

EFFECT OF THE 7/21 ROBERTSONIAN TRANSLOCATION ON FERTILITY IN JAPANESE BLACK CATTLE

H.Hanada¹, A.Onishi¹, K.Takeda¹ and M.Geshi²

¹ National Institute of Animal Industry, Tsukuba Norindanchi P. O. Box 5,
Ibaraki-ken 305, JAPAN

² Tohoku National Agricultural Experiment Station, Morioka-shi 020-01, JAPAN

SUMMARY

To confirm the effect of the 7/21 Robertsonian translocation on fertility in Japanese Black Cattle, semen characteristics of the carrier bulls were analyzed. Further cytogenetic studies were carried out on spermatozoa and embryos derived from bulls heterozygous for the 7/21 translocation. The 7/21 translocation seemed to cause no adverse effects on semen characteristics and the fertilization in *in-vitro* culture system. In bulls heterozygous for the 7/21 translocation, however, unbalanced spermatozoa resulting from adjacent meiotic segregation were detected but only at a rate of 4.1%. A few unbalanced embryos, due to the 7/21 translocation, were also observed in the chromosome analysis of the embryos derived from 7/21 translocation heterozygous carriers. These results indicate that the 7/21 Robertsonian translocation in a heterozygous state brings about only a slight reduction in reproductive efficiency. In Japanese Black Cattle, the 7/21 translocation appeared to be under little or no negative selection due to the slight disadvantage in reproduction.

Keywords: cattle, 7/21 Robertsonian translocation, chromosome analyses, reduced fertility

INTRODUCTION

In addition to the 1/29 translocation, the 7/21 translocation has been observed at a frequency of about 10% due to continuous use of the carrier bulls in artificial insemination in Japanese Black Cattle (Hanada, 1994). The 1/29 translocation has been reported in many breeds throughout the world and its effect on fertility has been studied in depth. This translocation apparently causes a reduction in reproductive efficiency due to the early death of unbalanced embryos. On the other hand, the 7/21 translocation is an individual chromosome aberration observed only in Japanese Black Cattle and its effect still remains to be solved. However, we have recently reported that the carrier bulls also produced chromosomally unbalanced spermatozoa and the subsequent abnormal embryos (Hanada *et al.*, 1995a,b). Probably, the 7/21 translocation leads to a reduction in fertility similar to the case of the 1/29 translocation though our results were obtained only from a limited number of spermatozoa and embryos we have analyzed.

To confirm the effect of the 7/21 translocation on fertility in more detail, we have carried out a semen analysis of bulls heterozygous regarding the 7/21 translocation and further cytogenetic studies of spermatozoa and embryos derived from the carrier bulls.

MATERIALS AND METHODS

Semen analysis. Samples for semen analyses were obtained from 32 Japanese Black bulls used for artificial insemination; 26 of them had a normal karyotype and 6 were heterozygotes for the 7/21 translocation. The parameters investigated were: volume, concentration of spermatozoa, abnormal sperm, motility before and after freezing. Testis materials for histological study were obtained from two carrier bulls at slaughter.

Chromosome analyses of spermatozoa and embryos. Frozen semen samples from five Japanese Black bulls were used in the chromosome analysis of spermatozoa. Of these bulls, three were heterozygotes. Chromosome analysis of spermatozoa was carried out using an inter-specific *in-vitro* fertilization system with zona-free golden hamster oocytes as described by Tateno and Mikamo (1987).

In *in-vitro* fertilization study, semen samples from three normal bulls and from three bulls with the 7/21 translocation were used. Fertilization rate was examined 20 h after insemination according to the method described previously (Geshi *et al.*, 1996). Bovine embryos for the chromosome analysis were produced by *in-vitro* fertilization. Maturation of oocytes *in-vitro*, sperm capacitation, insemination and culturing of fertilized ova were performed by a modification of the method described previously (Geshi *et al.*, 1993). Embryos collected at the 8-cell and blastocyst stages were used in this study. Chromosome analysis was performed on the diploid embryos.

RESULTS AND DISCUSSION

Semen characteristics of bulls with the 7/21 translocation. The 7/21 translocation carrier bulls were compared with chromosomally normal bulls concerning semen characteristics and the histological picture of testicular. Semen characteristics such as volume of the ejaculate and concentration of semen in the 7/21 translocation bulls appeared normal. Semen quality and freezability were not adversely affected by the translocation. The seminiferous tubules seemed to be normally developed with a histologically normal spermatogenesis as regards the carrier bulls.

As to the effect on semen characteristics in cattle, there are only a few pieces of data about the 1/29 translocation. Some studies indicate that the 1/29 translocation heterozygous bulls, unselected from the viewpoint of fertility, revealed a small reduction in the concentration of semen and in the total number of spermatozoa (Dyrendahl and Gustavsson, 1979; Gray *et al.*, 1991). However, we have been unable to detect any deleterious effect in semen analysis of the 7/21 translocation carrier bulls.

Results of chromosome analyses of spermatozoa and embryos. In normal bulls, one out of a total of 61 spermatozoa karyotyped was presumptive nullisomic sperm with 29, Y, resulting from nondisjunction. In bulls heterozygous for the 7/21 translocation, chromosome analysis

was successfully carried out on a total of 148 spermatozoa. Most of spermatozoa were normal and balanced cells from alternate segregation of a trivalent chromosome. However, unbalanced spermatozoa with 30, Y, t and 30, X, t chromosome complements, due to the 7/21 translocation, were detected, but only at a rate of 4.1%. The incidence corresponds to that of aneuploid secondary spermatocytes in the meiotic study of the 7/21 translocation heterozygous bulls (Hanada and Muramatsu, 1989).

In *in-vitro* culture system, the 7/21 translocation carrier bulls (76.8%) had a similar fertilization rate when compared to that of normal bulls (75.3%).

The results of the chromosome analysis of embryos can be seen in Table 1. In the group sired by normal bulls, all of the analyzable embryos collected at the 8-cell and the blastocyst stages showed normal chromosome complement of 60, XY and 60, XX. However, a few unbalanced embryos, resulting from the fertilization of unbalanced gametes, were observed in the group sired by the 7/21 translocation carrier bulls. In cattle, similar results were also obtained in the chromosome analysis of embryos recovered from cows inseminated with semen from the 1/29 translocation heterozygous bulls (King *et al.*, 1980; Schmutz *et al.*, 1991). The formation of unbalanced embryos in cattle with the 7/21 Robertsonian translocation appeared to be similar to that of the 1/29 translocation.

Table 1. Chromosome analysis of embryos derived from the 7/21 translocation carrier animals^A

No. of embryos	8-cell stage		Blastocyst stage	
	Karvotype of bull		Karvotype of bull	
	Normal	Hetero.	Normal	Hetero.
Processed	178	173	100	233
Analyzed	57	54	38	113
Balanced				
Normal	57	28	38	57
Hetero.	0	23	0	49
Unbalanced				
Monosomy	0	1	0	2
Trisomy	0	2	0	5

^A The results include those analyzed in previous studies (Hanada *et al.*, 1995a, b).

In conclusion, the 7/21 translocation is unlikely to affect spermatogenesis as much as it causes fertilization failure. In bulls heterozygous for the 7/21 translocation, however, the formation

of unbalanced spermatozoa and the subsequent embryos were detected though their incidence was low. The 7/21 translocation in a heterozygous state apparently leads to reduced fertility. The present study supports the conclusion that any reduction in fertility related to the 7/21 translocation would be due mostly as a result of the early death of unbalanced embryos rather than fertilization failure. The finding of a presumptive 60,XX,rob(7;21) zygote in the cytogenetic study of aborted fetuses indicates the possibility of fetal survival for several weeks following implantation (Hanada and Geshi, 1995). However, monosomic embryos for either chromosome 7 or 21 are probably eliminated at a much earlier stage. Considering the low incidence of chromosomally abnormal embryos and the time of their loss, the 7/21 translocation in a singly heterozygous state seems to bring about only a slight reduction in reproductive efficiency. In Japanese Black Cattle, the 7/21 translocation appeared to be under little or no negative selection due to the slight disadvantage in reproduction.

In mice, a severe breakdown was observed in mice with heterozygosity for two translocations with a common arm (Aranha and Martin-DeLeon, 1995). Recently, we have found a rare case with monobrachial compound involving the 7/21 translocation. To understand the presence of monobrachial homology for chromosome 21 in compound translocation heterozygotes and its consequences in more detail, further studies are now in progress.

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