Giemsa Banding Patterns of Bovine Chromosomes

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The modal chromosome number of cattle is 60. The autosomes are acrocentric and the sex chromosomes are submetacentric (MELANDER¹⁾). The size of the autosomes of cattle gradually decreases within the karyotype. Therefore, it has not been possible to identify the individual chromosome pairs by means of the conventional staining methods. Recently, SCHNEDL (1972)²⁾ identified most of the cattle chromosomes by the special Giemsa staining method based on treatment with NaOH.

The purpose of this experiment was to identify the individual chromosome pairs in bovine karyotype by their banding patterns after proteolytic digestion.

Three clinically normal Holstein bulls (3~5 years old) maintained at the National Institute of Animal Industry were used. They have been used for artificial insemination and normal daughters were produced. 10 ml of blood was drawn from jugular vein into a sterile tube containing heparin. Each 2~2.5 ml of the stabilized whole blood was splitted into four culture tubes and mixed with 7.5~8.0 ml NCTC-109 medium (Difco), 0.1 ml PHA (Phytohemagglutinine, Wellcome) and 0.2~0.4 ml antibiotics solution (Penicillin-G-potassium 500 I.U., Streptomycin sulfate 500 µg/0.1 ml), and then cultured at 37°C for 72 hours. Two drops of 4×10^{-6} M colchicine solution were added to each 10 ml of the culture 2 to 3 hours before harvesting. Hypotonic treatment was performed with 0.075 M KCl solution at 38°C for 15 to 20 minutes. The treatment with KCl solution helped to obtain elongated and well-spread chromosomes in the cells. After hypotonic treatment the cells were fixed with acetic acid-alcohol (1:3) for 20 to 30 minutes. Chromosome slides were prepared by means of the flame-drying technique and stored over a day before proteolytic digestion. The slides were treated with 0.1% trypsin solution for 3 to 10 minutes at 5°C and then stained for 15 minutes with Giemsa solution (Merck) diluted with phosphate buffer, pH 6.4. The time of trypsin treatment for getting clear bands varied with preparations. If trypsin treatment was too short, no clear bands were seen and if too long, the chromosomes were completely distorted.

The Giemsa banding patterns of the individual chromosome pairs are presented in Fig. 1. On the basis of the number of their bands and their extent and position, almost all the chromosomes of cattle can be identified. There were no differences between bulls. The banding pattern of pair No 20 very much resembled pair No 21, so it was difficult to distinguish between them with certainty. Pair No 25 did not show any clear bands. This may be due to errors in fixation process of the chromosomes. The banding pattern shown in Fig. 1. well resembled the result of SCHNEDL²⁾.

The Giemsa banding pattern of chromosome will be valuable for cytogenetics, genetic pathology and reproduction research of the domestic animals.

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Fig. 1. Karyotype of male cattle. The preparation was made by the technique described in the text. The banding patterns are characteristic for each chromosome.

It is to be desired that the standard Giemsa banding pattern for the arrangement of the cattle chromosomes into karyotype will be determined internationally.

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