92. Notes on XX/XY Mosaicism in Cells of Various Tissues of Heterosexual Twins of Cattle^{*)}

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Following the work of Keller and Tandler (1916), and Lillie (1916), it has been reported that in cattles (*Bos taurus*) the twin brother of dizygotic twins enclosed within a common chorionic membrane consistently affects the development as well as the differentiation of the gonads of its female co-twin. The female co-twin generally develops into an imperfect calf, phenotypically intersex and sterile, usually referred to as freemartin. Thanks to rapid advances in cytogenetic techniques which facilitated the precise analysis of mammalian chromosomes in general, the chromosome condition of the bovine freemartin has recently attracted the attention of many cytologists. Makino *et al.* (1962) have failed to detect, in cultured leucocytes and lung cells, any apparent change or significant variation from the normal female complement in the chromosomes of the freemartin, reporting the diploid number of 60 in $2n$, characterized by two large submetacentric X's. On the other hand, Ohno *et al.* (1962) reported the cytological detection of XX-cells and XY-cells in both the testicular tissue and bone marrow specimens of two newborn bull calves, and in the bone marrow of three newborn freemartins. In the present paper, are presented chromosomal data from several tissues of bovine freemartins recently collected by the authors.

Material and Methods: Out of 13 cases of heterosexual twins chromosomally studied, 4 cases designated as case A, B, C, and D which is non-freemartin, were dealt with in this paper. Some essential accounts of these subjects are given in Table I. In cases A and B, cultured cells from blood and various organs provided the materials for study. In case C, blood and marrow cultures were used for study, while blood cultures alone supplied material for case D. Chromosomal slides were made according to the routine air-drying technique. Details of technical methods will be given in

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another paper.

Normal chromosomes: The diploid chromosome number in domestic cattle (*Bos taurus*) is 60. The X chromosome is one of the larger chromosomes, characterized by a submedian centromere, while the Y approximates in size the smallest autosomes, being submetacentric in nature and positively heteropycnotic at metaphase. All the autosomes are of acrocentric nature (cf. Sasaki and Makino 1962).

Table I. Materials under study and chromosomal data obtained

	Case	Material	Age	No. of cells observed	Tissue origin
				XX-cell : XY-cell	
A {	♀(1) (freemartin)	Blood	5 m.*****	98 : 13	mesoderm
		Kidney		101 : 6	"
		Gonad*		192 : 3	"
	♂(2)	Blood	133 : 13	"	
B {	♀(3) (freemartin)	Blood	5 m.**	22 : 2	mesoderm
		Gonad*		136 : 2	"
		Lung		22 : 2	endoderm
		Skin		116 : 0	ectoderm
	♂(4)	Kidney	40 : 0	mesoderm	
		Blood	2 : 2	"	
		Gonad*	0 : 139	"	
		Liver	0 : 64	"	
		Kidney	2 : 40	"	
		Skin	0 : 108	ectoderm	
		Lung	0 : 71	endoderm	
C {	♀(5)	Blood	1 m.*****	7 : 232	mesoderm
	(freemartin)	Bone marrow		2 : 48	"
	♂***	—		—	—
D	♀(6)****	Blood	7.2 yr.	75 : 0	mesoderm

* Interstitial or connective tissue cells.

** 5-month-old foetus.

*** Twin brother of case C was not cytologically observed.

**** A co-twin from the non-freemartin heterosexual twins.

***** 5 and 1 months after birth, respectively.

Chromosomes of heterosexual twins: In a total of 13 studied cases involving freemartins or their twin brothers, 11 cases were cytologically confirmed to show XX/XY cell mosaicism, leaving 2 ambiguous cases in which the chromosomal survey was insufficient. In Table I are presented chromosomal findings from the four cases (A-D), specially on the ratio of XX-cells to YX-cells obtained from various organs of bovine twins under study.

Case A-1 (freemartin) and A-2 (♂): Cytological observations of

case A-1 (freemartin) revealed that the ratio of the number of 2A-XX cells to 2A-XY cells was 98:13 for leucocytes, 101:6 for kidney-tissue cells, 192:3 for gonadal tissue cells. Blood cultures from A-2 (♂) supplied the ratio, 2A-XX cells:2A-XY cells, as 133:13 (Figs. 1-4).

Figs. 1-2. Karyotypes of a freemartin (case A-1), from blood culture. 1, from a 2A-XX cell. 2, from a 2A-XY cell.

Figs. 3-4. Metaphase chromosomes (case A-1), Fig. 3, corresponding to Fig. 1 and Fig. 4 to Fig. 2.

Case B-3 (freemartin) and B-4 (♂): Cultures established from blood, gonad, lung, skin, and kidney in case B-3 (freemartin) presented the ratio, 2A-XX cells:2A-XY cells, as 22:2, 136:2, 22:2, 116:0, and 40:0, respectively. In case B-4 (♂), cultures derived from blood, gonad, liver, kidney, skin, and lung provided the ratio, 2A-XX cells:2A-XY cells, as 2:2, 0:139, 0:64, 2:40, 0:108, and 0:71, respectively.

Case C (C-5, freemartin): It was shown that the ratio, 2A-XX cells:2A-XY cells, was 7:232 for blood cultures and 2:48 for mar-

row cultures.

Case D (D-6, normal ♀): Based on blood cultures, the results were obtained that the 2A-XX:2A-XY cell ratio was 75:0.

Remarks: There are several reports about the XX/XY chimerism of bone marrow, blood and gonad of heterozygotic twins: Benirschke *et al.* (1962) in marmoset monkey; Benirschke and Brownhill (1962, 1963) in marmoset monkey; Ohno *et al.* (1962) in cattle; Uchida *et al.* (1964) in human beings. In this study, the authors observed in bovine dizygotic twins the chromosome constitutions of various organs. The results are shown in Table I. Generally, 2A-XX/2A-XY cell mosaicism is detected in both freemartins and their male co-twins, with various ratios of cell mosaicism in tissues. Organs of the endodermal and the mesodermal origin consisted of 2A-XX cells and 2A-XY cells in both freemartins and co-twins, but in those of the ectodermal origin no XX/XY mosaicism (or chimera) was detected (Table I). A co-twin (case D, ♀) from the nonfreemartin heterosexual twins showed no such cell mosaicism. Observed facts may be consistent for the assumption that cell mosaicism occurring in the freemartin and its co-twin suggests an interchange of cells between them as a result of vascular anastomosis. 2A-XX/2A-XY mosaicism detected in organs of endodermal and mesodermal origin alone seems to indicate that the vascular anastomosis occurs generally in certain embryonic stages after the completion of the blastoderm differentiation. Probably, the precocious maturation of gonads in a male co-twin may occur prior to vascular anastomosis. According to Lillie (1916, 1917), the bovine freemartin was produced by the effect of hormone on sex-differentiation and sexual development. The existence of XX/XY mosaicism in the freemartin does not always exclude the hormone theory of Lillie (1916, 1917) for the rise of bovine freemartins. However, Fechheimer *et al.* (1963), on the basis of recent information that freemartins and male co-twins are mosaics containing both male and female cells, have postulated that the freemartin condition may be caused directly by the sex-chromosome mosaicism. Particularly noticeable XX/XY ratios occurring in case A (♂) and in case C (♀) remains cytologically unexplicable at present, because of insufficient materials for conclusive statements. Considering the fact that the Y chromosome of mammals generally bears an effective masculinizing factor (or factors), it is likely that the sex-chromosome mosaicism may participate to a great extent in the rise of the freemartin condition.

Summary: The chromosome constitutions of three cases of bovine freemartins were observed in blood cultures as well as in tissue cultures from various organs. In both freemartins and their

co-twins (case A-C), tissue cells of mesodermal and endodermal origin generally showed 2A-XX/2A-XY mosaicism. A normal cow from the non-freemartin dizygotic twins possessed a normal female complement of chromosomes (case D).

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