A new centric fusion translocation in cattle: rob (13;19)

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A new Robertsonian translocation has been found in cattle. A bull from Marchigiana breed (central Italy) was found to be a heterozygous carrier of a centric fusion translocation involving cattle chromosomes 13 and 19 according to RBA-banding and cattle standard nomenclatures. CBC-banding revealed the dicentric nature of this new translocation, underlining the recent origin of this fusion. In fact, both the bull's parents and relatives had normal karyotypes. In vitro fertilization tests were also performed in the bull carrying the new translocation, in two bulls with normal karyotypes (control) and in four other bulls carrying four different translocations.

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About at the same time, two very original studies showed the importance of centric fusion translocations in bovids from both an evolutionary (WURSTER and BENIRSCHKE 1968) and clinical (GUSTAVSSON 1969) point of view. By analyzing the karyotypes of many bovid species, WURSTER and BENIRSCHKE (1968) concluded that the differences in the diploid number were essentially due to the common use of Robertsonian translocations which reduced the diploid number and conserved, with few exceptions, the NF. GUSTAVSSON (1969) demonstrated that the presence of rob (1;29) in Swedish cattle reduced fertility in the carriers when compared with cattle showing normal karyotypes.

After these two important studies, many others demonstrated a high degree of banding homologies among bovids by using banding techniques, confirming the importance of centric fusion translocations in the autosomal karyotype evolution of bovids which originated from one common bovid ancestor (BUCKLAND and EVANS 1978; HAYES et al. 1991; GALLAGHER and WOMACK 1992; IANNUZZI and DI MEO 1995).

Rob (1,29) has been found in more than 60 different cattle breeds (POPESCU and PECH, 1991) throughout the world and its correlation with reduced fertility in the heterozygous carries has been confirmed (DYRENDHAL and GUSTAVSSON 1979; RANGEL-FIGUEIREDO and IANNUZZI 1993; MOLTENI et al. 1996). With the systematic cytogenetic screening of cattle populations, especially for the bulls, other centric fusion translocations involving different chromosomes were discovered (LONG 1985).

In this study we report the cytogenetic characterization of a new centric fusion translocation involving cattle chromosomes (BTA) 13 and 19 in a bull from Marchigiana cattle (central Italy), as well as data on in vitro fertilization tests in both carriers and normal bulls.

MATERIALS AND METHODS

Cytogenetic analysis

Peripheral blood lymphocytes from both the carrier bull, its parents and related individuals (all from the Marchigiana breed) were cultured in McCoy's medium (Gibco) enriched with L-Glutamine (1%), FCS (15%), Concanavalin A (Sigma, 1.5%), Penicillin-Streptomicin (1%) and Fungizone (1%). BrdU (15 μg/ml) and colcemid (0.01 μg/ml) were added seven and two hours before harvesting, respectively. Slides were stained with Giemsa (4% in phosphate buffer, pH = 7.0) for normal metaphase scoring and treated for CBG- (SUMNER 1972) and RBA-banding (0.1% acridine orange in phosphate buffer, pH = 7.0). Chromosomes were identified according to the ISCNDA89 (1990).

Semen analysis and 'in vitro' fertilization

Semen from both the bull carrier and two bulls from the Friesian breed with normal karyotype (control) was analyzed to detect sperm motility and progression according to BRYAN and AKRUK (1977) and LENZ et al. (1983a,b). In vitro fertilization of oocytes, collected from several female donors, with sperms of both carrier and control, was carried out according to PARRISH et al. (1988) and FIRST and PARRISH (1987). Then embryos were recovered in TC medium for 7 days by changing the

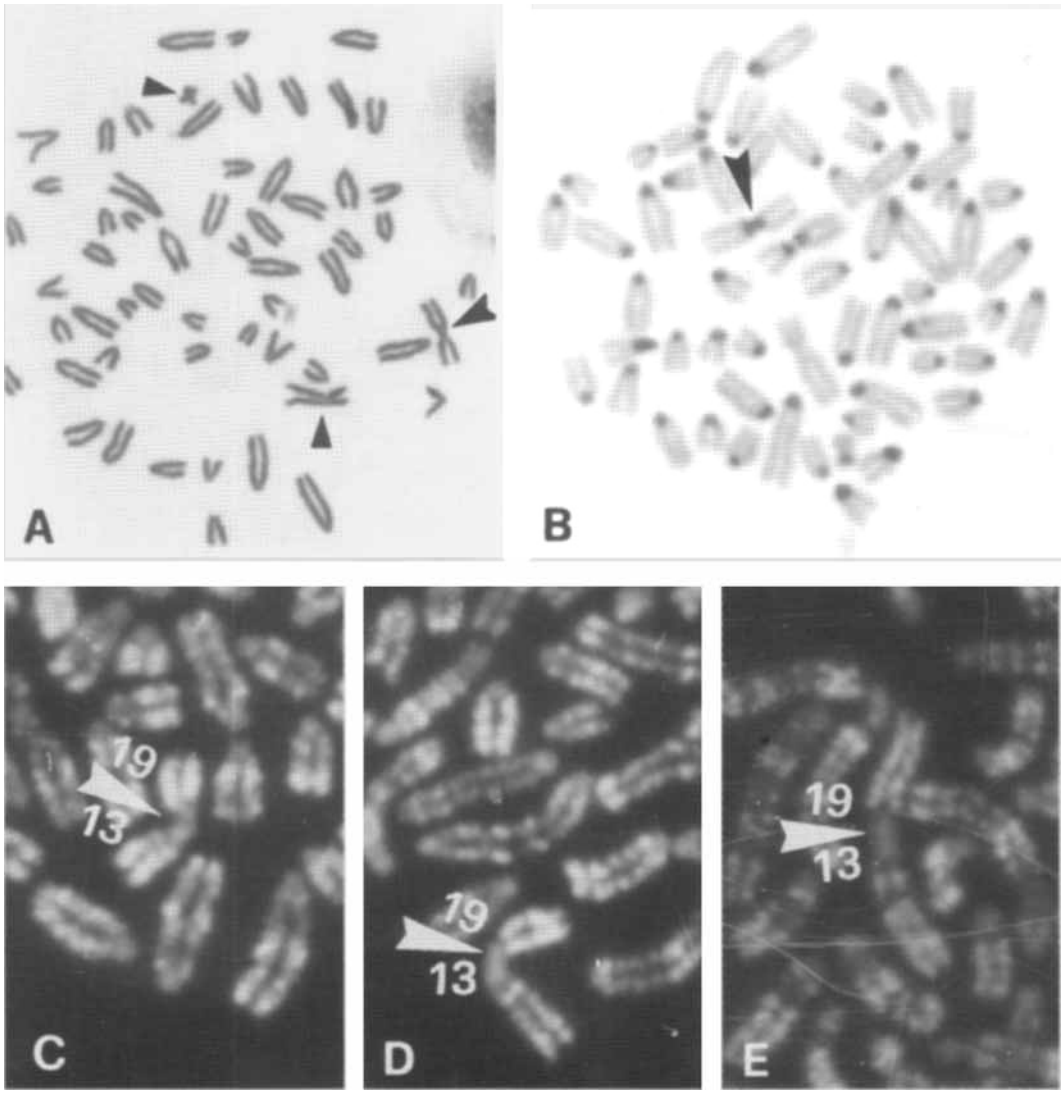

Fig. 1. A–E, A Conventional stained metaphase plate with 59 chromosomes for the presence of a biarmed chromosome (large arrow) in addition to X and Y (small arrows). **B** CBG-banded metaphase plate showing two clear HC-blocks in both arms of translocated chromosome (arrow). **C-E** Particulars of RBA-banded rob (13;19) (arrows) drawn from preparations with different degree of chromosome contraction.

medium every day. Of the total embryos, only those at the blastocyst state were considered as living. The same tests were applied to three bulls carrying three different centric fusion translocations: rob (1;29) and rob (14;17) from the Marchigiana breed and rob (25;27) from the Grigio Alpina breed.

RESULTS AND DISCUSSION

Cytogenetic investigations

All the 130 Giemsa stained metaphases we examined had 59 chromosomes with a biarmed chromosome, in addition to the X and Y (Fig. 1A). The CBG-banding patterns revealed the dicentric nature of this translocation (Fig. 1B), as observed in almost all other translocations involving different cattle chromosomes (LONG 1985). Dicentric translocations are known to be unstable due to the presence of both centromeres which can lead to the formation of a normal diploid number in few generations. Hence, dicentric translocations are considered of recent origin, unlike monocentric translocations, such as the rob (1;29), which is considered to be ancient. In fact, both parents and related animals of the bull carrying the new translo-

cation had a normal karyotype, suggesting the de novo origin of this translocation. The chromosomes involved in this translocation were identified as 13 and 19, according to the RBA-banding (Fig. 1C–E) and ISCNDA89 (1990) standard.

BTA19 has recently been involved in another centric fusion translocation with BTA21 (PINTON et al. 1997), which was also found to be dicentric.

Rob (13;19) involves two chromosomes which map the syntenic groups U11 (BTA13) and U21 (BTA19) (Texas nomenclature 1996). Thus, this marker chromosome could be used to obtain specific chromosome libraries to be used in Zoo-FISH mapping applications and cloning of specific DNA sequences of bovine U11 and U21. BTA19 is homologous to river buffalo chromosome 3p which also maps bovine U21 (IANNUZZI et al. 1997).

All cattle translocations could be attempts to obtain new genetic associations or simple recurrent chromosomal mutations. However, it is evident that only rob (1;29) seems to be stable in cattle populations, even if selection programs have progressively reduced its frequency in cattle breeds.

Semen analysis and in vitro fertilisation

To ascertain the effect of this translocation on fertility, in vitro fertilization tests on both carrier and two normal bulls (control) were performed. Compared to the control, we found a significant reduction in fertility (percentages of both in vitro fertilized oocytes and blastocysts of total zygotes) in the carrier. However, on analyzing the quality of the semen (sperm motility and progression) in both carrier and control, we found damage in the carrier acrosoma sperms, while the control sperms were normal. Thus, the reduced fertility in the carrier was probably due only to the poor conservation of frozen semen. To check this hypothesis, semen analysis and in vitro fertilization tests, as reported above, were performed on other bulls carrying different centric fusion translocation (robs 1-29, 14-17, and 25–27) previously found in the same and other breeds. Both semem analysis and in vitro fertilization tests gave normal values when compared with normal bulls. Thus, we concluded that the presence of centric fusion translocations does not affect the fertility in the carriers, at least in the early stages of an embryo's life. Since reduced fertility in the centric fusion translocation carriers is well documented (DYRENDHAL and GUSTAVSSON 1979; RANGEL-FIGUEIREDO and IANNUZZI 1993; MOLTENI et al. 1996), this study shows that it is not possible to select the bulls on the basis of in vitro fertilization tests only, and underlines the importance of cytogenetic analysis to eliminate the carriers from reproduction.

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