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1;25 centric fusion recognition in the cattle chromosome by fluorescence in situ hybridization

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Abstract. Fluorescence in situ hybridization (FISH) experiments with specific probes for chromosome 29 and 25 were carried out on a Brown Swiss bull, previously diagnosed as a carrier of 1;29 centric fusion. The hybridization of the chromosome 29-specific probe (BMC 4216 – already located on 29q13), produced signals on two small acrocentrics, but not on the translocated chromosome. The signals appeared on the translocated chromosome and on a single chromosome 25 after hybridization of the chromosome-specific probe (BMC 3224 – previously located on 25q24). According to the actual nomenclature, the analysed aberration is a robertsonian translocation involving chromosomes 1 and 25.

Key words: cattle, centric fusion, fluorescence in situ hybridization.

Introduction

The robertsonian translocation 1;29, diagnosed for the first time in cattle by GUSTAVSSON and ROCKBORN (1964), has turned out to be the most frequently occurring chromosome aberration in this species. It was described in numerous, mainly beef, cattle breeds (LONG 1985, POPESCU, PECH 1991). This aberration was usually diagnosed with the use of banding techniques, however, many cases were identified as the 1;29 translocation following conventional staining of chromosomes. On the other hand, there are reports indicating that

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in case of some small acrocentric chromosome pairs, difficulties arise in distinguishing them (Reading Conference 1980, EGGEN et al. 1994, IANNUZZI, Di MEO 1995).

Fluorescence in situ hybridization (FISH) using biotinylated chromosome–specific DNA probes provides a powerful tool for the analysis of numerical and structural chromosomal aberrations. Molecular studies of breakpoints in specific aberrations have now reached a watershed.

The aim of this study was to verify the previous diagnosis recognising the 1/29 centric fusion in a Brown Swiss bull by the use of FISH with chromosome-specific probes.

Material and methods

The probes

DNA specific probes (BMC 3224, BMC 4216) were derived from cosmids. The probes were developed and already located on chromosomes 25 (25q24) and 29 (29q13) at USDA, MARC, Nebraska (personal information).

In situ hybridization

In situ hybridization was performed essentially as described by LICHTER et al. (1990) with a modification by SOLINAS-TOLDO et al. (1993). Chromosomes were prepared from a fibroblast culture established from male bovine foetuses. Banding of the chromosomes was performed prior to hybridization by dipping the slides in a solution of 0.005% quinacrine mustard for 30 s at room temperature (QFQ-banding). Well spread metaphases were photographed prior to in situ hybridization with a Leitz Diaplan microscope equipped for fluorescence. After hybridization, the chromosomes were counterstained for 4 min with 4,6-diamidino-2-phenylindole (DAPI, 200ng/ml) in 2x SSC. To prevent fading of the signal, a few drops of antifade solution (JOHNSON et al. 1982) were used to cover the slides. FITC and DAPI were excited simultaneously with a BP 340-380 and BP 450-490 filter (Leitz Diaplan microscope). The two gray-scale fluorescence images were acquired with IPLab Spectrum and recorded in PICT format. Images were processed using IPLab and GeneJoin software package (RIED et al. 1992). Chromosomes with hybridization signals were identified following the standard karyotype (ISCNDA 1989) by using electronically stored pictures of QFQ-banding, taken prior to in situ hybridization.

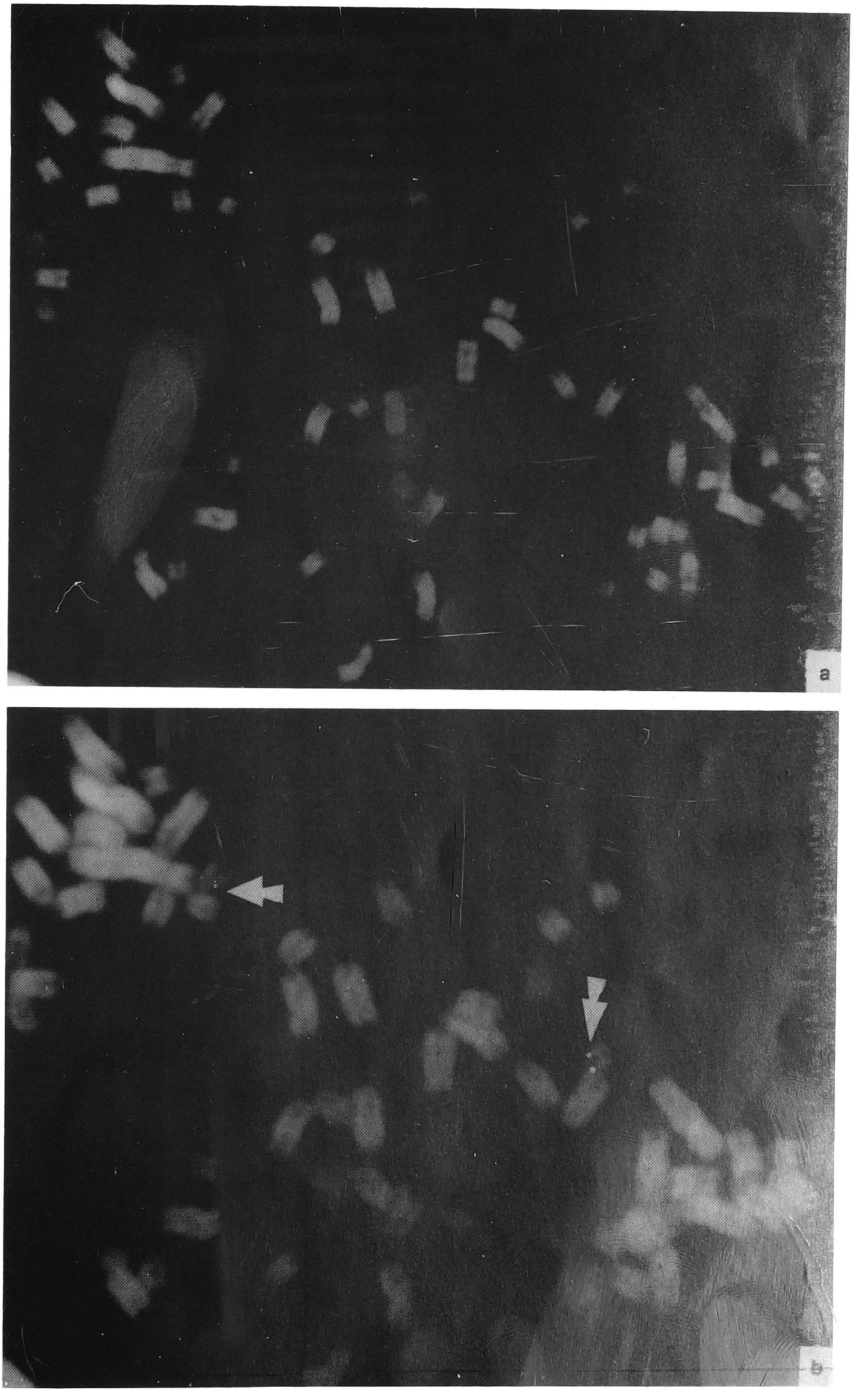

Fig. 1. Fluorescence in situ hybridization of cosmid probe BMC 4216, (29q13)
a – QFQ-banded partial metaphase prior to in situ hybridization, b – the same metaphase spread after in situ hybridization, stained with DAPI (the signal is indicated by arrow)

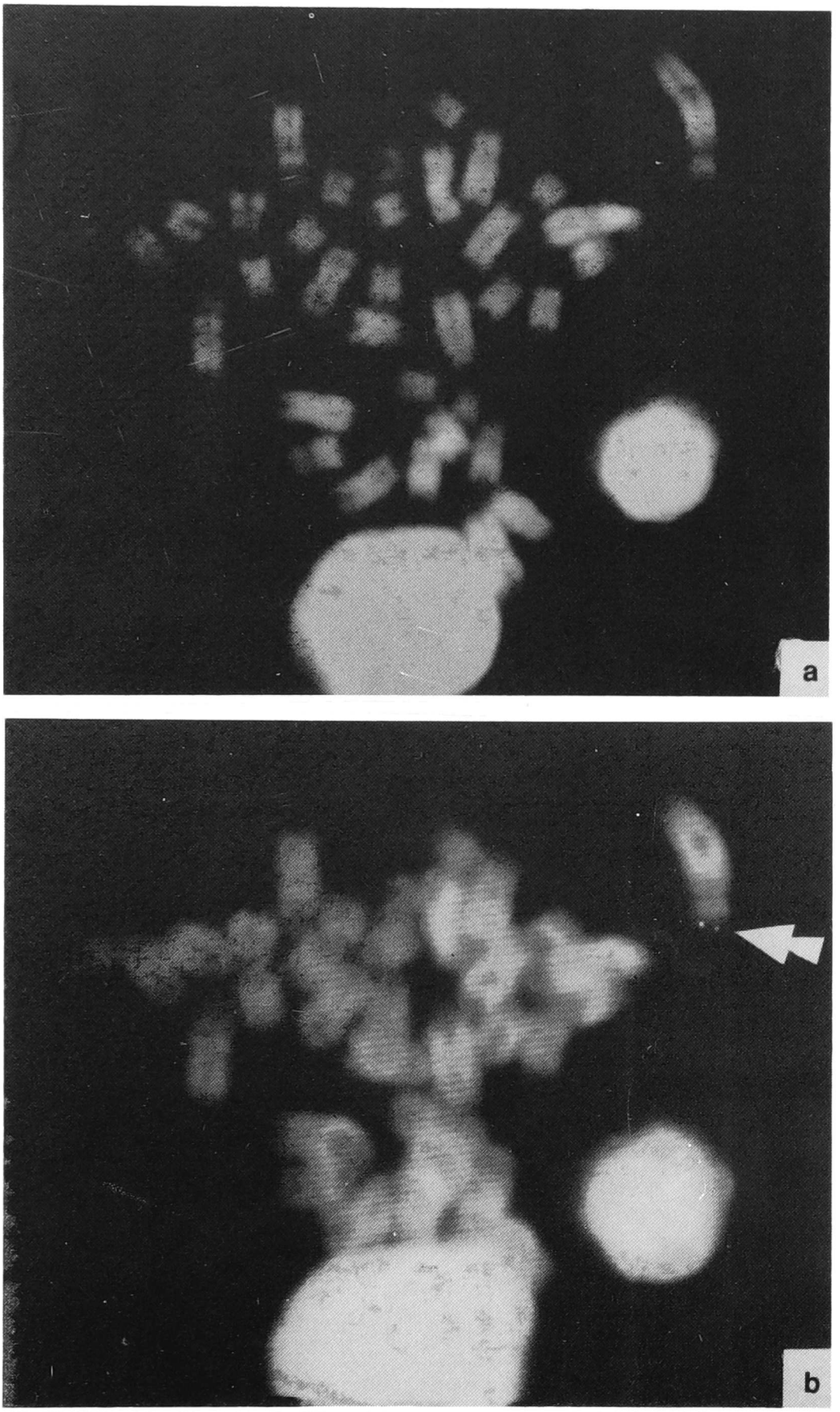

Fig. 2. Fluorescence in situ hybridisation of cosmid probe BMC 3224, (25q24)
a – QFQ-banded partial metaphase prior to in situ hybridisation, b – the same metaphase spread after in situ hybridisation, stained with DAPI (the signal is indicated by arrow)

Results and discussion

The in situ hybridization experiment with the use of the chromosome 29-specific probe (BMC 4216), showed no signals on the translocated chromosome. Among 10 spreads with preidentified QFQ banded chromosomes, fluorescence signals appeared in eight metaphases. In all of them, double-dot signals were identified on the both chromosomes 29 (Fig. 1).

The second experiment, carried out with the use of the chromosome 25-specific probe (BMC 3224) revealed hybridization signals on the p arm of the translocated chromosome and on a single chromosome 25. Again, among 11 spreads with preidentified QFQ banded chromosomes, double-dot signals were found in eight metaphases on the translocated chromosome and on a single chromosome 25 (Fig. 2).

These results showed that the translocation involved chromosomes 1 and 25, instead of previously described chromosomes 1 and 29.

Small acrocentrics in the bovine karyotype were difficult to recognize. It was already indicated on the Reading Conference (1980) that G-banding patterns of chromosomes 25 and 27 are quite similar.

IANNUZZI and Di MEO (1995) suggested that chromosomes 25 and 29 were inverted in the GTG and RBA standard karyotypes (ISCNDA 1989). The same difficulties were discovered by EGGEN et al. (1994), who applied molecular markers for recognition of chromosomes 25 and 29. They found that the molecular marker (IDVGA 7), previously mapped to G-banded chromosome 25, was localized on R-banded chromosome 29.

It shows that molecular studies are very useful in the diagnosis of chromosome aberration.

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