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FURTHER CHROMOSOMAL AND CLINICAL STUDIES ON THE XY/XYY MOSAIC BULL

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Cytogenetical investigation of the blood (4 times), skin (2 times), spleen and kidney was performed on a bull with unilateral cryptorchidism. This abnormal bull had two cell lines, XY cell line and XYY cell line, in all tissues examined, but the ratio with XYY cell line in the blood and skin samples became significantly lower with aging.

The father bull of this case, and the 9 half-sib males and 11 daughter cows sired by the bull were cytogenetically examined. Except for one half-sib male with sex chromosomal chimerism, which was a heterosexual twin male, there was no chromosomal abnormality detected.

Semen collected from this case was extremely lower in quality than that of normal bulls. Despite of lower semen quality, this case showed a similar conception rate to normal bull in Japan.

From the examination of the testes obtained at the time of slaughter, the descended right testis showed severe calcinosis, and its seminiferous tubes were filled with calcareous materials. The left testis was small and did not show any spermatogenesis.

Key words: chromosome, XY/XYY mosaic, bull, cryptorchidism

The establishment of techniques for preparing blood leucocyte cultures (MOORHEAD et al., 1960) for cytogenetical studies in human beings and domestic animals has greatly benefitted works on various congenital malformations and cases of sterility in domestic animals. Recently many reports have become available on the relations between chromosomal aberrations and abnormal reproductive developments in the horse,[2~4,10,12~14,19,26,28,29,32] pig,[5,27,34] cow[15,21,25,30,31,33,35,36] and others.[6,8,17,23]

We reported previously the case of XY/XYY mosaic bull (MIYAKE et al., 1981). This paper describes further studies of chromosomal and clinical features including reproductive efficiency of the bull. In the present study, the reproductive organs were macro- and microscopically examined at the time of slaughter.

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MATERIALS AND METHODS

This case was a Holstein-Friesian bull born in 1977 and characterized by an unilateral cryptorchidism in the left side. The other genital organs were well developed as in normal bull, but the sexual desire of this bull was inferior to that of normal bulls. The blood, a piece of the skin, spleen and kidney were aseptically collected for chromosomal investigations of this bull. In addition, blood samples were collected from 9 of its male half-sibs and 11 daughter cows. Chromosomal analysis was performed by the blood leucocyte culture methods and the long term tissue culture methods (HARE & SINGH, 1979), and was conducted counting 50 metaphase plates or more in all cases. To examine the changes of mosaic ratio *in vitro*, fibroblast cells derived from the skin, spleen and kidney were subcultured for periods of from 25 days to 171 days, and the mosaic ratio was estimated at each subculture.

This bull was slaughtered at the age of 5 years and 8 months old because its semen quality gradually decreased. Testes and other reproductive organs were macroscopically examined at the time of slaughter. Furthermore, some pieces of each testis were fixed with Bouin solution, embedded in paraffin and sectioned at 7 μ. After being stained with hematoxylin and eosin, the sections were histologically examined.

Semen quality and the conception rate of this bull were evaluated from records kept for about 3 years at an artificial insemination center in Japan.

RESULTS

The results of chromosomal analysis in this bull are shown in table 1. Blood samples were cytogenetically analyzed 4 times at proper distances, and the metaphase plates had two cell populations : an XY cell line (71.1%), and an XYY cell line (28.9%).

TABLE 1 *Chromosomal constitution of XY/XYY mosaic bull in blood samples*

NO.	TIME OBTAINED MATERIALS	XY	XYY	RATIO[1)]
1	September, 1978	56	51	47.66
2	January, 1979	32	27	45.76
3	November, 1982	81	16	16.49**
4	February, 1983	89	11	11.00**
	total (%)	258 (71.1)	105 (28.9)	28.93

1) Ratio was calculated with $\frac{\text{XYY cells}}{\text{total cells}} \times 100$.

** Ratio was significantly different ($p<0.01$).

The metaphase plates with XY and XYY are shown in figures 1 and 2. No XO cell line was detected in the animal. The ratios with XYY cell line (XYY cells / total cells $\times 100$) at 4 time examinations from 2 to 5 years old were 47.66, 45.76, 16.49 and 11.00, respectively. The ratios at the time of the third and fourth examinations were significantly lower ($p<0.01$) than those shown at the time of the first and second examinations (tab. 1). A piece of the skin, spleen and kidney were cultured for chromosomal analysis, and the metaphases of these also had two cell lines similar to those of blood samples (tab. 2). In the skin, the ratio with XYY cell line of second examination was significantly different from that of the first examination ($p<0.01$). From the blood type examination, no chimerism was detected.

The results obtained from the subculture of the skin, spleen and kidney *in vitro* are shown in table 3. In the skin and kidney, the XYY cell line disappeared from the culture at the time of the 3rd subculture (for 25 days) and the 16th subculture (for 79 days), respectively. In the spleen, however, neither the XY cell line or the XYY cell line disappeared from the cultures until the time of 29th subculture (for 171 days), and the mosaic ratios were kept unchanged.

The father bull and 9 half-sib males were chromosomally investigated (tab. 4). It was clear that the father bull and the 8 half-sibs had a normal 60,XY cell line. On the other hand, a male half-sib of heterosexual twins showed a sex chromosomal chimerism (XX/XY). The results of chromosomal analysis of the 11 daughter cows did not show the cytogenetical aberrations or clinical abnormalities (tab. 5).

Semen of the XY/XYY mosaic bull was collected and examined once a week and 2 or 3 times a day (tab. 6). Records of semen examination of once a month were kept and summarized. Semen volume was 1.8 ± 9.5 ml (3.73 ± 1.25 ml, mean$\pm$SD), and spermatozoan concentration was $1.5 \pm 11.2 \times 10^8$/ml ($4.85 \pm 1.98 \times 10^8$/ml). Viability indexes of sperm before and after freezing (NISHIKAWA, 1958) were 65.00~85.00 (79.23 $\pm$3.76) and 10.00~50.00 (40.47$\pm$8.27), respectively.

TABLE 2 *Chromosomal constitution of XY/XYY mosaic bull in tissues*

NO.	TIME OBTAINED MATERIALS	SKIN			SPLEEN			KIDNEY		
		XY	XYY	ratio	XY	XYY	ratio	XY	XYY	ratio
1	January, 1979	32	29	47.54		—			—	
2	February, 1983	74	3	3.90**	33	17	34.00	35	4	10.26

** Ratio was significantly different ($p<0.01$) compared with that of previous examination.

— Not observed.

TABLE 3 *Changes of XY/XYY mosaic ratio in vitro*

SUBCULTURE	SKIN		KIDNEY		SPLEEN	
1st	74 : 3 (3.9)	↓	35 : 4 (10.3)	↓	33 : 17 (34.0)	↓
3rd	110 : 0 (0)	25 days	44 : 6 (12.0)		36 : 14 (28.0)	
5th			44 : 6 (12.0)		18 : 7 (28.0)	
10th			24 : 3 (11.1)		30 : 13 (30.2)	
13th			38 : 3 (7.3)			
15th			33 : 0 (0)	↓	18 : 7 (28.0)	
16th			*56 : 0 (0)	79 days		
20th					35 : 23 (39.7)	
23th					35 : 24 (40.7)	
25th					28 : 14 (33.3)	
27th					32 : 21 (33.3)	
29th					34 : 23 (40.4)	171 days

The numbers in parentheses show the percentage of the XYY cells.

* Analysis was conducted to confirm the disappearance of the XYY cells, because the previous analysis consisted of a lower number of cells.

TABLE 4 *Chromosomal constitution of father bull and the half-sibs*

CASES	KARYOTYPE		
	60,XX	60,XY	61,XYY
father bull	—	*	—
this case	—	258	105
half-sibs			
No. 1	0	50	0
2	0	50	0
3	0	50	0
4**	5	45	0
5	0	50	0
6	0	50	0
7	0	50	0
8	0	50	0
9	0	50	0

* Personal communication by P. K. Basrur (1981)
** Heterosexual twin male

TABLE 5 *Chromosomal constitution of daughter cows sired by XY/XYY mosaic bull*

CASE NO.	KARYOTYPE
	60,XX
1	50
2	50
3	50
4	50
5	50
6	50
7	50
8	50
9	50
10	50
11	50

TABLE 6 *Semen examination in XY/XYY mosaic bull*

TIME OF EXAMINATION	VOLUME	SPERMATOZOAN CONCENTRATION	VIABILITY INDEX OF SPERM*	
			before freezing	after freezing
	ml	10^8/ml		
November–December, 1978	2.38±0.41**	6.31±1.96	80.00±0.00	45.00± 0.00
January–June, 1979	3.36±0.85	3.89±1.19	78.53±4.24	41.67± 2.58
July–December, 1979	3.23±0.64	4.52±1.76	79.38±3.10	37.50±10.84
January–June, 1980	3.62±0.98	5.24±2.22	79.29±4.32	35.00±13.78
July–December, 1980	3.79±1.22	5.64±2.16	78.44±4.73	44.17± 4.92
January–June, 1981	5.09±1.42	4.62±2.08	80.31±2.87	42.50± 2.74
total	3.73±1.25	4.85±1.98	79.23±3.76	40.47± 8.27

Semen was collected with a frequency of once a week and of twice or 3 times a day.
* by Y. Nishikawa (1958)
** mean ± SD

The results of the conception rate are shown in table 7. A total of 516 cows was inseminated for 3 years, and 363 cows (70.3%) were fertilized by the XY/XYY mosaic bull. For the same years, the average conception rate for about 350 bulls in Japan was 64.0~65.4%. The conception rates for this case and of 19 bulls in the same area in 1981 were 77.6% and 69.4%, respectively (tab. 8).

The testes obtained at the time of slaughter were macroscopically examined (fig. 3). In the descended right testis, calcinosis was observed widely. The left testis was small, and did not produce sperm. In figure 4, the microscopic figure of the descended right testis is shown. Many seminiferous tubes were filled with calcareous materials, and germ cells were absent from the seminiferous tubes. However, a few tubes of the right testis had normal spermatogenesis and produced scant amounts of sperm (fig. 5). In the seminiferous tubes of the left testis, only sustentacular cells and some spermatogonia were observed (fig. 6). Other male reproductive organs showed normal developments.

TABLE 7 *Conception rate of XY/XYY mosaic bull*

YEAR OF EXAMINATION	NUMBER OF COWS INSEMINATED	NUMBER OF PREGNANT COWS	CONCEPTION RATE	AVERAGE CONCEPTION RATE IN JAPAN*
			%	%
1979	142	98	71.5	64.0
1980	267	182	69.2	65.0
1981	107	83	77.6	65.4
total	516	363	70.3	65.3

* The results were calculated from a conception rate of about 350 bulls.

TABLE 8 *Conception rate in the same area (1981)*

CASE	NUMBER OF COWS INSEMINATED	NUMBER OF PREGNANT COWS	CONCEPTION RATE
		%	
this case	107	83	77.6
others*	1073	738	69.4

* The results were calculated from the conception rate of 19 bulls.

DISCUSSION

Until now, cases with an XYY sex chromosomal complement have been reported in human beings (SANDBERG et al., 1961; MAKINO, 1979) and mice (CATTANACH & POLLARD, 1969; EVANS et al., 1978). In the field of domestic animals, an XY/XYY cat (LOUGHMAN & FRYE, 1974), a case of 63,XO/65,XYY in equine (HÖHN et al., 1980), three cases of 60,XY/61,XYY mosaic bulls in Bulgaria (DOBRYANOV & KONSTANTINOV, 1970) and in Japan (HANADA & MURAMATSU, 1981; MIYAKE et al., 1981) have been reported.

It is unknown how this XY/XYY mosaic is formed; however, it is conjectured that this case is formed by chromosomal non-disjunction in a normal XY zygote during subsequent cell division in the early embryonic stage. HANADA & MURAMATSU (1981) reported a case of an XY/XYY bull in which two cell populations within an individual were formed by means of chimeric occurrence together with the possibility of mosaicism. However, it is clear that the occurrence of the XY/XYY in this case was due to mosaicism, because the results of blood type examination did not show the chimerism.

It has been reported in human chromosomal mosaicism that the proportion of complement cell types changes with aging in both *in vivo* (TAYLOR, 1968; NEU et al., 1969; TAYSI et al., 1970) and *in vitro* (TAYSI, et al., 1970; KULIEV, 1971; IKEUCHI & SASAKI, 1975). In this case, the ratio of the XYY cell line also tended to decrease with aging. It has been suggested that there exists a selective advantage of one cell type over the other in the mosaic cell population. Regarding this, we attempted long term tissue cultures of the skin, spleen and kidney *in vitro* from periods of from 25 days to 171 days and examined whether or not the mosaic ratios changed with the passage of subculture. Based on the result of the examinations, the XYY cell line was selected from the skin and kidney in the subculture; however, in the spleen, both cell lines have remained unchanged until now. It appears that some selective conditions, for example, the difference of the rate of cell growth between the two cell lines, has an effect upon the disappearance of the XYY cell line. But, it is unknown why the XYY cell line is maintained in the spleen cells for a long time *in vitro*. We are trying to maintain the cultivation of the spleen cells, although the rate of cell growth has become gradually lower.

No chromosomal aberrations were detected in the father bull, the 8 half-sib males, excluding the male with XX/XY born to a heterosexual twin, and the 11 daughter cows sired by the XY/XYY bull. As yet, we have not received any communication that abnormal characteristics have been detected in the appearance of the calves sired by the XY/XYY bull. In the case of XYY males in humans, only one case of XYY appearing also in the father and the son has been reported (SUNDQUIST & HELLSTROM, 1969). It is not yet clear in human and domestic animals that the YY sperm is significantly produced in XYY males.

The present case showed unilateral cryptorchidism in appearance, and the semen volume and number of sperm were extremely lower than those in normal bulls. The lack of spermatogenesis in the left testis probably had an injurious effect on the lower volume and concentration of sperm. Actually, when the left testis, obtained at the time of slaughter, was microscopically examined, no spermatogenetic figures were observed in the left testis. Despite the lower semen quality, this case showed a conception rate similar to that of normal bulls in Japan. This was due probably to the fact that the right testis of the bull produced a large number of sperm. This case had severe calcinosis in the descended right testis, which possibly had a direct effect upon the lower production of sperm, for which reason the bull was slaughtered. However, there did not seem to be any correlation between XY/XYY mosaicism and this pathological changes in the testis.

Few cases of XY/XYY mosaicism in domestic animals have been investigated in detail due to the fixed relations between chromosomal aberrations and the clinical features, with the exception of bovine freemartins with sex chromosomal chimerism (MARCUM, 1974). The scarcity of reports on this subject is due probably to the small number of cytogenetical investigators, the few cases of XY/XYY mosaicism per a certain particular chromosomal abnormality, or the difficulty of obtaining materials containing chromosomal aberrations.

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EXPLANATION OF PLATE

Fig. 1 Metaphase plate with XY obtained from peripheral leucocyte cultures of an XY/XYY mosaic bull
X : X chromosome, Y : Y chromosome

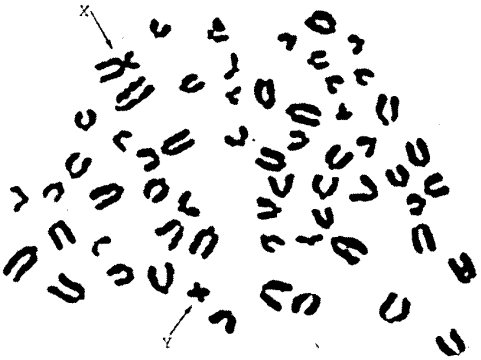
Fig. 2 Metaphase plate with XYY obtained in the same manner described above

Fig. 3 A photograph of the cut testes obtained at the time of slaughter It is clear that calcinosis was spread widely throughout the right testis The left testis was very small compared with that of normal bulls

Fig. 4 Cross section of the right testis Seminiferous tubes without any germ cells were filled with calcareous materials. (H & E) ×170

Fig. 5 Cross section of the right testis A few seminiferous tubes showed normal spermatogenesis. (H & E) ×170

Fig. 6 Cross section of the left testis In the seminiferous tubes, only Sertoli cells and some spermatogonia (Sgo, arrow) were observed. (H & E) ×330



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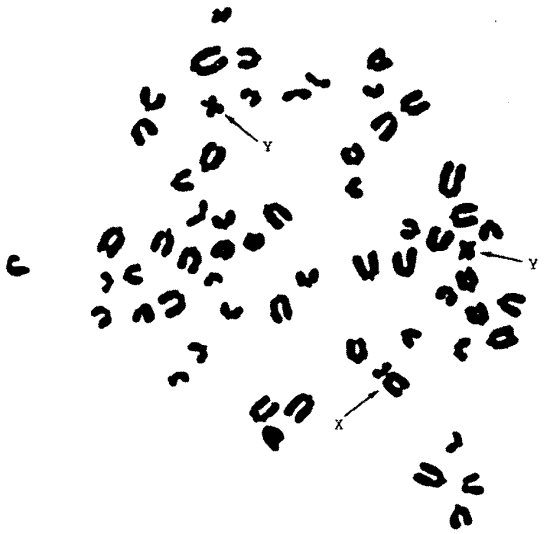

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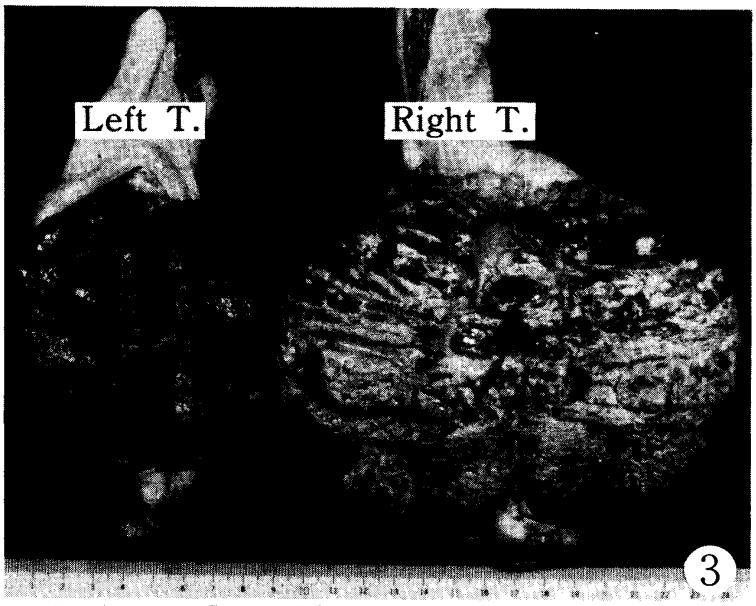

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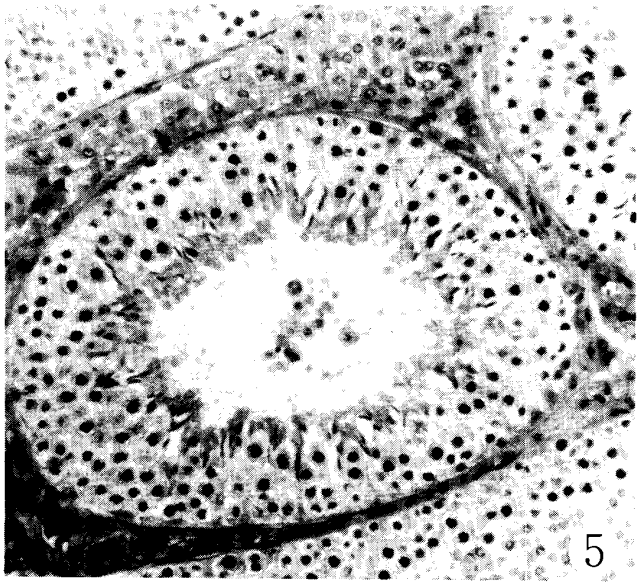

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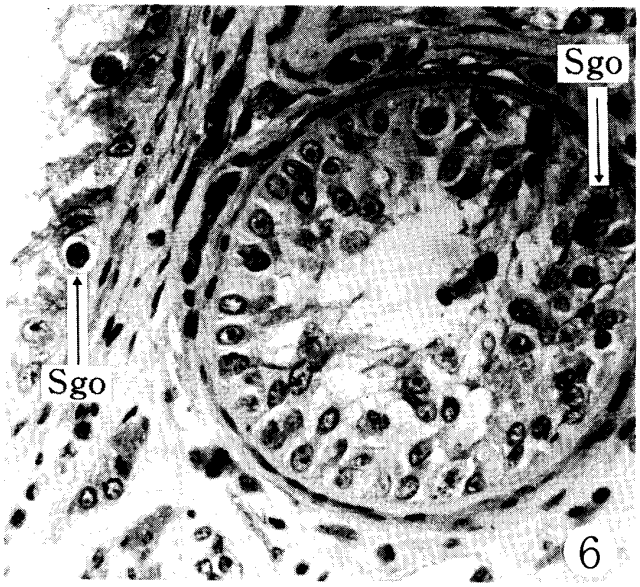

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