Genet. Sel. Evol. 32 (2000) 589–598

Original article

A new reciprocal translocation in a subfertile bull

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(Received 27 April 2000; accepted 19 July 2000)

Abstract – Three bulls of the Montbéliarde breed that exhibited fertility rates lower than 30% following more than 400 artificial inseminations were examined. Semen quality (sperm motility and morphology) from these bulls was normal. Fertilizing ability estimated from *in vitro* embryo production results was studied for two of them. *In vitro* production rate was very low for one bull (A) and normal for the other (B). Cytogenetic analyses were carried out on the three bulls using chromosome banding techniques. These analyses revealed a reciprocal translocation (12;17)(q22;q14) in bull B. Based on family analyses, the hypothesis of a *de novo* origin of this rearrangement is proposed.

cattle / chromosome abnormality / fertility / semen quality / cytogenetics

Résumé – Une nouvelle translocation réciproque chez un taureau hypofertile. Trois taureaux de race Montbéliarde présentant une très faible fertilité (< 30 %) après plus de 400 inséminations artificielles ont été étudiés. Les trois individus présentaient une qualité de semence (motilité et morphologie des spermatozoïdes) normale. La fécondance de la semence (aptitude à la production *in vitro* d'embryons) a été étudiée chez deux individus. Celle-ci a été faible pour un taureau (A) et normale pour l'autre (B). Des analyses cytogénétiques ont été réalisées dans tous les cas à l'aide de techniques de coloration en bandes des chromosomes. Elles ont révélé une translocation réciproque (12 ; 17)(q22 ; q14) chez le taureau B. Les analyses familiales réalisées permettent de faire l'hypothèse d'une apparition *de novo* du remaniement.

bovin / anomalie chromosomique / fertilité / qualité de la semence / cytogénétique

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1. INTRODUCTION

The first chromosomal abnormality in cattle was demonstrated as early as 1964 (Robertsonian translocation 1/29, [9]). Its negative effects on reproductive performances [6,8] led to the creation and development of control systems in numerous countries. In bovines, about 50 000 chromosomal analyses have been carried out up to now. This species, after man and mice, has been the most widely investigated from a cytogenetic point of view [7]. Most of the analyses have been carried out with simple chromosome staining techniques (conventional Giemsa staining). All 58 autosomes of the bovine karyotype also have the particularity of being acrocentric. These two elements help to explain why most of the abnormalities identified in cattle are Robertsonian translocations, *i.e.* centric fusions (42 described to date, [7]). This type of abnormality is characterised by a reduced number of chromosomes per cell (-1 for heterozygotes) and by the existence of an additional (sub)metacentric chromosome that is easily detected by conventional staining. The other types of structural rearrangements (inversions or reciprocal translocations for instance) generally have little effects on the shape of the chromosomes and can most often be visualised only by chromosome banding techniques. Only 15 cases including 10 reciprocal translocations (some of them being associated with subfertility) have been reported to date [4,7,12,16,19]. In this paper, we describe a new case of reciprocal translocation identified in a subfertile bull belonging to the Montbéliarde breed.

2. MATERIALS AND METHODS

This study aiming to detect individuals likely to present a karyotypic abnormality was carried out on bulls belonging to the Montbéliarde breed subjected to progeny testing during the 1996–1997 and 1997–1998 insemination campaigns. Selection of the bulls submitted subsequently to karyotypic analysis was based on the "fertility indicators" of the bulls given by the French national genetic evaluation program for fertility traits carried out twice a year for all dairy bulls [3]. These values are estimated using all available artificial insemination (AI) records and are corrected for effects such as herd, lactation number, month of insemination, inseminator and additive genetic values of the bull's mates. They are expressed as a rate of success after AI and in terms of deviation from the mean of the population. They are published only if 300 AI per bull and per year on dams of the same breed are performed. Thus a sample of 358 Montbéliarde bulls, which made 300 to 600 AI over two insemination campaigns, was studied. From these, three bulls (A, B and C) could be clearly distinguished from the population (with fertility indicators deviating respectively by -24 (A), -22 (B) and -16% (C) from the mean of the population).

The semen quality of the three subfertile bulls was assessed by thawing three straws of each ejaculate in hot water at 37 °C for about 30 s. After thoroughly mixing semen, the subjective motility of sperm cells was determined at $\times 400$

using negative phase contrast illumination. Sperm morphology was assessed at $\times 1\,000$ using differential interference contrast illumination.

Fertilizing ability *in vitro* was studied for bulls A and B, but not for bull C which presented a higher rate of sperm abnormalities. Bovine ovaries were obtained from the slaughterhouse and were transported to the In Vitro Embryo Production (IVEP) U.N.C.E.I.A. laboratory in phosphate buffered solution at 35 °C. Cumulus-oocyte complexes were aspirated from 2–6 mm follicles. Oocyte maturation was performed in TCM 199 (25 mM Hepes Gibco) supplemented with 15% fetal calf serum (FCS), 10 mg $\cdot$ L^{-1} FSH/LH, 1 mg $\cdot$ L^{-1} estradiol 17 β at 38.5 °C under an atmosphere of 5% CO_2 in air for 26 h. Modified Tyrode media (TALP) were used for capacitation and fertilization [2,13]. Frozen-thawed semen derived from a single ejaculate was swim-up separated in TALP medium. Motile sperm from the upper fraction were centrifuged and the sperm concentration was adjusted to 1×10^6 cells $\cdot$ mL^{-1} in fertilization medium. A final volume of 0.5 mL of sperm suspension was placed in the test tubes. Matured oocytes (117 for bull A and 150 for bull B) were washed 3 times in the fertilization medium and placed in fertilization tubes in the presence of 2 mg $\cdot$ L^{-1} heparin, 10 μM hypotaurine, 1 μM epinephrine and 20 μM penicillamine. After 18 h, the presumed zygotes were vortexed for 2 min, washed and cultured for 6 days in 50 μL SOF micro-droplets covered with mineral oil [18]. FCS (10%) was added to SOF micro-drops 24 h after beginning the culture. On day 7 (day 0 = day of *in vitro* fertilization) the embryos were morphologically evaluated and classified as developed (morulae and blastocysts) or degenerated. The results of embryo production are expressed as fertilization rate, cleavage rate, blastocyst development rate and blastocyst yield (blastocyst number/oocyte number).

Blood samples were taken from all three bulls. An additional skin sample was taken subsequently from the bull carrying the chromosome rearrangement. Other blood samples were taken from animals related to the carrier bull (the mother, 8 daughters, 10 paternal half-sibs) so as to determine the origin of the abnormality and its transmission rate.

Mitotic chromosomes were prepared from non-synchronised cultures of peripheral blood lymphocytes, or from synchronised cultures of skin fibroblasts. Whole blood was cultured at 37 °C for 72 h in RPMI-1640 medium supplemented with 20% fetal calf serum, 500 UI Heparin, 1% antibiotic-antimycotic solution (Gibco), and stimulated with Concanavalin A (final concentration: 0.3 μg $\cdot$ L^{-1}). Colcemid (final concentration: 0.03 mg $\cdot$ L^{-1}) was added to the culture 60 min before harvesting. Primary fibroblast cultures were initiated from skin fragments, disrupted and digested in a trypsin solution (2.5 g $\cdot$ L^{-1}), and grown at 37 °C in a CO_2 incubator in Falcon dishes (75 cm^2 containing a medium comparable to that previously described for lymphocyte cultures (RPMI 1640 replaced by Ham'F12). Hypotonic treatment (10 mL 1/6 calf serum) was followed by pre-fixation and fixation in ethanol:acetic acid (3:1). Chromosome preparations were spread on cold wet slides and air dried. After overnight incubation at 60 °C, the preparations were treated for GTG banding as described in [17].

The chromosomes were arranged according to the standard nomenclature [10].

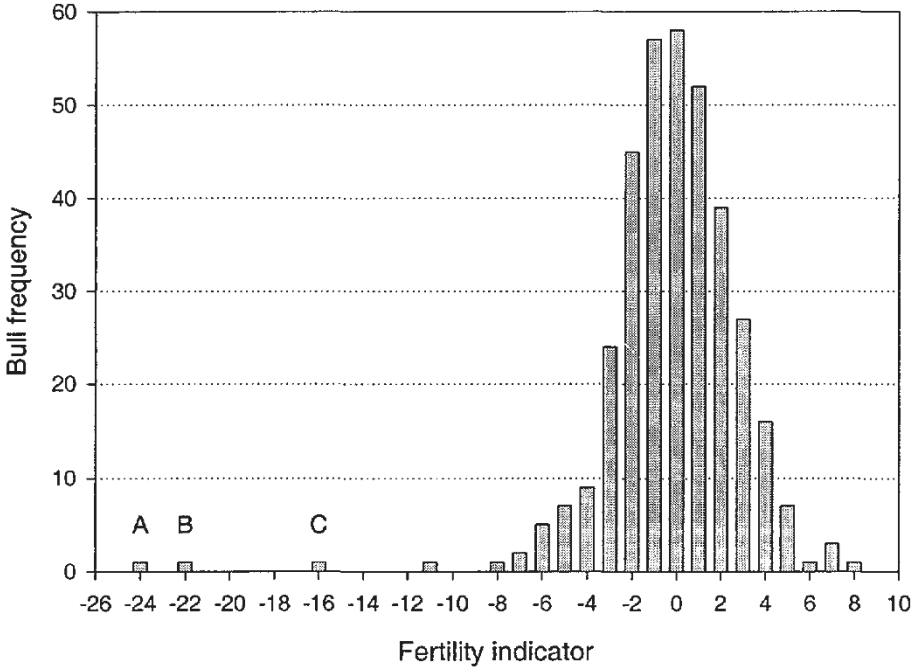

Figure 1. Distribution of 358 Montbéliardes testing bulls according to their fertility indicator during the AI periods 1996–1998.

3. RESULTS

The distribution of the progeny-tested bulls according to their fertility indicator was apparently similar to a Gaussian distribution, varying from −8 to +8% with an average of −0.19 and a standard deviation of 3.20 (Fig. 1). Bulls A, B and C, with fertility rates −24, −22 and −16% below the mean value, respectively, were clearly outside this distribution.

Semen quality estimated from frozen-thawed semen could not explain the poor fertility of bulls A, B and C, since the percentage of progressive sperm cells was over 45% for all three bulls (Tab. I) and the percentage of sperm cells with a major abnormality did not differ from that of fertile bulls, *i.e.* low for bulls A and B (12.9 and 16.0%) and still acceptable for bull C (22.6%).

Bulls A and B were evaluated for *in vitro* fertilization and IVEP. Fertilization and embryonic development rates observed with the semen from bull A were very low (56 and 8% respectively). In contrast, the fertilization and development rates obtained with semen from bull B (88 and 34% respectively, Tab. I) were comparable to the average values regularly obtained with routine procedures.

Initial examination of metaphasic chromosomes of bulls A, B and C revealed different abnormalities for bull B only: (1) presence of one chromosome 12 with an abnormal distal portion representing approximatively 1/3 of the chromosome (presence of a large light band at the tip); (2) apparent lack of one

Table I. Semen quality, *in vitro* embryo production and fertility results for the three subfertile test bulls.

	Semen Quality			*In vitro* embryo production					Fertility	
Bull	Number of ejaculates	Sperm motility	Sperm with major abnormality	Inseminated oocytes	Fertilization rate	Cleavage rate	Developmental rate	Blastocyst yield	Number of AI	Fertility indicator
A	10	46.5%	12.9%	117	56%	72%	8%	3%	435	−24%
B	4	50.0%	16.0%	150	88%	82%	34%	27%	436	−22%
C	4	48.8%	22.6%	–	–	–	–	–	581	−16%

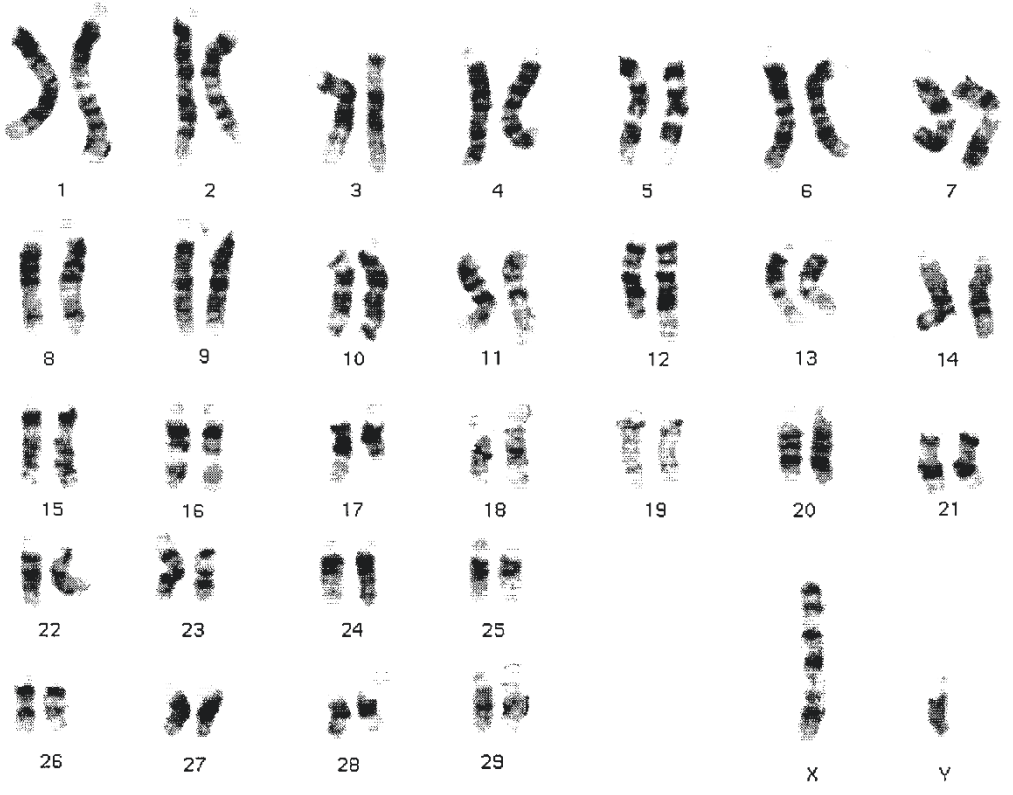

Figure 2. GTG-banded karyotype of the bull heterozygous carrier of the 12/17 reciprocal translocation (normal chromosomes are on the left, tranlocated chromosomes on the right).

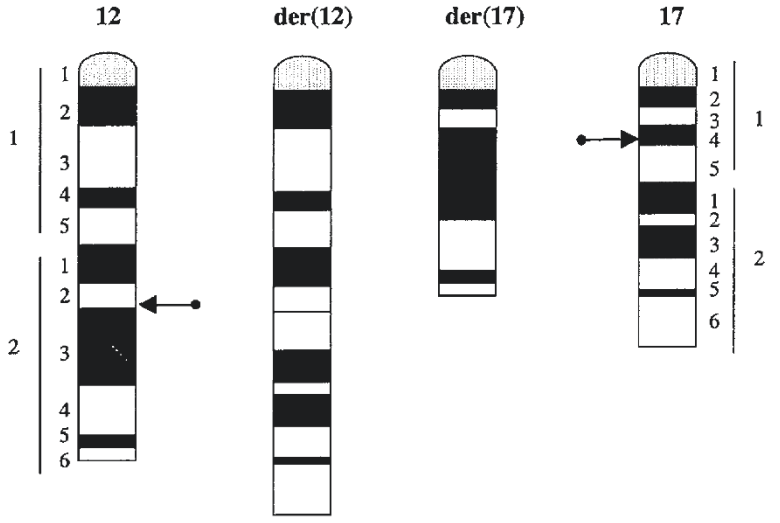

Figure 3. Diagrammatic representation of the GTG-banded normal and translocated chromosomes No. 12 and No. 17 (the arrows represent the breakpoints on the chromosomes.

chromosome 17 (the banding of which is usually well characterised by a thick black band in the medial portion); (3) presence of a small chromosome comparable in size and band profile to a chromosome 27 (Fig. 2). It became apparent from more thorough analysis that the fragment identified in the distal portion of the abnormal chromosome 12 in fact presented a band profile similar to the q14 → qter portion of chromosome 17. Reciprocally, the small supernumerary chromosome exhibited a terminal banding profile equivalent to bands 12q23 → 12qter. We therefore propose the hypothesis of a reciprocal translocation between chromosomes 12 and 17. The chromosomal rearrangement could be described, according to the standard nomenclature, as 60,X,rcp(12; 17)(q22; q14), Figure 3.

The dam of bull B was found to be a non-carrier of the abnormality. The sire was already dead at the time of the study and could not be karyotyped. However, 10 progeny of the sire could be analysed (6 males and 4 females — half-sibs of bull B) and were all non-carriers. Two of the eight karyotyped daughters were carriers of the abnormality.

The estimated fertility indicator of the sire of bull B was excellent (+7.6%) whereas the estimated values for the 16 paternal half sibs of bull B varied within a normal range (between −5% and +6%; +0.9% on average).

4. DISCUSSION

The analyses carried out on the animals related to bull B, carrier of the chromosome rearrangement, led us to hypothesise a *de novo* appearance of the abnormality in this bull. The dam of bull B was proven to be a non-carrier of the translocation, as well as the 10 offspring of the sire of bull B

that were analysed. The probability for 1 offspring of a heterozygous carrier to be free of the translocation being 0.5, the probability of paternal origin was less than 0.001 or $\left(\left(\frac{1}{2}\right)^{10}\right)$. This hypothesis was coherent with the fertility indicator values estimated for the sire and the half-sibs of the bull. This result is original since the source of chromosome abnormalities reported in the literature for livestock species is relatively rarely indicated, due to the absence of the necessary familial information.

Examination of the literature shows that in the bovine, as in man or in other species, a balanced reciprocal translocation carried in the heterozygous state by an individual generally leads to the production of genetically unbalanced gametes (partial monosomies or trisomies) with unaltered fertilizing capacity, thus resulting, after fertilization with normal gametes, in non-viable embryos [16, 20]. Our observations about the quality and fertilizing ability of semen, as well as *in vitro* embryonic development of bull B clearly confirm this hypothesis. These results also indicate that the abnormality identified in this bull does not alter the early stages of embryonic development up to the blastocyst stage (8 days). Embryonic mortality due to chromosome imbalance would therefore occur beyond this stage. The proportion of unbalanced gametes in the semen of the translocated bull could, for example, be determined by fluorescent *in situ* hybridization on sperm nuclei [11].

Apart from describing a new abnormality of a type very rarely demonstrated in cattle (only 10 cases of reciprocal translocation have been described in bovines), the case reported here illustrates the value of systematic chromosome control in livestock in cases of male subfertility. Such a control policy has been successful for several years in pigs [5,15] and has made the prevention of the diffusion of chromosome abnormalities with unfavourable economic effects possible. The only official objective of routine cytogenetic controls carried out up to now in bovines has been to detect the Robertsonian translocation 1/29 in certain breeds [5]. More detailed analyses involving chromosome banding techniques, should be recommended in individuals that exhibit considerably reduced fertility so that possible chromosomal abnormalities, of the type described in the present article, can be identified early on. This recommendation was recently applied in two other breeds of cattle (Blonde d'Aquitaine and Holstein Friesian). Neither of the two bulls tested exhibited a chromosome abnormality. The reduced fertility of these two bulls, as those of bulls A and C studied in the present paper, could be due to modifications at the gene-level, non-detectable with cytogenetic techniques, but also to non-genetic factors.

Our current knowledge of the overall frequency of chromosome abnormalities in the bovine is rather limited, due to the relatively low number of banding analyses carried out on animals that have not been selected *a priori* in relation to predisposition criteria (malformations, sterility, ...) In our laboratory, 750 banding analyses have been carried out on animals chosen at random, without any particular distinguishing signs. Only two abnormalities have been characterised: a Robertsonian translocation 19/21 in a Holstein Friesian cow [14] and a complex rearrangement not yet characterised in a Charolais bull. This estimated frequency (less than 0.3%) seems much lower than that reported in man (0.62%, [1]) or in pigs (about 1%, [5]). Broader knowledge

in this domaine will be required to judge the relevance of controlling the karyotypes of bulls with negative fertility indicators but less distant from the mean than those reported in the present article, which should have been eliminated anyway (potential carriers of abnormalities with a moderate effect on reproductive performance).

ACKNOWLEDGEMENTS

We wish to express our gratitude to the staff of the insemination centres and of the selection organisations for the blood-sampling and various arrangements concerning the animals.

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