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Journal Article**Author(s):**

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Publication date:

1981

Permanent link:

<https://doi.org/https://doi.org/10.3929/ethz-b-000422814>

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Originally published in:

Journal of Heredity 72(5), <https://doi.org/10.1093/oxfordjournals.jhered.a109527>

Some rare cases of chimerism in twin cattle and their proposed use in determining germinal cell migration

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ABSTRACT: Three dizygotic, heterosexual twins with chimerisms carrying marker chromosomes are described. Phenotypic and cytogenetic methods were used to identify these animals. The occurrence of germinal cell migration causing gonad chimerism can be detected by the marker chromosome event under conditions described in this report.

THE PHENOMENON of chimerism has been studied and discussed frequently in recent years¹. In embryo transfers for twin production in cattle chimerism is a potential problem because females with male twins are likely to become sterile¹⁰. Such females are unsuitable for breeding and have subnormal weight-gain efficiency compared to males. Freemartins among twins are generally identified by rectal palpation of adult animals⁹, as well as by the

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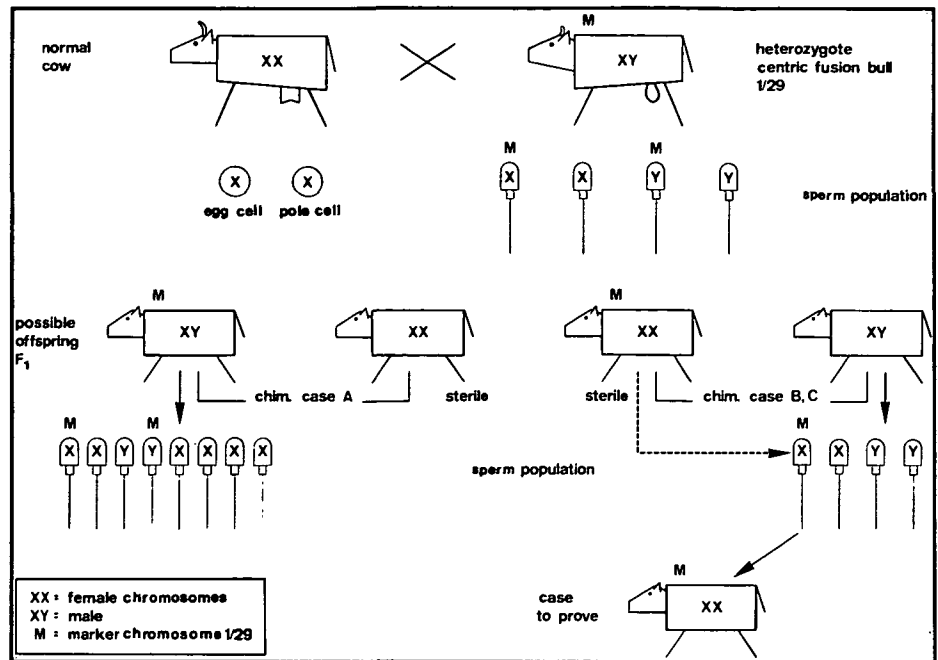


FIGURE 1 Fertilization of a normal egg cell with sperm carrying the centromere fusion chromosome. Also shown are the potential combinations of various germ cells (see text).

size and form of the clitoris. A blood group analysis is successful only if a sufficient number of factors are tested so as to distinguish the two erythrocyte populations. Such studies become more reliable if additional biochemical markers¹³ or an H-Y antigen test¹² are included. A further diagnostic possibility is a cytogenetic examination as described by Herschler and Fehheimer^{4,5}. Given sufficient cell material one can determine the origin of cells on the basis of sex chromosomes. However, the cell proportion

is of little value when considering the degree of virilization.

A high proportion of affected females are freemartins. The sex differentiation of the male partner seems to be quite normal, but according to various reports¹ fertility troubles do occur causing increased rates of culling among affected males. Thus, the presence of XX primordial germ cells in testes^{9,16} could lead to disturbances in spermatogenesis and thus alter reproductive fitness as well as sex ratio among offspring. However, the time and

Table I. Metaphases counted from short-term lymphocyte cultures and one long-term fibroblast culture (muscle cells) of chimeric twin pairs A, B, and C and repeat investigations in bulls B and C

Material	Date of examination	Pair A*				Pair B				Pair C			
		male		female		male		female		male		female	
		cell	type	cell	type	type	type	cell	type	cell	type	cell	type
		XY _M	XX	XY _M	XX	XX	XX _M	XY	XX _M	XY	XX _M	XY	XX _M
Blood	24-9-79	27(20)	110(80)	28(24)	90(76)								
	9-10-79	23(22)	82(78)	24(24)	77(76)								
	22-4-80					89(89)	11(11)	93(93)	7(7)	32(44)	41(56)	23(35)	42(65)
	17-7-80					85(85)	15(15)			32(39)	61(61)		
	14-8-80					84(84)	16(16)			34(34)	66(66)		
	25-9-80					97(97)	3(3)			38(38)	62(62)		
	9-10-80					95(95)	5(5)			51(51)	49(49)		
	20-11-80					98(98)	2(2)			28(28)	72(72)		
	29-1-81					93(93)	7(7)			50(50)	50(50)		
	12-2-81					97(97)	3(3)			57(57)	43(43)		
26-2-81					92(92)	8(8)			54(54)	46(46)			
Fibroblast	15-5-81					21(87)	3(13)			11(48)	12(52)		

* X, Y = sex chromosomes; M = marker chromosome; () = percentage

ways of migration of XX cells into an XY male gonad is very uncertain. For one thing, implantation of the embryo occurs relatively late, approximately 20 days after fertilization, and the development of placental vessels is even further delayed. According to Jost et al.⁷, gonadal differentiation is nearly complete at that time, thus preventing the entry of female cells into the gonad from a twin.

The positive identification of chimeric cells in the male gonad and their subsequent development into spermatozoa that fertilize and give rise to offspring can be achieved by coat color marker genes¹¹, or more easily by marker chromosomes such as the 1/29 centric fusion chromosome found quite often in cattle populations.

Materials and Methods

An experiment was conducted using mixed semen from three bulls of the Angus, Simmental, and Bavarian Brown breeds and having diploid numbers of 60, 59, and 58 chromosomes, respectively. The last two bulls are carriers of the 1/29 centric fusion either heterozygously or homozygously. As a first step, twin calves of different sex sired by the heterozygous translocation carrier (59) were produced. Second, a cytogenetic investigation was carried out to find animals with the necessary chromosome composition to prove gonadal chimerism. Only male calves were used, since the female partners were more likely to be sterile. Several heterosexual twin pairs were obtained sired by the heterozygote Simmental bull known to carry the centric fusion 1/29. In all cases, the males appeared

normal, while females showed definite symptoms of freemartinism.

Standard techniques for leucocyte culture and chromosome preparations were used¹⁴ to study the three twin pairs A, B, and C. Repeated cultures (pair A, two times; the bulls from B and C, nine times) were made to assure accurate results especially as related to the possible influence of cell selection in vitro. Finally, muscle biopsies were taken from bulls B and C and fibroblast cultures established to get indications of chimerism from the chromosome preparations.

Results and Discussion

The cytogenetic analysis showed that in twin pair A the male resulted from the fertilization of a normal egg cell with sperm carrying the centromere fusion chromosome (designated as M in Figure 1), while the female developed from fertilization with a normal sperm. In the twin pairs B and C the reverse situation prevailed with females carrying the marker chromosomes. The diagram in Figure 1 illustrates the initial situation and the potential combinations of the various germ cells. For the sake of simplicity, all autosomes that are not essential in this diagram have been omitted. The diagram includes gametes expected from male twins with hypothetically chimeric gonads including those of animals B and C that may eventually lead to conclusive progeny tests. In Table I the results of the number of cell populations from the three twin pairs and the results of repeat analyses of bulls B and C from blood cultures and one fibroblast study are presented. All

three cases show consistent results. The white blood cells from the bull of pair C varies in the male cells from 28 to 57 percent and in the female cells from 41 to 72 percent. The bull in pair B had a very low proportion of female cells (2-16 percent); therefore, the animals represent, to some extent, both possibilities of dominating cells. Figure 2 shows the metaphases of the four different cell types and two karyograms including the sex chromosomes and marker chromosomes of the chromosome situation in pair B. In the fibroblast cultures the ratio of the two cell types are in the range of the lymphocytes (bull B, 87/13 percent; bull C, 48/52 percent). Therefore, the chimeric cell mixture is comparable in both tissues. The bulls from twin pairs B and C can thus be used in subsequent matings to further investigate gonadal chimerism because if their testes also are chimeric they should transmit the marker chromosome to some female offsprings derived from their twin sister.

The important factor in this unusual case of chimerism is the use of translocation chromosome 1/29, as first suggested by Herschler and Fehcheimer⁴. Their marker chromosome in a set of bovine triplets and our case is the 1/29 centric fusion type that was studied extensively by Gustavsson⁹ and others².

Since twinning in cattle occurs in less than 5 percent of births⁶ and the frequency of centric fusions also is low in most breeds, special matings involving bulls known to be translocation heterozygotes are essential for investigations of chimerism, as proposed in this paper. Numerous biochemical and genetical phenomena may be associated with sexual chimerism of cattle twins, but these

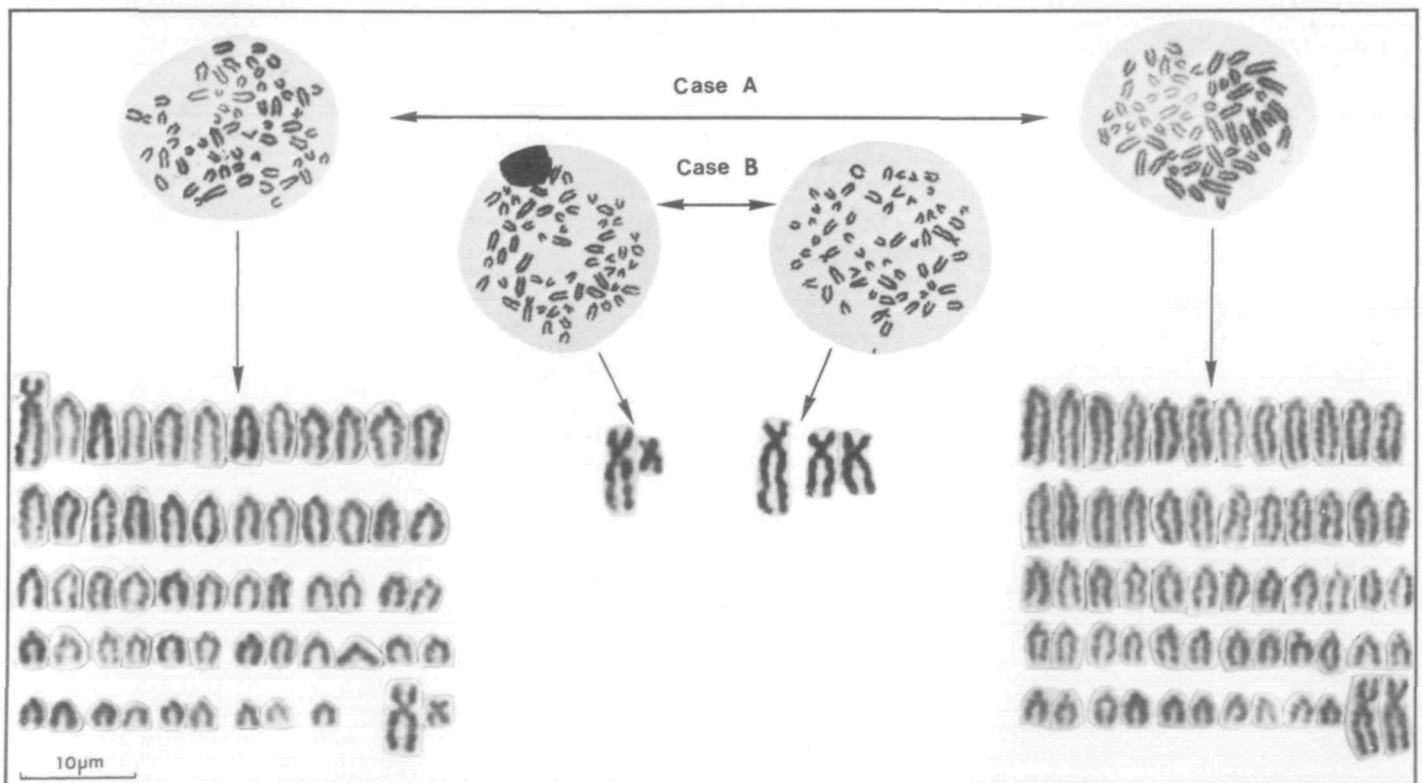


FIGURE 2 Metaphases (case A and B) and two karyotypes (case A) and additional significant chromosomes (case B) of the chimeric cattle twins.

may be masked by embryonic mortality. Therefore, the described cases could serve as model examples with which to better understand certain events involved in sex differentiation or genetic expression. Since chimerism is commonly found among heterosexual, dizygotic twins we believe that chimerism between like-sexed twins is equally frequent and should receive further attention in future studies, especially with animals from embryo transfers.

A single offspring with a centromeric fusion chromosome from the described males B and C would support the theory of germ cell chimerism. Such studies are now in progress.

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Left ostium straight: an inherited anomaly of the female genital tract in the rabbit

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ABSTRACT: An inherited anomaly resulting in the left oviduct extending lateral to the kidney with the ostium opening upward in an otherwise normal animal is described. This may be the result of a mechanical problem caused by the persistence of early embryonic mesenteries. Inheritance appears to be due to an autosomal recessive gene with incomplete penetrance. We propose the symbol *los* for the gene responsible for this condition.

ABNORMALITIES of the female genital tract are relatively rare in wild and domestic animals and most are associated with abnormalities of the urinary tract. The Müllerian

ducts are formed from invaginations of the coelomic epithelium of the urogenital ridges⁷. Details of their growth are not well known but early stages of their development suggest a close relationship between Müllerian and Wolffian ducts⁷. When a portion of the Wolffian duct is absent, the Müllerian duct will grow only as far as the terminal end of the Wolffian duct.

The developing uterine tubes fail to match the elongation of the trunk as a whole and the flaring ostial ends finally lie some 13 segments posterior to the level of their origin¹. Normally the ostium is tied to the ovary by the fimbriae⁷ and is pulled around mechanically when the ovary migrates posteriorly so that the open end lies over the anterior end of the gonad.

In 1955 when an adult rabbit of strain AX/J was necropsied, it was shown to be normal in every respect except that the anterior end of the left oviduct projected anteriorly and laterally to the kidney with the ostium projecting straight up instead of curling around the ovary. In 1958 six more cases were observed. Since that time many more such animals have been seen both in strain AX/J and in its subline AXBU/J. We know of no similar condition in any other animal. In this communication we report the mode of inheritance and describe the pathology of this new condition in the rabbit.

Materials and Methods

Our data were obtained from the necropsy records of the rabbit colony of the Jackson Laboratory. All rabbits that die or are killed for experimental purposes are necropsied and

checked for gross abnormalities of all major organ systems³. The data for this report concern two partially inbred strains, AX/J and AXBU/J⁵.

Standard genetic and statistical analyses were used to determine the mode of inheritance.

Results

Description. Figure 1 shows the relative position of the oviducts and ostia in a rabbit with left ostium straight. The right oviduct and ostium are normal with the ostium tubae curving around its ovary. The left oviduct is straight and projects cranial lateral to the kidney. The ostium tubae opens as a straight extension of the oviduct. In some specimens a thin mesentery tied the ostium tubae to the spleen, but most lacked such a mesentery. It may have existed temporarily in earlier development. The left ovary is in its normal position, but the left ostium tubae remains closer to its primitive location.

Litter size is significantly reduced in affected animals (Table I) of both strains. However, we have evidence that at least some of the eggs from the left ovary of affected females can make the long journey to, and be picked up by, the straight left ostium tubae.

Genetics. From September 1969 through December 1977, 1909 strain AX/J females were examined and 106 (5.6 percent) were found with straight left ostia. Similarly, among 1397 strain AXBU/J females, 99 (7.1 percent) had the left ostium straight. In contrast, of 12,133 females from all other strains examined during the same time period, only 5 (0.0004

The authors are, respectively, research associate, and senior staff scientist, The Jackson Laboratory, Bar Harbor, Maine 04609. This investigation was supported in part by NIH research grant RR-00251 from the Division of Research Resources to the Jackson Laboratory, and in part by institutional funds of the Jackson Laboratory. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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