A New Type of Robertsonian Translocation (1/26) in a Bull with Unilateral Cryptorchidism, Probably Occurring *de novo*

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ABSTRACT. The karyotype of a Holstein-Friesian bull with unilateral cryptorchidism was investigated according to the G-, C- and Cd-banding techniques. By G-banded karyotyping, it was shown that this bull had 1/26 Robertsonian translocation. Furthermore, there were 2 dark dots at the centromere of 1/26 translocated chromosome by C- and Cd-banded karyotyping. Although the chromosomes of the parents of this bull were not examined, 12 half-sib cows from different sires or dams did not show any chromosomal aberrations. The semen quality of the father and the conception rate of the parents were within the normal range in Japan. From the results obtained, it appears that this translocation resulted from a centric fusion at the early embryonic stage and had occurred *de novo*.—**KEY WORDS:** bull, chromosome, cryptorchidism, Robertsonian translocation (1/26).

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Since it has been reported that many types of chromosome translocation frequently show unfavorable effects on the fertility of domestic animals [4, 6–11], this chromosomal abnormality has gained an interest among cytogeneticists involved in the field of animal reproduction as well as theriogenologists. The 1/29 Robertsonian translocation reported by Gustavsson & Rockborn [6] was the trigger of subsequent findings of various types of chromosome translocation, especially in the bovine because of the decrease in fertility that was observed. Presently, it has been found that this is true in at least 38 breeds, distributed throughout the world, where cytological studies have been made [4]. In this study, we report a new type of translocation, 59, XY, t(1;26), in a bull with unilateral cryptorchidism.

MATERIALS AND METHODS

A Holstein-Friesian bull was born in September of 1985, and has been grown for beef. At the time of castration (10 months

old), it was found that the right testis did not descend into the scrotum, and thus this bull was diagnosed as a case of unilateral cryptorchidism.

For chromosome analysis, heparinized blood and a fragment of skin were aseptically collected, and were cultured in a medium supplemented with fetal calf serum and phytohemagglutinin (in the case of blood culture) at 38°C. In addition, the heparinized blood samples of 9 half-sib cows from different mothers, and 3 half-sib cows from different fathers were also cultured. After 72 of blood culture and at the time of the highest frequency in cell division of the fibroblasts from the skin, the cultures were treated with colcemid and chromosome preparations were made according to routine procedures. Over fifty metaphase plates per case were examined under a microscope and analyzed for karyotyping. For further studies, the chromosomes were analyzed by the G-banding [4], C-banding [11] and Cd-banding [1] techniques.

The semen quality of the father of this bull, and the conception rate of the father

Fig. 1. A metaphase plate with the translocated chromosome (arrow) ($2n=59$) derived from blood culture. X and Y show the sex chromosomes.

Table 1. Chromosome analysis from leucocytes and fibroblasts in bull with unilateral cryptorchidism

	Karyotype	
	59, XY, t (1; 26)	60, XY
Leucocytes	82 ^{a)}	0
Fibroblasts	51	0

a) Number of cells counted.

and the mother were evaluated from the records at the artificial insemination center and of their owners in Japan.

RESULTS

The results of chromosome analysis from the blood and fibroblasts of this case are shown in Table 1. Blood samples were cytogenetically analyzed 2 times at proper intervals. The chromosome number of all 81 metaphases examined was 59 which was smaller than that of normal cattle ($2n=60$) and the sex chromosome complement showed XY. In the metaphases stained with giemsa solution, there was a larger submetacentric chromosome than X chromosome, which was not normally observed

Fig. 2. G-banded karyotype. The dotted arrows show the partner of chromosomes, No. 1 and No. 26, that form the translocated chromosome (1/26).

(Fig. 1). The 51 cells from the fibroblast culture showed the same karyotype as in the results obtained from the leucocytes (Table 1). There were no 60, XY cells in any of the metaphases examined from both tissues. From these results, it was surmized that this large submetacentric chromosome resulted from a centric fusion of 2 different acrocentric chromosomes. By G-banded karyotyping, an abnormal submetacentric chromosome was clearly identified to be formed by Robertsonian translocation between chromosomes No. 1 and No. 26 (Fig. 2), according to the standard agreed upon at the 1976 Reading Karyotype Conference [3]. The centromeric regions of 1/26 translocated chromosome were stained with C-banding and Cd-banding techniques, and had two dark dots (Figs. 3 & 4).

Although the chromosomes of the father and mother of this case was not investigated because they were already slaughtered, it

Fig. 3&4. Fig. 3 shows the C-banded metaphase plate. The translocated chromosomes of A-E which were derived from 5 different metaphases are dicentric. Fig. 4 shows the Cd-banded metaphase plate. The translocated chromosome (left) and chromosome No. 1 (right) were derived from 5 different metaphases (A-E). Note the dicentricity in the translocated chromosome, and the monocentric one in chromosome No. 1.

Fig. 5. Chromosome analysis of 12 half-sib cows from different parents. P1-P9: Half-sib cows from different mothers. M1-M3: From different fathers.

was ascertained that the 9 half-sib cows from different mothers and the 3 half-sib cows from different fathers had normal karyotypes (Fig. 5).

The semen of the father was collected and examined once a week and once or twice a

day. From a total of 53 records of semen examination, the semen volume was 7.5 ± 2.8 ml (mean $\pm$ SD), spermatozoan concentration was $14.8 \pm 2.6 \times 10^8$ /ml, pH was 6.3 ± 0.1 and the viability index was estimated as excellent (Table 2).

The conception rate of cows inseminated with semen from the father is shown in Table 3. For a period of 2 years, a total of 210 cows including the mother of this case were inseminated with semen from the father, and 147 cows (70.0%) conceived. For the same years, the average conception rate for 291–313 bulls in Japan was 61.3–62.0%. On the other hand, the mother had 9 calves including this case during her life, and the conception rate was excellent.

DISCUSSION

The following 3 hypotheses are considered as for the possible cause of this Robertsonian translocation: first, by the heritable translocation from either ancestor; second, by the fertilization of gamete with translocated chromosome that was formed

Table 2. Semen examination record of the father of 1/26 bull

n	Volume (ml)	Spermatozoan concentration ($\times 10^8/\text{ml}$)	pH	Viability
53	7.5 $\pm$ 2.8 ^{a)}	14.8 $\pm$ 2.6	6.3 $\pm$ 0.1	+++

a) mean $\pm$ SD.

Table 3. Conception rate of cows artificially inseminated with semen from the father of 1/26 bull

Years of examination	Number of cows inseminated	Number of pregnant cows	Conception rate (%)	Average conception rate in Japan (%)
1984	59	45	76.3	62.0 (313) ^{a)}
1985	151	102	67.5	61.3 (291)

a) The numbers in parentheses show the number of bulls bred for the artificial insemination in Japan.

at the time of meiosis of either ancestors; third, by the centric fusion at the time of early embryonic stage *per se*.

The chromosomes of the father and mother of this case could not be examined, and it was definitely unknown whether this translocation resulted from heritable translocation of either parent or not. If either parent has a translocation, it is expected that half the number of half-sib cows from different sires or dams will have the same type of translocation as that in this case. However, a total of 12 half-sib cows examined did not show any abnormal karyotypes. Therefore, the first hypothesis would be denied as the cause.

Similarly, the second hypothesis would be denied because of the normal karyotypes in 12 half-sib cows, normal semen quality of the father and the excellent conception rate of both parents. If either parent produced a gamete with translocated chromosome, the calves with translocated chromosome would be detected. Furthermore, it seems that the disturbances of fertility would be noted from the poorer semen quality or fewer

number of calves.

In this study, we have confirmed that this translocation resulted from a centric fusion between chromosome No. 1 and No. 26 at the early embryonic stage. This was verified from the C-banded and Cd-banded karyotypes. In the case of the famous 1/29 translocated chromosomes, the single C-band observed has been accepted by some authors as the evidence of the old, well-established translocation. Robertsonian translocations of a more recent origin seem to have larger, or dicentric centromeres as shown by C-banding or Cd-banding [2]. Therefore, our translocation (1/26) has not been transmitted for many generations, but has occurred *de novo*. MÄKINEN & REMES [10] reported one A. I. boar with reduced litter size and reciprocal translocation, probably of a *de novo* origin.

However, it is not clear whether there is any correlation between the chromosome translocation and the unilateral cryptorchidism. This will be discussed later in another paper.

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要 約

片側性潜伏精巢を有する雄牛の染色体転座 (1/26) 例：三宅陽一，金田義宏¹⁾（岩手大学農学部寄生虫病学教室，¹⁾家畜病院）——片側性潜伏精巢を示したホルスタイン種の雄牛の培養白血球について，染色体を分析した。観察されたすべての細胞分裂中期像（82個）中に，常染色体性の転座染色体1個認められ，染色体数は59個であった（正常牛は2n=60）。異常染色体では，2個の異なる常染色体対の1個ずつが，その動原体部位で融合し，Gバンド分染法により，染色体 No.1 と No.26 の転座が明らかにされた。皮膚由来の染色体構成分析によっても白血球と同様の染色体転座が認められた。また C, Cd バンド分染法では，転座染色体の動原体部位に2個の濃染ヘテロクロマチンが観察された。両親牛の染色体分析は実施できなかったが，異母兄妹の雌牛9頭と異父兄妹雌牛3頭には染色体異常は見出されなかった。これらの結果から，転座染色体は両親牛に由来するものでなく，おそらく受精卵ないし早期胚における染色体異常に起因するものと思われた。