A chimaeric calf with XX/XXY mosaicism and intersexuality

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Summary. A Friesian calf with an elongated urethra and without a vulva was born twin to a dead bull calf. Red cell chimaerism and XX/XY/XXY lymphocytes were found in the blood. XX/XXY mosaicism was found in the skin and the minute gonads which some showed signs of early testicular development.

Introduction

In the classic freemartin calf, the clitoris shows various degrees of enlargement while the vulva and urethra generally keep a female shape. The present investigation was prompted by the appearance of a urethra of masculine form in an intersex twin calf.

Animal and Methods

The intersex Friesian calf was born in the milking herd of an Essex farmer. The bull twin was still-born but was said to be of normal appearance. On noticing the abnormal genitalia of the other calf, the farmer consulted his veterinary surgeon and in due course the calf was offered to the Department of Clinical Veterinary Medicine, University of Cambridge. The dam was 11 years old at the time of the birth: she had previously produced 4 bull calves, 3 heifer calves and one set of twins of mixed sex (a bull and a freemartin). The sire was an A.I. bull who was dead at the time of the present study and whose records were not available.

Examination of the calf at the age of 3 months showed it to be well grown and feminine in appearance. There was no vulva in the normal position but between the hind legs behind and just above the level of the teats there was a urethral opening surrounded by a tuft of long hair. Neither penis nor clitoris was palpable and no gonads were present in the inguinal region. Later when the calf was bigger, rectal examination failed to find any evidence of a tubular genital tract in the genital fold. No behavioural signs of oestrus were ever seen.

During life, blood samples were collected for typing of red cell antigens and transferrin, testosterone assay and lymphocyte culture. Skin biopsy was also performed to obtain cells for culture.

Blood lymphocytes were cultured with phytohaemagglutinin and prepared by a modification of the whole blood method of Basrur & Gilman (1964). Tissue from the skin and the gonads was cultured by a modification of the method of Hyman & Poulding (1972). The blood groups and transferrin types were analysed from heparinized blood samples at the Animal Breeding Research Organization, Edinburgh.

The concentrations of testosterone in the blood were measured by the radioimmunoassay method of Main, Davies & Setchell (1978). The specificity of the testosterone assay relative to testosterone (100%) was only significant with respect to two other androgens: dihydrotestosterone (52.0%) and 5 α -androstane-3 α ,17 β -diol (34.4%). The sensitivity of the assay is 80 pg. Organs for histological examination were fixed in 10% formal saline and examined by light microscopy.

Results

Anatomy

The animal was slaughtered at the age of 15 months and post-mortem examination was carried out. The tubular urethra curved round the sciatic arch, showed some dilation within the pelvis and ran

forward to the bladder. No evidence of penis or clitoris was found. Two minute spindle-shaped gonads each about 5 mm long, were found in the genital fold. The cortex in both cases was disgenic and flattened to form a layer of the tunica albuginea type. The medulla was better developed and showed some evidence of abortive growth of a tubular rete. No Wolffian or Müllerian structures could be seen.

Blood groups

The erythrocyte antigens of the dam and her calf were as follows:

dam	A	G	E ₂	Q'	WX ₁	F	H'
calf	A	G	O ₃	E ₂	I' Q' C ₂	± X ₂ F V ± L ± Z H' E ₁ ± U ₁	

The blood types of the sire were not available but it can be assumed that the 9 antigens which occurred only in the calf (O₃, I', C₂, X₂, V, L, Z, E₁ and U₁) were of paternal origin. Four of these showed partial lysis (X₂, L, Z and U₁). The inheritance of plasma transferrin types appeared to be normal since only one of each parental genotype (dam, D₁, D₂; sire, A, E) was contributed to the calf (D₂, E).

Two cultures of blood from the dam were attempted but both failed. The sex chromosome constitution of cultured somatic and gonadal tissues from the calf is given in Table 1. All the tissues examined had small clones of XXY cells: in somatic cell cultures they reached about 10%. The percentage of XX cells in blood and skin was about 66%. The gonads contained about 90% XX cells while the remainder were XXY with the exception of one aneuploid cell from the right gonad which had an XY constitution. Among the skin cells 6 appeared to have small fragments about the size of a centromere or short-arm region of an autosome. In one of these cells the 'fragment' could be paired with one small autosome while an extra small submetacentric may have represented the product of the fusion of the long arms with another acrocentric autosome.

Table 1. Sex chromosomes in somatic and gonadal tissues from the calf

Tissue	No. of cells	XX	XO	XY	XXY	XXX	Polyploid	Uncertain
Blood lymphocytes	67	44	2	7	7	1	5 { XXYY (2) XXYYY XXY X	1
Skin (primary culture of 7 days)	62	41			6			15 { 6 with 'fragments' 2 60,XY?2X 7 sex chromosomes missing or obscure
Left gonad (primary culture of 7 days)	50	46			4			
Right gonad (primary culture of 7 days)	50	44			5			1 41,XY

Testosterone

The level of testosterone in the circulating blood was less than 200 pg/ml.

Discussion

Partial lysis of the red cells in respect of four antigens from the calf (X₂, L, Z and U₁) demonstrated red cell chimaerism. These groups must have been a paternal contribution because they were absent

from the maternal genotype, but it is difficult to attach any other significance to this particular occurrence which may have been coincidental. This and the XX/XY lymphocyte chimaerism were probably due to a placental anastomosis with the male twin. The genotypes of the skin and the gonads established the animal as an XX/XXY mosaic. The XXY clone of lymphocytes might have come from either or both twins. The low level of circulating testosterone, the male-type urethra, the indifferent gonads with a tunica albuginea and the absence of Wolffian or Müllerian structures combine to suggest a sequence of events different from those in classic freemartinism. The nature of the influence of the male twin on the freemartin gonad is still in debate (Vigier, Locatelli, Prépín, du Buisson & Jost, 1976) but freemartins with elongation of the urethra are very rare (Jost, Vigier & Prépín, 1972). It is possible that the male gonadal cells induced testicular growth at the normal time, but that this soon failed because of the sparsity or abnormality of the Y-bearing cells.

Artificial sex chromosome chimaeras have shown that fertile testes can consist of somatic cells of both sexes (Mystkowska & Tarkowski, 1968; Mintz, 1968; McLaren, 1971). Recent work on the H-Y (Y histocompatibility) antigen which is generally present on Y-bearing cells (Wachtel, Ohno, Koo & Boyse, 1975) suggests that the H-Y antigen induces a testis by exerting a local action on the embryonic gonad. Ford (1970) described work on the proportions of Y-bearing cells in XO/XY human mosaic gonads which indicates that the mesenchymal stroma of the ridge may specify the sex of the gonad and a threshold proportion of XY cells be essential for a testis. Some cytogenetic findings in mosaic gonads of cattle are summarized in Table 2. Among these animals the proportion of Y-bearing cells in testicular tissue varied between 20 and 50%. Ovarian and disgenic tissue contained up to 25% of Y-bearing cells. If these proportions are those of the original gonadal ridge, the observations support the possibility of a threshold proportion of cells below which a testis does not develop. In these few cases it seems to be about 20–25%, but the estimate takes no account of abnormalities in the Y-bearing genome or of differential growth in culture. In our Friesian calf the minute indifferent gonads with a tunica albuginea and slight tubular development of the medulla are evidence of early testicular differentiation. The disgenic structure and small size suggest a very early failure of development. Neither the complement of 10% and 8% 61,XXY cells in right and left gonads respectively nor the influence of the male twin was effective in maintaining testicular differentiation.

Jost, Vigier, Prépín & Perchellet (1973) and Vigier, Prépín & Jost (1976) presented the sequence of responses in the undifferentiated genital system under the influence of the embryonic testes in the calf. The first part of the tract to respond was the genital sinus. At the 47th day it began to elongate to form the penile urethra; the genital papilla reached its position behind the umbilicus and at the 58th day the scrotum and prostate gland were formed. Our animal and three others with disgenic gonads in Table 2 show the effects of this early stimulus on the form of the genital sinus. It seems likely that they all had mosaic gonads. There were probably some XY cells in the gonads of Animal B (XX/XY) and their sparsity may have accounted for the failure in development. The stimulus appears to have been enough to initiate but not to maintain development beyond the stage reached between the 40th and 58th days.

Jost *et al.* (1972) and Jost, Perchellet, Prépín & Vigier (1975) contrasted development in freemartins with that of normal males and females. They showed growth in the undifferentiated gonads of freemartins and normal females up to the 48th day of gestation. After this, growth in the freemartins stopped and the upper Müllerian ducts began to degenerate. These were the first signs of fraternal influence. At 90 days, growth was resumed in those freemartin gonads which would become masculinized. Seminiferous cords appeared, but the anogenital distance was not affected and only rarely varied from that of normal females. In our calf the penile urethra and absence of Müllerian ducts indicate testicular development at least to the 47th day but absence of scrotum, prostate and penis suggests that development ceased before the 58th day. This would explain the absence of Wolffian ducts which cannot persist without androgens (Jost *et al.*, 1975). The freemartin, by contrast, can maintain the Wolffian ducts, probably by androgens from her own gonads (Short *et al.*, 1969).

The mosaicism in our calf could have arisen in a number of ways. Either the egg or the spermatozoon may have carried an extra sex chromosome as a result of non-disjunction at meiosis. Non-disjunction in the XXY zygote could then have accounted for the four clones of cells 61,XXY, 60,XX, 60,XY and 59,XO. Origin in digyny or dispermy is not supported by the cytogenetic evidence or by

Table 2. Genotype and genitalia of mosaic cattle

Animal, sex of twin, somatic genotype	Gonads	Gonadal genotype	Internal genitalia			External genitalia	Reference
			Wolffian duct	Müllerian duct			
A Holstein, single, XX/XY	Right: ovary Left: ovotestis Ovarian Testicular	? ? 20% XY 80% XX	Left epididymis and ductus deferens Seminal vesicles	Uterus Right oviduct Cervix Vagina		Penis Scrotum	Dunn, Kenney & Lein (1968)
B German 'Simmental', female twin, XX/XY	Disgenic ovaries with tunica albuginea-like transformation of germinal epithelium	100% XX	Epididymis Ductus deferens Seminal vesicles	Vestigial uterus		Extended urethra Clitoris	Rieck (1973)
C Holstein, single, 60,XX/90,XXY	Right: ovotestis Ovarian Testicular Left: ovary	75% 60,XX 25% 90,XXY 50% 60,XX 50% 90,XXY 89% 60,XX 11% 90,XXY	Right epididymis and ductus deferens	Uterus Oviducts Cervix Vagina		Penis Scrotum	Dunn, McEntee & Hansel (1970)
D German "Simmental" (single) 60,XX/90,XXY	Disgenic ovaries stimulated medullary rete	75% 60,XX 25% 90,XXY	Absent	Uterus Cervix Vagina		Extended urethra No penile structures	Rieck (1973)
E ? (single) XX/XY	Rudimentary ovotestes	? ?	? ?	Hypoplastic uterus		Extended urethra Hypertrophic clitoris	Lojda (1968)
F Friesian, male twin, XX/XY/XXY	Minute disgenic gonads, tunica albuginea Right Left	(2% ?XY) 88% XX 10% XXY 92% XX 8% XXY	Absent	Absent		Extended urethra No penile structures	Present case

the plasma transferrin types of the calf, which showed the normal inheritance of a haploid contribution from each parent. Meiotic non-disjunction may be the explanation of the mosaicism. The age of the dam (11 years) or some genetic reason could be the cause of the aneuploidy. There is some indication in the work of Rieck, Hohn & Hertzog (1970) that an inclination to meiotic non-disjunction can be inherited. The breakages and translocation in the skin cells may be a sign of such abnormality.

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