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**CHROMOSOME STUDIES  
ON HETEROSEXUAL TWINS IN CATTLE**

**IV LONG-TERM OBSERVATIONS OF SEX-CHROMOSOME  
CHIMERA RATIO IN CULTURED LEUKOCYTES\***

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Since OHNO et al. first reported sex-chromosome chimerism in heterosexual bovine twins in 1962, FECHHEIMER et al., MURAMOTO et al., KANAGAWA et al.<sup>5-9)</sup>, GOODFELLOW et al. and MAKINO et al. have studied the same subject. Recently, OHNO & GROPP, and KANAGAWA et al.<sup>7)</sup> reported the same findings in a set of heterosexual triplets and BASRUR also reported similar findings in a set of heterosexual quintuplets in cattle. Sex-chromosome chimerism appeared in cultured leukocytes and bone marrow cells of heterosexual twins or more multiples involving freemartin cattle, and thereby gives us an important tool in the early diagnosis of freemartinism<sup>5,6)</sup>. However, it has never been proved chromosomally whether the chimerism is an essentially invariable nature for life. With these aspects in mind, a considerable long-term (1~15 months of age) observation was made on sex-chromosome chimera ratio in cultured leukocytes from heterosexual twins.

TABLE 1 *Materials used and observation periods*

NO.	ANIMAL NUMBER	SEX	OBSERVATION PERIODS
1 } 2 }	F 8	Male Freemartin	1~ 5 <sup>mo</sup> 1~ 3
3 } 4 }	F 26	Male Freemartin	2~ 6 2~15
5	F 3	Freemartin	1~15
6	F 34	Freemartin	1~15
7	F 2	Male	1~14

\* A part of this work was communicated at the 61st Meeting of the Japanese Society of Veterinary Science on the 1st April, 1966 in Tokyo.

## MATERIALS AND METHODS

Two pairs of Holstein cattle heterosexual twins, as well as 2 freemartins and 1 male co-twin with a freemartin were used in this study. Some of them were used in previous studies<sup>5,6,8,9</sup>). The observation periods of each animal are given in table 1.

Chromosomal study was carried out by the routine leukocyte culture method described previously<sup>5</sup>).

After the observation periods, the freemartins were sacrificed and examined macroscopically to ascertain their sexual organs.

## RESULTS AND DISCUSSION

In tables 2 and 3 are the findings of sex-chromosome chimera ratio from cultured leukocytes of these heterosexual twins during the observation periods.

TABLE 2 *Results of long-term observations of sex-chromosome chimera ratio in cultured leukocytes of 2 pairs of heterosexual twins*

OBSERVATION PERIODS	NO. 1-MALE (F 8)			NO. 2-FREEMARTIN (F 8)		
	No. of cells counted	2A-XX	2A-XY	No. of cells counted	2A-XX	2A-XY
1 <sup>mo</sup>	58	30 (51.7) %	28 (48.3) %	54	29 (53.7) %	25 (46.3) %
2	62	33 (53.2)	29 (46.8)	53	28 (52.8)	25 (47.2)
3	55	28 (50.9)	27 (49.1)	68	39 (57.4)	29 (42.6)
4	68	35 (51.5)	33 (48.5)	—	—	—
5	53	27 (50.9)	26 (49.1)	—	—	—
	NO. 3-MALE (F 26)			NO. 4-FREEMARTIN (F 26)		
1	—	—	—	—	—	—
2	50	12 (24.0)	38 (76.0)	61	17 (27.9)	44 (72.1)
3	48	11 (22.9)	37 (77.1)	—	—	—
4	53	14 (26.4)	39 (73.6)	42	13 (31.0)	29 (69.0)
5	—	—	—	—	—	—
6	25	6 (24.0)	19 (76.0)	30	8 (26.7)	22 (73.3)
7	—	—	—	—	—	—
8	—	—	—	48	12 (25.0)	36 (75.0)
9	—	—	—	—	—	—
10	—	—	—	25	7 (28.0)	18 (72.0)
11	—	—	—	—	—	—
12	—	—	—	—	—	—
13	—	—	—	42	12 (28.6)	30 (71.4)
14	—	—	—	—	—	—
15	—	—	—	55	14 (25.5)	41 (74.5)

Note —: Not examined

TABLE 3 *Results of long-term observations of sex-chromosome chimera ratio in cultured leukocytes*

OBSERVATION PERIODS	NO. 5-FREEMARTIN (F 3)			NO. 6-FREEMARTIN (F 34)			NO. 7-MALE (F 2)		
	No. of cells counted	2 A-XX	2 A-XY	No. of cells counted	2 A-XX	2 A-XY	No. of cells counted	2 A-XX	2 A-XY
		%	%		%	%		%	%
1 <sup>mo</sup>	219	6 (2.7)	213 (97.3)	51	19 (37.3)	32 (62.7)	60	24 (40.0)	36 (60.0)
2	153	5 (3.3)	148 (96.7)	62	24 (38.7)	38 (61.3)	—		
3	228	8 (3.5)	220 (96.5)	—			—		
4	171	5 (2.9)	166 (97.1)	17	6 (35.3)	11 (64.7)	—		
5	177	5 (2.8)	172 (97.2)	52	18 (34.6)	34 (65.4)	—		
6	160	5 (3.1)	155 (96.9)	55	20 (36.4)	35 (63.6)	—		
7	212	8 (3.8)	204 (96.2)	58	22 (37.9)	36 (62.1)	—		
8	235	7 (3.0)	228 (97.0)	53	18 (34.0)	35 (66.0)	—		
9	197	6 (3.0)	191 (97.0)	54	19 (35.2)	35 (64.8)	—		
10	226	6 (2.7)	220 (97.3)	—			—		
11	—			—			—		
12	—			25	9 (36.0)	16 (64.0)	—		
13	155	5 (3.2)	150 (96.8)	—			—		
14	—			50	17 (34.0)	33 (66.0)	52	22 (42.3)	30 (57.7)
15	158	5 (3.2)	153 (96.8)	48	17 (35.4)	31 (64.6)	—		

Note —: Not examined

Both the freemartins and the male co-twins showed 2A-XX/2A-XY chimerism without exception. The chimera ratio varied with each individual, but no significant difference was statistically observed between each pair of co-twins (Nos. 1 vs. 2 & Nos. 3 vs. 4,  $P < 0.05$ ).

Furthermore, there was no significant difference in the chimera ratio of each individual throughout the observation periods ( $P < 0.05$ ).

KANAGAWA et al.<sup>5-7)</sup> and HERSCHLER et al. reported that early diagnosis of bovine freemartinism by leukocyte culture technique based on sex-chromosome chimerism is significant. The results in this study could indicate that the sex-chromosome chimerism may be invariable in nature at least during one year or so after birth. Therefore, it can be said that this gives further fundamental basis on diagnosis of bovine freemartinism by chromosomal analysis.

One of the interesting findings in the present study is the fact that the chimera ratios tend to parallel each other among co-twin individuals. In order to ascertain the diagnosis, it is recommended that all twins should be individually subjected to chromosomal examination.

It should be a requirement in the future, to study whether such invariability of sex-chromosome chimerism may exist also in other tissues or cells besides blood leukocytes, although continual collection of such specimens for a long time perhaps might not be an easy task. Bone marrow biopsy might be available for this purpose<sup>8)</sup>.

#### SUMMARY

Sex-chromosome chimera ratio in cultured leukocytes was examined regularly during 1~15 months after birth on 7 heterosexual twins involving the freemartin.

All freemartins and male co-twins showed 2A-XX/2A-XY chimerism. The chimera ratio varied in each animal, but between co-twin pairs, the difference of the ratio was insignificant. In addition, no significant difference was detected in the chimera ratio in each individual throughout the observation periods (1~15 months). This suggests that the sex-chromosome chimerism may be of an invariable nature for life.

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## EXPLANATION OF PLATE

Fig. 1 Chromosome preparation from leukocyte cells cultured for 72 hours  
Arrows show metaphase.  
Air-drying method and stained with Giemsa  
×500

Figs. 2 & 3 Metaphase figures with sex-chromosome chimerism from a blood  
culture of a freemartin (No. 2)  
Note XX (fig. 2) and XY (fig. 3).  
×2,400

