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Characterization of a Q-banded karyotype of a crossbred bull

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ABSTRACT

With the objective of characterizing the Q- banding pattern of cattle chromosomes, peripheral blood lymphocyte metaphase chromosomes of a bull calf (*Bos indicus* x *Bos taurus* L.) were studied using quinacrine fluorescence banding technique. A karyotype of the Q-banding pattern of bovine chromosomes, together with a diagrammatic representation and a complete diploid cell is presented. The haploid set of metaphase chromosomes showed 125 positive bands. A detailed description of the positive bands were given in the idiogramme, providing accurate description of the banding pattern on individual chromosomes of this species. This technique is simple and reproducible and can be used in routine chromosome analysis.

Key words: Bovine chromosomes, karyotype, Q-banding

INTRODUCTION

Cytogenetics is a relatively new science applied to animal production. It has many diverse applications in livestock management, breeding and veterinary medicine (Fechheimer 1979). Therefore, it has become an important aspect in farm animal breeding in many developed countries. Fertility problems are one of the major constraints in economic animal production.

Much of the pregnancy wastage in farm animals may be caused by abnormal chromosome complements in breeding animals. Such abnormalities will lead to embryonic losses at very early stages of the embryo and can only be detected by cytogenetic analysis (Gustavson 1971). In European countries cytogenetic analysis of breeding animals, particularly in stud bulls is compulsory and their karyotype should be free from any abnormality (Gutavson 1979). This will help to avoid spreading chromosomal abnormalities among the breeding populations, thus preventing undetectable fertility problems among the domestic animals. However, in developing countries, the importance of use of this techniques has not been recognized in veterinary profession and Sri Lanka is no exception.

Fertility problems are common in exotic breeds under local conditions partly due to poor management and feeding. This may be attributed to chromosomal abnormalities among the breedable animals too. Therefore, routine cytogenetic analysis of breeding herds, especially bulls used for artificial insemination purposes will help to avoid such problems.

The chromosome identification is the most important aspect in cytogenetic analysis. However, the inherited morphology of cattle chromosomes hinders their reliable identification in routine laboratory analysis. Therefore, a quick and reliable banding technique is indeed helpful to overcome this problem.

The karyotype of cattle have been studied by many workers using different banding techniques and, G and R banding techniques are widely used by them (Gustavson and Hageltorn 1976, Diberadino and Iannuzi 1982, Lin *et al.* 1977, ISCNDA 1989, Iannuzi 1990).

The Q-banding method is comparatively a simple, reproducible technique, which can be used efficiently in routine chromosome analysis. The aim of this study was to use Q-banding technique for identification of cattle chromosomes and to characterize their individual Q-banded patterns. Attention was focused on the main identification features of the individual chromosomes.

MATERIALS AND METHODS

Heparinized peripheral blood (0.5 ml) from a healthy crossbred bull calf was cultured in 10 ml RPMI 1640 medium, supplemented with 20 % foetal calf serum and containing 0.2 ml pokeweed metogen. 0.2 ml of L-Glutamin and Antibiotics were

added to the culture medium. Cultures were incubated for 96 hours at 37.8° C. Colcemid was added 20 min. before harvesting and no BrdU was added. Following colcemid exposure, cells were immediately centrifuged at 1500 rpm for 10 min., resuspended in 0.075 m KCl, and incubated at 37°C for 25 min. Cells were then spun for 8 min. at 1000 rpm and resuspended in fresh 3:1 Methanol: Acetic acid fixative. The fixative was added drop by drop while shaking the cell suspension.

After cells were allowed to remain for few hours in the first fixative, fixative was changed several times. Slides were prepared by drawing the cells resuspended in fixative in to a micro-pipette and dropping them on to a glass slide placed at 30° angle.

Chromosome preparations were allowed to age for 2-3 days at 5°C in a refrigerator and then stained with 0.005 % Quinacrine mustard solution dissolved in distilled water for 25-35 sec. and were thoroughly rinsed with double-distilled water. Sorenson's buffer (pH = 6.8) was applied on to the stained slides and each slide was covered with a glass coverslip. Metaphases were observed under fluorescence microscope and photographed using a Kodak Pan technical film. Chromosomes were arranged to prepare a karyotype according to ISCNDA recommendation (ISCNDA 1989). Idiogram was constructed for Q-bands, most prominent land marks and regions were defined and bands were numbered. The numbering of regions and bands was based on ISCNDA (1989).

RESULTS AND DISCUSSION

For the purpose of chromosome analysis, most mitoses can be readily classified by relative length and banding patterns in to four stages; late prophase, prometaphase, early metaphase and mid metaphase (Yunis *et al.* 1978). In this study, chromosomes were arrested at early metaphase to avoid condensation of the chromosomes during late metaphase. Further to that, most of the prominent Q-bands are clearly visible in early metaphase chromosomes than prophase chromosomes. Early metaphase of a cell and a karyotype representing Q-banded cell is shown in figures 1 and 2 respectively.

A careful examination of the different chromosomes reveals that their banding patterns are consistent at any given cell and the general appearance of a specific chromosome usually remains similar through out as shown in Fig. 3. This makes possible their characterization and schematic representation. In addition, certain bands remain quite conspicuous serving as useful markers for identification of individual chromosomes. They are shown by the idiogram. As shown in Figs.2 and 3 the banding patterns of cattle chromosomes in early metaphase can be identified with accuracy, and all the chromosomes in the karyotype of crossbred animals are similar to that of European cattle. Y chromosome is a sub metacentric chromosome, which shows a clear difference from Y chromosome

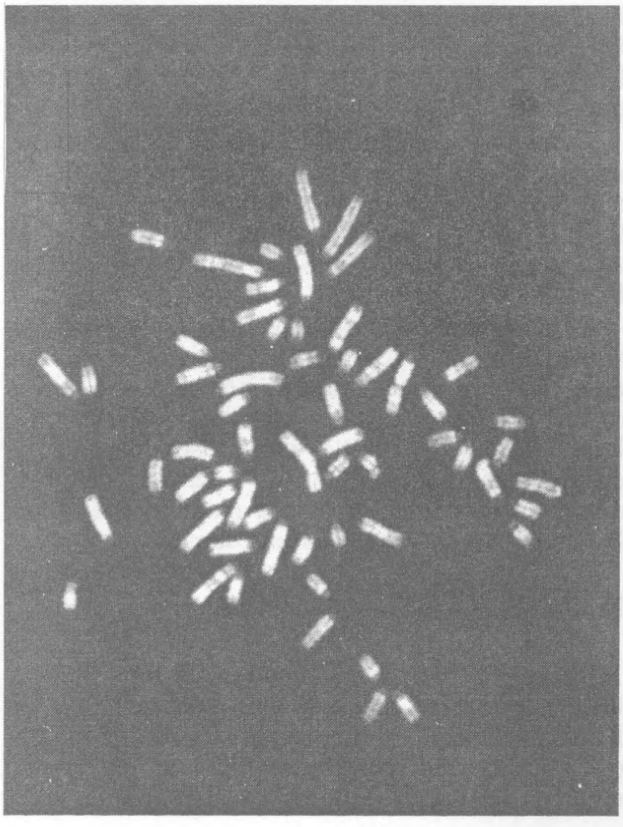

Fig.1. Q-banded diploid cell (2n=60).

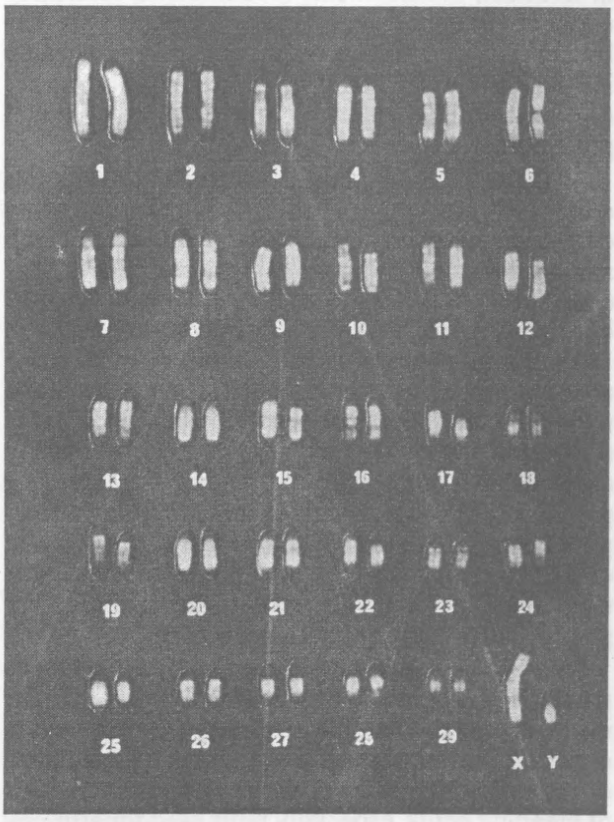

Fig. 2. Karyotype of the crossbred bull demonstraing Q- banding pattern.

of *Bos indicus*.

The schematic representation of cattle chromosome corresponds approximately to 125 bands. They can be described as follows;

Chromosome 1: Four positive bands in region one close to centromere and three positive bands in the telomere region 3 separated by a clear negative band. Telomere has a prominent negative band.

Chromosome 2: Four regions can be identified. A broad positive band adjacent to the centromere separated from the region 2 with two positive bands by a prominent negative band. Center of the chromosome has a negative band. However, it is not prominent as in the chromosome described earlier. Region four consists of two positive bands and a negative telomere.

Chromosome 3: Three regions can be identified with two distinguishable bands, one on the

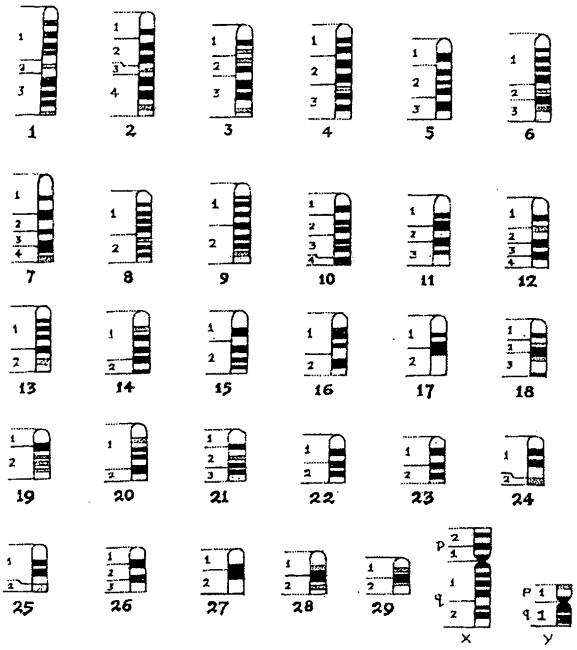

Fig. 3. Diagrammatic representation of Q-bands.

centromere region and in the regions 2 and 3. It has a very clear negative band in between regions 1 and 3. Telomere is negative.

Chromosome 4: Three regions equally banded with positive bands from centromere to telomere.

Chromosome 5: Three regions banded positively and separated by two clear negative bands. Telomere region consists of a negative band.

Chromosome 6: Two clear positive regions 1 and 2 separated by a easily distinguishable negative band closer to the telomere region. The positive band in between the regions 2 and 3 is an important land mark to identify this chromosome.

Chromosome 7: Four regions demonstrated by two prominent positive proximal bands and two distal bands. The band in the region 4 is broad and more prominent than in the bands in the centromere region.

Chromosome 8: Two regions with prominent positive bands in proximal half and negative and slightly positive bands in the distal region.

Chromosome 9: Two regions equally banded 5 positive bands and a negative telomere.

Chromosome 10: Four regions with equally distributed positive bands separated by 3 negative bands.

Chromosome 11: Three regions with equally distributed positive bands separated by a prominent negative band. The telomere is a prominent negative band.

Chromosome 12: Four region can be identified. A prominent positive band adjacent to the centromere and two broad positive bands distal to the centromere are land marks of this chromosome.

Chromosome 13: Two regions with prominent positive band in proximal and a negative band in distal region.

Chromosome 14: Two regions. Positive band is just below the centromere in region 1 and a broad prominent band in region 2.

Chromosome 15: Two regions. A prominent band adjacent to the centromere and 3 bands in the region 2 separated by a prominent negative band.

Chromosome 16: Two regions. Two positive bands in region 1 and one prominent band in distal part. A prominent negative band in between the two regions close to the distal part.

Chromosome 17: Two regions. A central positive band and a negative telomere region.

Chromosome 18: Three regions. Positive band in centromere region is followed by a broad positive band in central region. Distal part consists of a positive band.

Chromosome 19: Two regions. A positive subcentremeric band is followed by slightly positive bands in the region 2.

Chromosome 20: Two regions. Similar to the chromosome 14, but smaller in size.

Chromosome 21: Two regions. A positive band in the proximal part followed by a broad negative band. In region 2 two positive bands are located close to each other.

Chromosome 22: Two regions. Consist of three positive bands, one of which is close to the centromere and the other two are close to the distal part of the chromosome.

Chromosome 23: Two regions with three positive bands, which are located in equal distances.

Chromosome 24: Two regions. Two positive bands in the proximal part followed by a negative band. Telomere is slightly positive.

Chromosome 25: Two regions. Positive band close to the centromere followed by a negative band in the telomere region.

Chromosome 26: Three regions. Two positive

bands separated by a broad negative band.

Chromosome 27: Two regions. One positive band in the proximal region.

Chromosome 28: Two regions. A central positive band followed by a slightly positive band.

Chromosome 29: Two regions. The smallest chromosome with a positive band in the central position.

Chromosome X: A large sub-metacentric chromosome with two regions in both arms p and q.

Xp : Two regions. Two positive bands located in central region.

Xq : Two regions. Three positive bands in region q1 followed by two positive bands in region q2.

Chromosome Y: A sub-metacentric chromosome with one region in both arms p and q.

Yp : Negative band in region one

Yq : Two positive bands in proximal and distal parts.

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