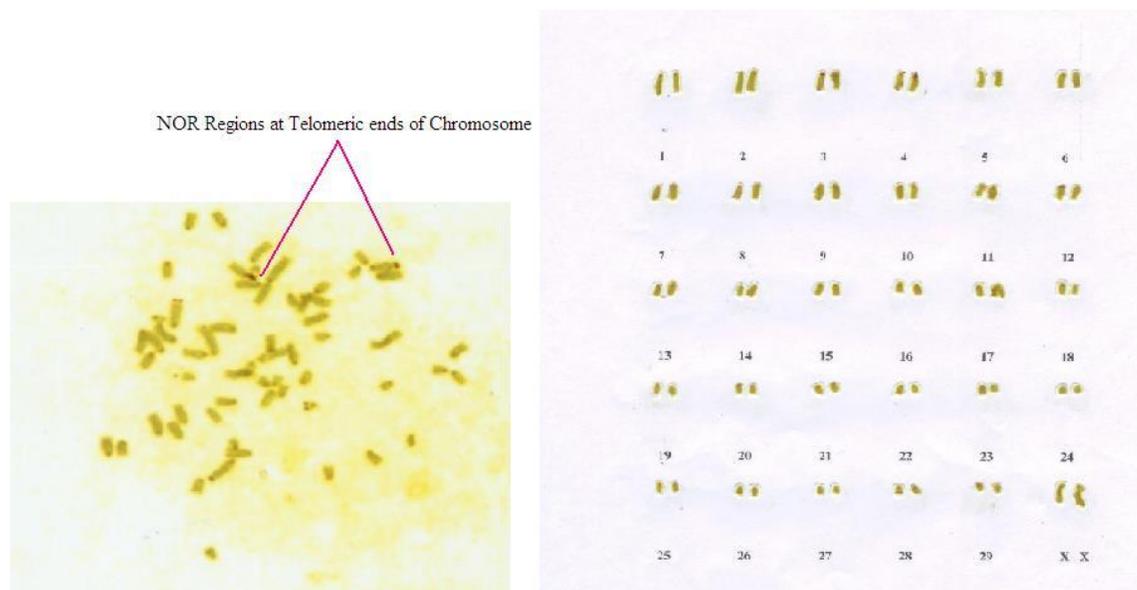
Table 1: No of metaphases examined, No. of NORs detected and Mean number of NORs per metaphase of Punganur Cattle.

Animal No.	No. of Metaphases examined	No. of NORs detected	Mean Number of NORs per metaphase
P6	15	48	3.2
P16	30	120	4.0
P32	21	79	3.76
P65	14	57	4.07
P69	18	69	3.83
Total	98	373	3.806

Table 2: Frequency distribution of NORs per metaphase in Punganur cattle.

Number of NORs per metaphase	Frequency (No. of metaphases)	Percentage
1	13	13.27
2	16	16.32
3	25	25.51
4	10	10.21
5	12	12.24
6	5	5.10
7	13	13.27
8	4	4.08
Total	98	100.00

NOR banded Mitotic Metaphase Spread (Left) and Karyotype (Right) of Punganur Cattle



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