

DISTRIBUTION OF SEX CHROMOSOMES (XY) IN LYMPHOCYTE METAPHASE SPREADS OF DAIRY BULLS

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Position of autosome and sex chromosomes in metaphase spreads is grate concerned of Cytogeneticians worldwide to understand cell biology. A few isolated studies have been conducted for the distribution of chromosomes in metaphase spread. Our studies reveal that most sex chromosomes (XY) remain on periphery and semi-periphery, 84.16% for X and 86.97% for Y respectively, in round metaphase spreads. The application of sex chromosome position in metaphase spreads is to easily find out sex chromosomes under microscope even without banding patterns. An another application is to identify or confirm sex chromosomes in unknown species on which cytogenetic studies have not been performed.

Key words: dairy bulls, methaphase, sex chromosomes

INTRODUCTION

For many years it was assumed that chromosomes may have fixed relative dispositions at Mitosis, meiosis and interphase (COMINGS, 1980). However, it was also assumed that Chromosomes *in vivo* are as they 'appear' in most metaphase spread preparations, that is, more or less randomly scattered, and with no fixed relationships in their positions relative to either cell structures or to any other chromosomes. There are a few well known cases where chromosomes lie in fixed relative positions. This is a very tight association, which remains during, and in spite of, the randomizing influences of fixation and spreading.

Consistency of optimum chromosome spreading during harvest of cytogenetic blood samples remains major concerned. Individual chromosomes are organized into discrete domains in the interphase nucleus (CREMER and CREMER, 2001; PARADA and MISTELI, 2002). In turn, there is order to the arrangement of chromosomal domains because the location of specific chromosome domains relative to the nuclear Centre and periphery correlates with gene density

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and chromosome size (NAGELE *et al.*, 1995; CROFT *et al.*, 1999; TANABE *et al.*, 2002). Various cases of position of different chromosomes in metaphase have been reported especially in humans (ANTOINE *et al.*, 1982; DUTRILLAUX *et al.*, 1981; AURIAS *et al.*, 1982). Because of paucity of literature in humans and animals, it is difficult to discern the relationship of sex chromosomes and their position in metaphase spreads. However, the present study was taken up to study the phenomenon of location of X and Y chromosomes in metaphase spreads prepared from the lymphocyte culture of various cattle breed and Murrah buffalo for routine cytogenetic investigation.

MATERIALS AND METHODS

Chromosome spreads were prepared from lectin stimulated peripheral blood by conventional cytogenetic techniques as described earlier (PATEL, 1999). For cytogenetic screening, metaphase spreads were prepared from heparinised blood samples of many breeds of cattle; Holstein, Jersey (*Bos taurus*), Jersey crossbreds (*Bos taurus x Bos indicus*), Gir, Red Kandhari, Khillar and Gaolao (*Bos indicus*) and Murrah buffaloes (*Bubalus bubalis*). To identify sex chromosomes in cattle and buffaloes, GTG- banding was performed (PATEL *et al.*, 1995). Round metaphase spreads were only considered for the study and noted the position or distribution of X and Y chromosomes. Number of metaphases considered for each breed for the present studies are shown in pie graph (Figure 1) and Table 1. Based on position of X and Y chromosomes in spread, the metaphases classified in to three groups; sex chromosome(s) in periphery, semi-periphery and centre of periphery as shown in Figure 2.

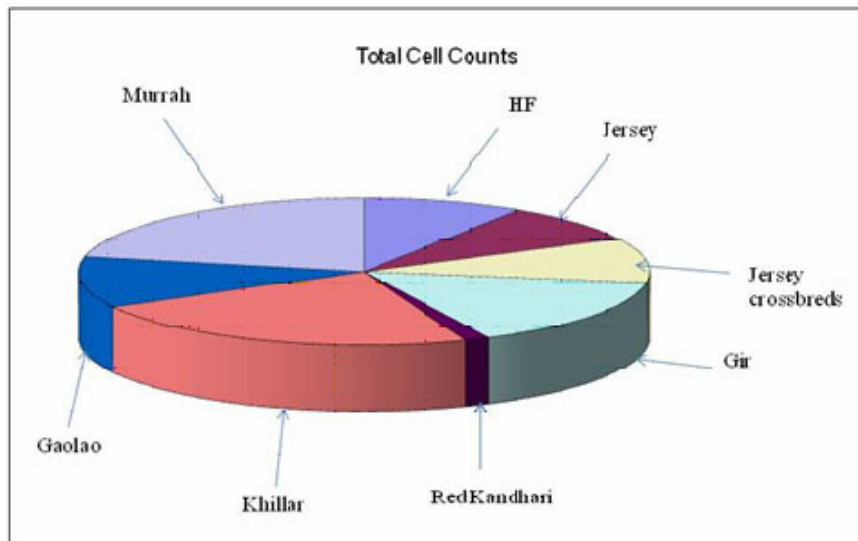


Fig1. Pie graph indicating the number of round cells considered from each animal breed.

Table 1. Position of X and Y chromosome in metaphase spreads (P for periphery, S for semi periphery and C for centre)

| BREED | Total Cell Count | X-chromosome | | | | | | Y-chromosome | | | | | |
|-----------------------------------|------------------|--------------|-------|-----|-------|-----|-------|--------------|-------|-----|-------|-----|-------|
| | | P | % | S | % | C | % | P | % | S | % | C | % |
| 1 HF | 108 | 42 | 39 | 59 | 54.62 | 7 | 6.48 | 36 | 33.33 | 65 | 60.18 | 7 | 6.48 |
| 2 Jersey | 100 | 34 | 34 | 57 | 57 | 9 | 9 | 32 | 32 | 63 | 63 | 5 | 5 |
| 3 jersey X Gir | 115 | 37 | 32.17 | 72 | 62.6 | 6 | 5.21 | 41 | 35.65 | 67 | 58.26 | 7 | 6.08 |
| 4 Gir | 183 | 58 | 31.69 | 97 | 53 | 28 | 15.3 | 70 | 38.25 | 91 | 49.72 | 22 | 12.02 |
| 5 Red Khandhari | 17 | 11 | 64.7 | 5 | 29.41 | 1 | 5.88 | 8 | 47.05 | 5 | 29.41 | 4 | 23.52 |
| 6 Khillar | 269 | 106 | 39.4 | 112 | 41.63 | 51 | 18.95 | 125 | 46.46 | 93 | 34.57 | 51 | 18.95 |
| 7 Gaolao | 133 | 45 | 33.83 | 67 | 50.37 | 21 | 15.78 | 51 | 38.34 | 74 | 55.63 | 8 | 6.01 |
| Total cell count of Cattle | 925 | 333 | 36 | 469 | 50.70 | 123 | 13.30 | 363 | 39.24 | 458 | 49.52 | 104 | 11.24 |
| 9 Murrah | 257 | 100 | 38.91 | 96 | 37.35 | 61 | 23.73 | 117 | 45.52 | 90 | 35.01 | 50 | 19.45 |
| 11 Grand Total | 1182 | 433 | 36.63 | 565 | 47.80 | 184 | 15.57 | 480 | 40.61 | 548 | 46.36 | 154 | 13.03 |

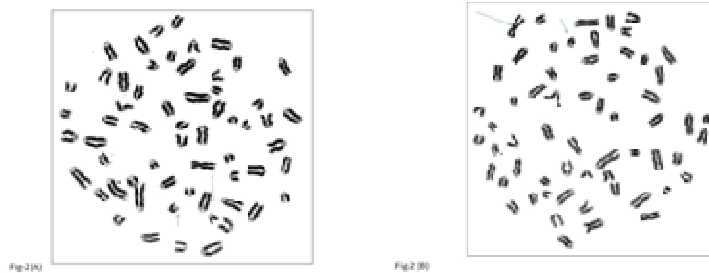


Fig2-1: Large arrows indicate X-chromosomes and small arrows Y chromosomes in all metaphase spreads of cattle and buffaloes. (A) Metaphase from *Bos indicus* cattle indicates X is almost in centre and Y at semi-periphery, (B) indicates position of X and Y chromosomes on periphery and semi-periphery respectively



Fig-2 (C)



Fig-2 (D)



Fig-2 (E)

Fig2-2: Large arrows indicate X-chromosomes and small arrows Y chromosomes in all metaphase spreads of cattle and buffaloes. (C) indicates X and Y on periphery, (D) indicates position of X and Y of *Bos taurus* cattle almost in centre and (E) indicates X and Y chromosomes of buffalo (*Bubalus bubalis*) on periphery position.

RESULTS AND DISCUSSION

X-chromosome distribution

On observation under the microscope, the position of X-chromosome in metaphase spreads of Red Kandhari was found mostly on periphery (64.7%) and semi-periphery (29.41%) whereas number of metaphase recorded were very less (17). Maximum cells recorded (269) in case of Khillar breed of cattle wherein X chromosome was found 39.4% in periphery and 41.64% in semi-periphery. The majority of cattle (all breeds) metaphase spreads exhibited X chromosome in periphery (36%) and semi-periphery (50.70%). In Murrah buffalo the X chromosomes were

found in periphery in 38.91% metaphase spreads which is slightly higher as compare to cattle whereas percentage of X chromosomes in semi-periphery was far less (37.35%). The ratio of X-chromosome distribution in buffalo at periphery and semi-periphery is more or less similar unlike in cattle breeds (Table-1, Figure 3A). The overall position of X-chromosomes was in periphery and semi-periphery (84.43%). The distribution of X-chromosome in centre was very less in most breeds and overall was only 15.57%.

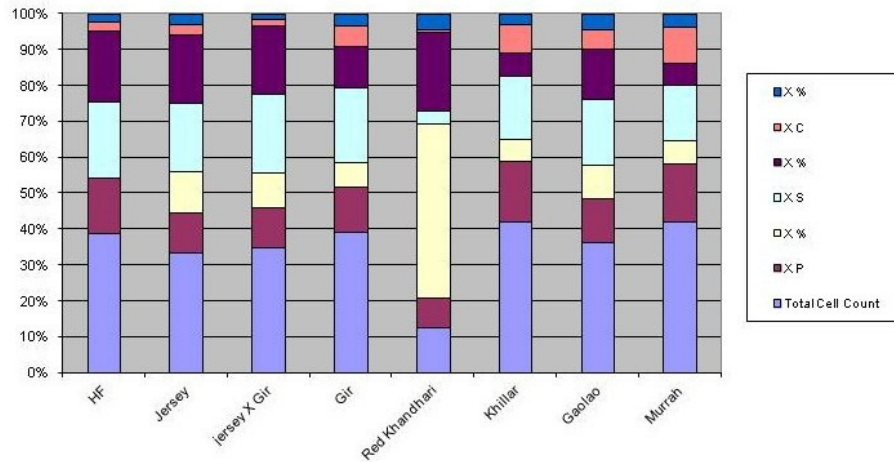


Fig 3A. Graphs (A) indicates % of X chromosome in periphery, semi-periphery and centre of metaphase plates.

Y-chromosome distribution

Y-chromosome was found in periphery highest in Red Kandhari cattle breed (47.05%) though number of metaphase considered was very less (17). The second highest (46.46%) was found in case of Khillar where 269 metaphase spreads were considered. The overall position of Y-chromosomes in case of cattle breeds was found to be 39.24% in periphery and 49.52% in semi-periphery. All together Y-chromosomes remain in periphery and semi-periphery (88.74%) which is higher than X-chromosomes of cattle and buffaloes. Murrah buffalo exhibited Y-chromosomes in periphery, semi-periphery and central as 45.52%, 35.01% and 19.45% respectively. The overall Y chromosomes in cattle and buffaloes mostly remain in periphery and semi-periphery (86.97%) (Table-1 and Figure 3B). The position of sex chromosomes mainly remain periphery and semi-periphery may be because of following hypothesis; the sex chromosomes contain more heterochromatin region especially in Y chromosomes (TREUS *et al.*, 1997), effects of dropping height of cell suspension, slide condition, drying time, fixative ratio, relative humidity on the quality of metaphase spreads and evaporation rate of fixative from fixed slides on room temperature (DENG *et al.*, 2003).

Heterochromatin consists of two types: constitutive heterochromatin, which is present in all cells and is characteristic of the Y chromosome, and facultative heterochromatin, which is present in the inactivated X chromosome of the mammalian female. Most of reports on position of normal or abnormal chromosomes are reported to be random. ANTOINE *et al.* (1982) explained except for one t(10;15) and for several t(X;autosome), the chromosomes and their normal homologues seem to have random positions in metaphase spreads. DUTRILLAUX *et al.* (1981) reported no random position of radiation induced metaphasic chromosomes. Similarly, AURIAS *et al.* (1982) reported no random distribution of translocated chromosomes; t(7;14)(p14;q12) and t(7;14)(q35;q12). However, KOWALSKI *et al.* (1978) studied the location of Y chromosome in metaphase spreads in groups of 96 normal males, 17 Down's syndrome patients, and 51 patients with Klinefelter's syndrome. The position of the Y is scored as either peripheral or no peripheral using several of the traditional methods found in the literature. They observed Y-chromosome on periphery in normal human. On the contrary, MILLER *et al.* (1963) observed non-random distribution of chromosomes in metaphase figures from cultured human leucocytes, but found the peripheral location of chromosomes 13, 17–18 and 21. No much more work has been done on this aspect; therefore, the position of sex chromosomes in metaphase spreads remains unclear. However, the application of sex chromosome position in metaphase spreads is to easily find out X and Y chromosomes under microscope even without banding patterns. Another application may be identification or confirmation of sex chromosomes in other species on which cytogenetic studies have not been attempted.

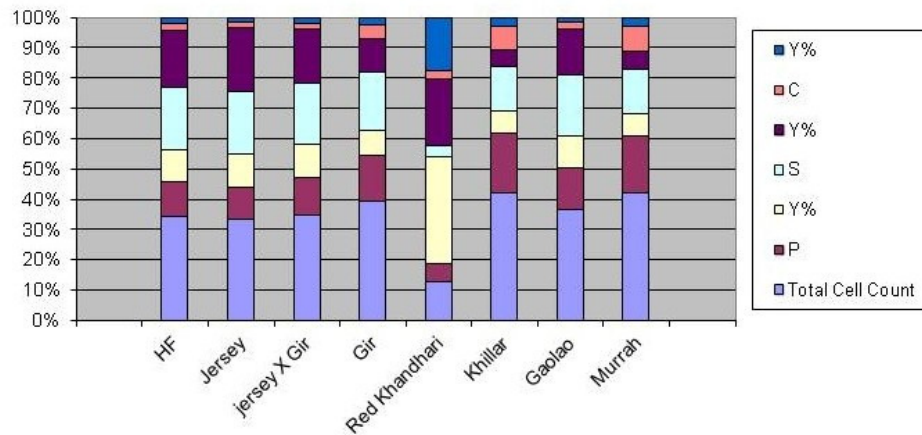


Fig3B. Graphs (B) indicates % of Y chromosome in periphery, semi-periphery and centre of metaphase plates.

CONCLUSION

Present study reveals that sex chromosomes (XY) of cattle and buffaloes are mostly positioned on periphery and semi-periphery in round metaphase spreads prepared from whole blood culture.

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**DISTRIBUCIJA POLNIH HROMOZOMA (XY) U METAFAZI LIMFOCITA MLEČNIH
KRAVA**

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Izvod

Pozicija autozoma i polnih hromozoma u metafazi je predmet citogenetičkih ispitivanja u cilju razumevanja biologije ćelije. Vršeno je nekoliko izolovanih studija distribucije hromozoma u preparatu ćelija u metafazi u toku deobe. Ova istraživanja su potvrdila da većina polnih hromozoma (XY) ostaju na periferiji ili semi – periferiji. 84.16 % X hromozoma i 86. 97 Z hromozoma. Pozicije polnih hromozoma mikroskopom u fazi metafaze je lako identifikovati i bez traka obojenosti. Druga primena ovog pristupa je identifikacija polnih hromozoma kod nepoznatih vrsta koje nisu ispitivane citogenetičkim metodama.

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