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**CHROMOSOME STUDIES
ON HETEROSEXUAL TWINS IN CATTLE
III SEX-CHROMOSOME CHIMERISM (XX/XY) IN
BONE MARROW SPECIMENS***

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In the previous papers^{1~4)}, the authors described chromosomal analyses of cultured leukocytes from both freemartins and their co-delivered males, clearly manifested a sex-chromosome chimerism (XX/XY), and that this is useful for a successful early diagnosis of the freemartin.

This article deals with sex-chromosome findings of bone marrow cells, one of the ancestors of leukocytes, in heterosexual twins in cattle.

MATERIALS AND METHODS

Four calves of Holstein breed, consisting of 3 freemartins and one male calf co-twin with a freemartin were subjected to the study. They, except one (No. 3), were those used in the previous studies^{1,2,4)}. Ages of the animals and source of materials, together with their chromosomal data, are given in the table.

Bone marrow specimens collected from three different bones, the sternal body, left and right iliums, received a colchicine treatment as mentioned previously^{1,4)}, then a water pretreatment was added. Chromosomal slides were made by the routine air-drying method and stained with Giemsa.

RESULTS AND DISCUSSION

All the freemartins and the male co-twin showed 2A-XX/2A-XY chimerism in bone marrow cells as well as in cultured leukocytes without exception. The chimera ratio varied with individuals, but it tended to parallel each other among different material sources in an individual and also between a set of co-twins (No. 1). The details of chromosomal findings are presented in the table.

As described in the previous papers^{1~4)}, sex-chromosome chimerism were

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TABLE *Materials and results*

NO.	ANIMAL NUMBER	AGE	SOURCE	MALE			FREEMARTIN			
				No. of cells counted	2A-XX	2A-XY	No. of cells counted	2A-XX	2A-XY	
		days			%	%		%	%	
1	F 8*	16	Bone marrow	Sternal body	29	17 (58.6)	12 (41.4)	22	12 (54.5)	10 (45.5)
				Left ilium	55	34 (61.8)	21 (38.2)	57	32 (56.1)	25 (43.9)
				Right ilium	26	14 (53.8)	12 (46.2)	21	11 (52.4)	10 (47.6)
			Blood	65	33 (50.8)	32 (49.2)	74	45 (60.8)	29 (39.2)	
2	F 3	25	Bone marrow	Sternal body	—	—	—	36	1 (2.8)	35 (97.2)
				Left ilium	—	—	—	50	2 (4.0)	48 (96.0)
				Right ilium	—	—	—	30	1 (3.3)	29 (96.7)
			Blood	—	—	—	239	7 (2.9)	232 (97.1)	
3	F 26	100	Bone marrow	Sternal body	—	—	—	31	9 (29.0)	22 (71.0)
				Left ilium	—	—	—	24	6 (25.0)	18 (75.0)
				Right ilium	—	—	—	26	7 (26.9)	19 (73.1)
			Blood	—	—	—	61	17 (27.9)	44 (72.1)	

Notes: * Both of the twins were examined.

— Not examined

always observed in cultured leukocytes from bovine heterosexual twins or triplets involving freemartins, and interestingly the chimera ratios were parallel other among co-twins or co-triplets. Thus, the results of this study showed the same tendency as those in cultured leukocytes. Furthermore, chimera ratios in bone marrow cells are similar to those in cultured leukocytes in the same individuals. This may lead us to assume that the leukocytic chimerism might be destined by the chimerism in bone marrow cells which are thought to be ancestral for leukocytes in the blood. In addition, if the lymphatic system is also proven being chimeric, the assumption will be more realistic.

In view of OHNO & GROPP's statement that in liver cell cultures from bovine heterosexual fetal triplets, only 12 mm in crown-rump length, cell chimerism was already established. Such a high similarity of sex-chromosome chimera ratios in marrow cells collected from three different bones within an individual would suggest that the transportation of primordial germ cells from a male fetus to a female or inversely through anastomosed chorionic blood vessels may take place in very early embryonic stages.

SUMMARY

The sex-chromosome constitution and the incidental rate of the 2A-XX/2A-XY chimerism were studied with colchicine treated bone marrow specimens, collected from three different bones in 3 freemartins and one male calf co-twinning with a freemartin.

Bone marrow cells in all cases manifested 2A-XX/2A-XY chimerism with varying degrees. But the chimera ratios tended to parallel each specimen in an individual and also with a set of co-twins. In addition, there is a similar tendency in the chimera ratios between bone marrow cells and cultured leukocytes.

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EXPLANATION OF PLATE

- Fig. 1 Slide from a bone marrow specimen of a freemartin (No. 1)
Arrows show metaphase cells.
Air-drying preparation, stained with Giemsa
×116
- Fig. 2 High magnification of metaphase figures from the same specimen
2A-XX (left) and 2A-XY (right) conformations
×790

