Cytogenetic and reproductive studies of bulls born co-twin with freemartins

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Summary. The XX lymphocytes in peripheral blood of 22 bulls born co-twin with freemartins ranged from 5 to 95%. The difference of 1.2% between the means of XX percentages at initial and final karyotyping of 14 bulls over periods of 1–5 years was not significant ($P > 0.25$). Seven (58.3%) of the 12 A.I. chimaeric bulls were reproductive culs as compared to only 5.4% of their 128 controls ($P < 0.001$). The culled chimaeras produced no semen, had a low sperm output or had a high incidence of abnormal spermatozoa. The means for motility, sperm concentration and motile spermatozoa per ejaculate were significantly lower for the 12 chimaeric bulls than for a random sample of 12 controls ($P < 0.025$). The 60- to 90-day non-return rate for 30 814 services to 8 chimaeric bulls was 70% with a -0.1% mean deviation from contemporary breed average ($P > 0.5$).

The 7 chimaeric bulls culled for poor reproductive performance had larger and more numerous focal areas of testicular degeneration. Areas of lymphocytic infiltration in testes of many of these bulls suggest a rejection phenomenon or the autoimmune response. No XX germ cells were detected in testes of 11 chimaeric bulls, aged 1–9 years. Differential haemolysis blood typing tests on one bull with 85% XX lymphocytes and 23 of his dam–daughter pairs provided no evidence of germ cell chimaerism. One bull with 33% XX cells sired an excess of daughters (71%) in a progeny group of 48, a significant deviation from a 1:1 ratio ($P < 0.01$).

A disturbance in normal spermatogenesis in prenatal and postnatal life by transplanted XX primordial germ cells may contribute to the marked reduction in reproductive fitness of many chimaeric bulls.

Introduction

The factors responsible for the virilization of the freemartin gonad have eluded discovery for over half a century (Ohno, 1969; Jost, Vigier & Prepin, 1972). Ohno, Christian, Wachtel & Koo (1976) have proposed that H-Y antigen, specified by a gene on the Y chromosome, is responsible for testicular-like transformation of the freemartin gonad.

The normality of chimaeric bulls born co-twin with freemartins went unchallenged until Ohno, Trujillo, Stenius, Christian & Teplitz (1962) detected XX germ cells in the testes of two newborn bulls. Later, XX spermatogonia and primary spermatocytes were detected in testicular specimens from three chimaeric bulls at 2, 10 and 11 months of age (Teplitz, Moon & Basrur, 1967). Dunn, Kenney, Stone & Bendel (1968) then reported that 5 XX/XY bulls were either sterile or below stud average in semen quality and fertility. Other reports of a reduction in reproductive fitness of chimaeric bulls include cryptorchidism and sterility (Abdel-Raouf, 1964; Gerneke, 1969), low fertility and poor semen quality (Stafford, 1972; de Giovanni, Popescu & Succi, 1975; Cribiu & Popescu, 1975), abnormal testosterone synthesis (Pierrepoint, Stewart &

Rack, 1969; Short *et al.*, 1969) and underdeveloped seminal vesicles and ampullae (Short *et al.*, 1969). Normal breeding performance of chimaeric bulls has been reported also (Hansen, 1968; Stafford, 1972; Cribiu & Popescu, 1975; Pollock, 1975; Gustavsson, 1977).

If transplanted XX germ cells could persist in the testes of a mature chimaeric bull and undergo meiosis as "disguised spermatocytes", there would be an increased output of X-bearing spermatozoa and an increase in the percentage of female calves in his progeny, many of which would receive genes from his freemartin twin (Ohno *et al.*, 1962; Teplitz *et al.*, 1967; Dunn *et al.*, 1968).

Three chimaeric bulls are known to have sired a significantly higher number of female calves (Dunn *et al.*, 1968; Lojda, 1972; Cribiu & Popescu, 1975; de Giovanni *et al.*, 1975), but there is no proof that any of these bulls had transmitted his twin sister's genes to any of his offspring via donor XX germ cells.

In the present study, observations were made on 22 XX/XY bulls to determine (1) their reproductive fitness, (2) whether donor germ cells differentiated into functional spermatogonia, and (3) the stability of the XX:XY ratios. A preliminary study of 5 of these 22 bulls has been reported previously (Dunn *et al.*, 1968).

Materials and Methods

Animals

Thirteen chimaeric XX/XY bulls in artificial insemination (A.I.) centres and 9 in a college experimental unit were studied from 1964 to 1978. Twelve bulls (11 Holstein and 1 Ayrshire) in the New York A.I. Centre had twin sisters. A Guernsey bull (in the Louisiana Dairy Herd Improvement Centre), registered as a singleton, was karyotyped as a chimaera following a report that 71% of his offspring were females (McSparrin & Patrick, 1967).

For the control group, 128 non-twin bulls of the same age and breed as the 12 New York A.I. chimaeric bulls were available. Each of the 12 control units comprised 3–22 bulls. Karyotypes of 260 non-twin bulls in this centre, including 48 of the 128 controls, were 60,XY. Based on this large sample, all the control bulls were assumed to be non-chimaeric.

Breeding records were available on 1–2-year-old A.I. sires and on those selected on the basis of their progeny tests. No records were available on sires awaiting proofs or on sires culled with unsatisfactory proofs at 6 years of age. (Only about 2.4% of 6-year-old bulls are culled for poor reproductive fitness.) There were no records on the 9 experimental bulls.

Semen samples with less than 50% progressively motile spermatozoa and/or less than 400×10^6 spermatozoa/ml were classified as discards. Bulls with poor libido, reproductive organ anomalies, unsatisfactory semen and low fertility were classified as reproductive culls. During the 14-year period, semen processing varied and included liquid- and frozen-semen techniques.

A comparison of reproductive fitness of chimaeric and non-chimaeric bulls was made utilizing all 128 control bulls, but for analyses of the data on semen characteristics, one bull from each control unit was selected at random for comparison with each of the chimaeric bulls. Since the sperm output of most bulls increases between 2 and 6 years of age (Coulter, Larson & Foote, 1975), a comparison was made between the sperm production of 7 chimaeric bulls at these ages. Successive ejaculates in each age group ranged from 9 to 32.

Cytogenetic and histopathological techniques

The leucocyte culture methods described by Dunn, Lein & Kenney (1967) and Greene, Dunn & Foote (1977) were used. Whenever possible at least 100 metaphases were scored to provide reliable estimates of the XX:XY ratios which were expressed as the percentage of XX. Each bull's code was determined by the percentage of XX cells in all metaphases examined.

Testicular tissues were obtained from live bulls under anaesthesia or at necropsy. Colcemid was not administered to any of the bulls. Sections from the dorsal, central and ventral regions of the left and right testes were fixed in Bouin's solution for histopathological examination. Meiotic preparations were made using the techniques of Evans, Breckon & Ford (1964) and Ohno (1965).

Differential haemolysis blood typing tests were made according to the technique of Stone, Berman, Tyler & Irwin (1960).

Results

Blood cell cytogenetics

The XX cells in blood of the 22 chimaeric bulls ranged from 5 to 95% (Table 1). The stability of the XX:XY ratios was examined on data from 14 bulls by comparing the percentage of XX cells at initial and final karyotyping. The two estimates for each bull, the differences and the interval in years between karyotyping are in Table 2. Percentages were converted to angles by arcsin transformation for analysis of variance (Snedecor & Cochran, 1971). The difference of 1.2% between the means was not significant ($F_{1,13} = 0.07$; $P > 0.25$). There was a highly significant difference among bulls ($F_{13,13} = 17.62$; $P < 0.01$).

Table 1. Mean percentage of XX lymphocytes in peripheral blood of chimaeric bulls (H = Holstein; G = Guernsey; A = Ayrshire)

A.I. bulls			Experimental bulls		
Bull	No. of metaphases	%XX	Bull	No. of metaphases	%XX
H 1	216	5	H 12	250	5
H 2	231	24	H 13	300	18
H 3	100	36	H 14	241	27
H 4	200	41	H 15	352	29
H 5	100	43	H 16	464	48
H 6	84	60	H 17	106	52
H 7	100	65	H 18	258	81
H 8	137	71	H 19	312	87
H 9	150	77	H 20	200	95
H 10	267	85			
H 11	90	93			
G 1	58	33			
A 1	313	13			

Semen characteristics and reproductive performance

Semen characteristics of ejaculates obtained at 1–2 years of age are summarized in Table 3. Seven attempts to collect semen from Bull 36 XX over a 9-week period failed because of the bull's poor libido and failure to serve the artificial vagina. No attempt was made to collect semen from Bull 43 XX, whose testes were globular and flaccid, and from Bull 71 XX, whose growth and physical condition were unsatisfactory. Semen from Bulls 13 XX and 60 XX did not meet A.I. standards and was not used for insemination; sperm concentration and motility were consistently low in the semen of Bull 13 XX while the semen of Bull 60 XX, after staining with India ink, revealed 63% abnormal spermatozoa which included 22% with abaxial midpieces and 22% with cytoplasmic droplets. The semen of Bull 65 XX froze poorly; during one season, 71% of his spermatozoa had abnormalities of the acrosomes.

Table 2. Differences in percentage of XX lymphocytes in peripheral blood of chimaeric bulls at initial and final karyotyping

Bull*	Interval (years)	% donor XX cells		
		Initial	Final	Difference
A.I.				
5 XX	3.6	6	4	-2
24 XX	5.2	29	20	-9
41 XX	3.7	52	29	-23
65 XX	3.5	63	68	+5
77 XX	4.4	68	82	+14
85 XX	1.5	82	87	+5
Experimental				
5 XX	5	14	2.5	-11.5
18 XX	3	21	12	-9
27 XX	3	37	14	-23
29 XX	5	33	41	+8
48 XX	2	49	44	-5
52 XX	2.2	52	52	0
81 XX	3	75	92	+17
87 XX	5	78	96	+18
Means (angles retranslated to %)		46.3	45.1	-1.2

* This number was allocated to indicate the percentage of XX cells.

Table 3. Semen characteristics of A.I. chimaeric bulls during their sampling period at 1-2 years of age

Bulls	No. of ejaculates	Volume (ml)	Motile sperm. (%)	Sperm conc./ml ($\times 10^6$)	Motile sperm./ejaculate ($\times 10^9$)	Ejaculates discarded (%)
5 XX	9	7.2	51	1216	4.6	11
13 XX	14	7.2	47†	552†	1.9†	100
24 XX	9	4.4	54	1505	3.6	33
36 XX	0	No semen				
41 XX	11	5.6	54	1013	3.1†	
43 XX	0	No semen				
60 XX	15	5.1	17†	1397	1.3†	100‡
65 XX	10	5.5	56	2439	7.5	
71 XX	0	No semen				
77 XX	10	6.3	55	1797	6.5	
85 XX	32	8.3	58	1304	6.3	
93 XX	11	4.3	53	1243	2.8	9
XX/XY ($n = 9$)§	121	6.0	49	1385	4.2	24**
Controls ($n = 9$)	98	5.6	55	1574	4.9	8
Differences		+0.4	-6	-189	-0.7	+16
XX/XY ($n = 12$)¶	121	4.5	37*	1039*	3.1*	24**
Controls ($n = 12$)	117	5.7	56	1665	5.2	7
Differences		-1.2	-19	-626	-2.1	-17

* These means significantly lower than the controls ($P < 0.05$).

** These percentages significantly higher than the controls ($P < 0.01$).

† These values are below those of control bulls.

‡ Excessive number of abnormal spermatozoa.

§ Means of the 9 bulls producing semen.

¶ Means of all 12 bulls.

A comparison of the 9 semen-producing chimaeric bulls with their 9 controls revealed no significant differences between the means for sperm volume ($F_{1,16} = 0.61$; $P = 0.45$), motility ($F = 1.90$; $P = 0.19$), concentration ($F = 0.77$; $P = 0.39$) and the number of motile spermatozoa per ejaculate ($F = 0.68$; $P = 0.42$). However, when the 3 impotent bulls and their 3 controls were included in the analyses, the differences were significant for motility, concentration and motile spermatozoa per ejaculate ($t_{(11)} = 2.6$, $P < 0.025$). The 12 control bulls produced, on average, 2.1×10^9 motile spermatozoa per ejaculate more than did the 12 chimaeric bulls. Only 8 (7%) of the 117 ejaculates of the controls were discarded, whereas 29 (24%) of the 121 ejaculates of the chimaeric bulls were discarded. The difference was highly significant ($\chi^2 = 12.02$; $P < 0.001$).

During the period from birth to 10 years of age, 7 (58.3%) of the 12 chimaeric bulls were culled for poor reproductive performance (Table 4), whereas only 7 (5.4%) of the 128 control bulls were culled, 3 with unsatisfactory semen and 4 with low fertility. The difference in culling rates was highly significant ($\chi^2 = 28.45$; $P < 0.001$).

Table 4. Reproductive performance and testicular pathology of XX/XY bulls

Bull	Age (years)	Reproductive performance		Testicular lesions
		Rating	Defect	
77 XX	7	Good	None	—*
41 XX	7	Good	None	—*
5 XX	6	Good	None	SF-DT†
24 XX	7.5	Good	None	SF-DT†
65 XX	6	Fair	Sperm quality‡	SF-DT†
93 XX	10	Poor	Sperm output; fertility	LF-DT
85 XX	8.5	Poor	Sperm output	LF-DT
13 XX	3	Poor	Sperm output	LF-DT
60 XX	2	Poor	Abnormal spermatozoa	LF-DT
36 XX	1.5	Poor	No libido, no semen	LF-DT
43 XX	1	Poor	Testicular anomaly, no semen	LF-DT
71 XX	1	Poor	Poor growth, no semen	LF-DT
Experimental bulls (3–5 years)				
5 XX and 29 XX				LF-DT
18 XX, 27 XX, 48 XX and 87 XX				SF-DT

SF-DT, small foci of tubules deficient in germinal cells; LF-DT, large foci of tubules deficient in germinal cells with primordial germ cells and multinucleated giant cells in lumina.

* Bulls still in service.

† Bulls culled for genetic proof.

‡ Poor freezability and abnormal acrosomes as a 2-year-old.

There was an increase in sperm output by 6 of 7 chimaeric bulls in service at 6 years of age when compared with their sperm output as young bulls (means = 3.6×10^9 motile spermatozoa/ejaculate). There was a significant decrease (1.81×10^9 motile spermatozoa/ejaculate) in sperm output of Bull 85 XX ($F_{1,62} = 7.87$; $P < 0.01$), and 18 of 32 successive ejaculates were discards ($\chi^2 = 22.34$; $P < 0.001$) with many abnormal spermatozoa which included 10–25% with abaxial midpieces as observed in semen of Bull 60 XX.

Histopathology and cytogenetics of testes

Histopathological examinations were made on testicular specimens from 16 chimaeric bulls (10 A.I. and 6 experimental) and 9 control bulls. All 16 chimaeric bulls had varied degrees of testicular degeneration as shown by tubules which were deficient in germinal cells (Table 4). The

most advanced stages of degeneration were observed in the testes of Bull 60 XX (Pl. 1, Fig. 1) and Bull 71 XX (Pl. 1, Fig. 2). Bulls with good reproductive performance had only a few seminiferous tubules which were deficient in germinal cells (SF-DT), whereas those with poor reproductive performance had larger and more numerous focal areas of testicular degeneration (LF-DT). Many of these bulls also had perivascular and peritubular foci of lymphocytes. Four of the 12 randomly selected controls had LF-DT in at least one testis. Two of these 4 bulls had low sperm output but the reproductive fitness of the other 2, culled for unsatisfactory proofs, was not known. The incidence of LF-DT in the XX/XY bulls (70%) was not significantly different from the 44.4% in the control bulls ($\chi^2 = 0.44$; $P > 0.5$).

Table 5. Meiotic chromosomes in testicular preparations from XX/XY bulls

Bull	Age (years)	Spermatocytes				Spermatogonia		
		Pachytene		Diakinesis		XY	XX	Degen.
		XY+	XY—	XY	XX(?)			
A.I.								
5 XX	6	13	0	1	0	0	0	0
24 XX	8	0	0	33	4	2	0	1
60 XX	2	275	51	4	1	3	0	21
71 XX	1	55	3	0	0	0	0	3
85 XX	9	21	4	0	0	0	0	13
Experimental								
5 XX	5	0	0	0	0	0	0	0
18 XX	3	0	0	0	0	0	0	0
27 XX	3	8	0	2	2	0	0	0
29 XX	5	119	6	19	1	1	0	0
48 XX	3	22	2	5	0	0	0	0
87 XX	5	36	0	33	5	0	0	0
Totals		549	66	97	13	6	0	38

XY+ = XY sex vesicle present; XY− = XY sex vesicle absent; XX(?) = absence of typical end-to-end association of X and Y.

Meiotic preparations were made from testes of 5 A.I. and 6 experimental chimaeric bulls. The 769 observations are summarized in Table 5. Only a few XY, and no XX, spermatogonial metaphases were observed, partly due to the advanced testicular degeneration in many of these bulls. Of 110 spermatocytes in diakinesis, 97 showed the terminal association of the X and Y chromosomes and the 29 autosomal bivalents typical of the XY spermatocyte (Pl. 2, Fig. 3). The remaining 13, classified as XX(?), lacked the typical XY bivalent, but being contracted and condensed, they were most probably atypical XY bivalents (C. E. Ford, personal communication; Ford & Evans, 1977). The typical heteropycnotic sex vesicle with the XY bivalent (Pl. 2, Fig. 4) was not observed in 66 pachytene nuclei (Pl. 2, Fig. 5). These, like the two reported by Short *et al.* (1969), may have been XX spermatocytes. On the other hand, they may be considered mid- to late pachytene in which the sex vesicle has disappeared, revealing the sex bivalent in ring-like forms (Pl. 2, Fig. 5).

Fertility and progeny tests

Semen of 8 A.I. chimaeric bulls was used for artificial insemination. Estimates of fertility are reported as 60- to 90-day non-return rates with deviations from corresponding 12-month breed averages. The non-return rate for 30 814 services to these 8 bulls was 70%. The mean deviation from breed average was −0.1% and not significant ($t_7 = 0.13$; $P > 0.5$).

Fig. 1. Section of testis of Bull 60 XX showing pycnotic germ cells and Sertoli cells. $\times 200$.

Fig. 2. Section of testis of Bull 71 XX showing seminiferous tubules deficient in active germinal epithelium. $\times 200$.

(Facing p. 26)

PLATE 2

Fig. 3. An XY spermatocyte at diakinesis from testis of Bull 60 XX showing the 29 autosomal bivalents and the end-to-end association of the X and Y.

Fig. 4. A pachytene nucleus from the testis of Bull 60 XX showing a typical XY sex vesicle, SV.

Fig. 5. A pachytene nucleus from the testis of Bull 60 XX showing absence of typical XY sex vesicle. Possible X-Y bivalents (arrows).

Sex ratio estimates were obtained on single-born offspring of 4 bulls (Table 6). Only the ratio for Bull 33 XX was significantly different from a 1:1 ratio ($\chi^2 = 8.33$; $P < 0.01$). Differential haemolysis blood typing tests were made on Bull 85 XX, 23 of his daughters and their 23 dams. The results showed that 10–15% of his blood represented his own genotype, whereas 85–90% represented the genotype of his twin sister. The erythrocyte and lymphocyte mixtures were essentially identical for this bull, although this is not generally true for cattle chimaeras (W. H. Stone, R. G. Cragle, H. O. Dunn, A. F. McFee & S. V. Rosser, unpublished data). Blood tests provided no evidence that Bull 85 XX transmitted any of his twin sister's blood group genes to any of his 23 progeny. A blood test on Bull 33 XX did not show erythrocyte chimaerism despite the clear chimaerism of the lymphocytes and tests on 3 of his daughters provided no evidence of abnormal transmission. There was no cytological evidence of germ cell chimaerism in any of the 4 bulls.

Table 6. Fertility and progeny sex ratios of A.I. chimaeric bulls

Bull	No. of first services	60–90 day		Progeny (no.)	Sex ratio %F:%M
		% non-return	Deviation†		
5 XX	402	66.9	–0.1		
24 XX	13 428	72.2	+3.3	126	48:52
33 XX	1153	60.0	–5.0	48	71:29*
41 XX	336	65.8	–0.2		
65 XX	344	66.9	–0.1		
77 XX	442	68.6	+2.6		
85 XX	3502	74.9	–0.1	266	55:45
93 XX	11 207	67.0	–1.3	297	52:48
Total	30 814				
Means		70.0	(–0.1)		

* Significantly different from a 1:1 ratio, $P < 0.01$.

† Deviations from 12-month breed averages.

Discussion

This study provides further evidence that there is a marked reduction in the reproductive fitness of many bulls born co-twin with freemartins. Based on our sample of 12 chimaeric A.I. bulls and their 128 controls in one A.I. centre, there is a 58% chance that a chimaeric bull will be culled for poor reproductive performance in the first 10 years of his life, compared with only a 5% chance for a single-born non-chimaeric bull. As expected, there was no association between the percentage of XX donor blood cells and the reproductive fitness of a chimaeric bull. For example, Bull 77 XX (23% XY) was superior to Bull 5 XX (95% XY) in both sperm output and fertility, the poorest quality semen was produced by Bulls 13 XX and 60 XX, while the fertility of Bulls 24 XX, 77 XX and 85 XX was essentially equal. This is consistent with the report by Greene *et al.* (1977) who found that the XX:XY blood lymphocyte ratio was not related to the degree of masculinization of the freemartin reproductive organs. Similarly, W. H. Stone (unpublished) found that the degree of blood group chimaerism was not correlated with the severity of the freemartin condition.

Three chimaeras with anomalies of the reproductive tract (cryptorchidism and under-developed seminal vesicles and ampullae) have been reported (Abdel-Raouf, 1964; Gerneke, 1969; Short *et al.*, 1969). In our study, one bull, No. 43 XX, had an anomaly of the reproductive tract (globular flaccid testes). One of the three chimaeras in the British Isles had poor semen quality (Stafford, 1972), but there were 7 chimaeras in our group which were culled for this

reason. Two chimaeric bulls with low fertility were observed in the British Isles and Europe (Stafford, 1972; Cribru & Popescu, 1975; de Giovanni *et al.*, 1975). Fertility of 7 of the 8 A.I. chimaeras listed in Table 6 was satisfactory; the Louisiana Guernsey, Bull 33 XX, was 5 percentage units below the breed average. Of the 7 chimaeras with satisfactory fertility, however, Bulls 85 XX and 93 XX were culled for low sperm output at 8.5 and 10 years of age, respectively. As mentioned above, normal breeding performance of chimaeric bulls has been reported. Most of the 34 bulls located in the British Isles or Europe were young bulls which may account for fewer reproductive problems. Nevertheless, there is no explanation for the marked difference in the sample described in this report and the good performance of many bulls in other locations. It seems very unlikely that there could be a breed or environmental effect. One can conclude that unlike the freemartins, which are sterile and have widely different degrees of virilization of the reproductive tract, the chimaeric bulls show a wide range in reproductive fitness but very little variation in the reproductive tract.

The aetiology of the reproductive failure and testicular degeneration in many of these bulls is only speculative. It may be associated with the embryonic and neonatal elimination of transplanted donor XX germ cells, which might be few or numerous (Ohno *et al.*, 1962; Teplitz *et al.*, 1967; Weiss & Hoffmann, 1969) and the failure of the XY germ cells to repopulate certain portions of the tubules monopolized by the XX germ cells. Lymphocytic infiltration in the areas of testicular degeneration in the XX/XY bulls with LF-DT lesions suggests a rejection phenomenon or autoimmune response. Testicular degeneration in 3 young XY/XY chimaeric bulls from a group of 8 pairs of like-sexed chimaeric twins (K. McEntee & H. O. Dunn, unpublished data) may be the result of autoimmune responses.

Our failure to find XX germ cells in testes of 11 chimaeric bulls, aged 1–9 years, accords with that of Ford & Evans (1977) who found no XX germ cells in spermatogonia and primary spermatocytes of 7 chimaeric bulls, aged 44–46 weeks, and with that of Short *et al.* (1969) who found no XX germ cells in the testis of an 11-month-old chimaeric bull, with the exception of two possible XX spermatocytes. However, proving that no XX germ cells are present in either testis of an XX/XY bull is a monumental task since the donor cells, if few in number, could be missed in the normal sampling procedure. The ease with which chimaerism can be overlooked in some cases was demonstrated by Dunn, McEntee & Hansel (1970) who found only diploid XX cells in 600 metaphases from skin, blood and bone marrow of a 60,XX/90,XXY bovine true hermaphrodite but detected many Y-bearing triploid cells in other tissues. The conclusion by Weiss & Hoffman (1969) that XX/XY chimaerism present in testes of a 2-month-old bull vanished by 18 months of age was based on only 72 cells and these from tissue culture.

Progeny tests on 2 sets of dizygotic twin bulls with erythrocyte chimaerism failed to detect transmission of donor genotypes to their offspring (Stone *et al.*, 1960; Ross & Thomas, 1978). Similarly, no evidence of abnormal transmission of blood group genes was detected in progeny of Bulls 85 XX and 33 XX. No progeny tests were reported on the European chimaeric bull with 78% females ($P < 0.001$) in a progeny group of 148 (Cribru & Popescu, 1975; de Giovanni *et al.*, 1975) or the chimaeric bull with 60% females ($P < 0.025$) in a progeny group of 168 (Lojda, 1972). Of the three chimaeric bulls with significant deviations in the percentages of female calves, two had low fertility (Bull 33 XX and the de Giovanni bull) but the fertility of the Lojda bull was not reported. A New York A.I. Jersey bull, which was not karyotyped but was probably non-chimaeric, had 58% females in a progeny group of 168 ($P < 0.05$) which was the largest sex-ratio deviation in a study of 35 423 single births by mates of 111 sires (Foote, 1978).

As yet there appears to be no satisfactory evidence of germ cell chimaerism in the testes of chimaeric bulls. Short *et al.* (1969) suggested that XX germ cells are unable to undergo spermatogenesis when placed in a testicular environment. Ohno (1977) states that “the possession of more than one X chromosome is simply incompatible with further differentiation of testicular primordial germ cells to definitive spermatogonia”. However, in the testis of sex reversed XX,*Sxr*⁻ male mice, aged 300 days, XO germ cells derived from XX primordial germ

cells complete spermatogenesis and spermiogenesis forming O- and X-bearing spermatozoa (Ohno, 1977). If similar events occurred in a chimaeric bull testis and the O-spermatozoa were not viable, an excess of X-bearing spermatozoa could account for the over-abundance of daughters among progeny of certain chimaeric bulls.

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