

CYTOGENETIC STUDY OF INTERSEX SWINE

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ABSTRACT

To clarify the etiology of intersexuality due to genetic factors, 33 intersex swine and 33 of their ascendants were studied karyotypically. Metaphases were obtained from peripheral blood lymphocytes and analyzed by chromosome G and C banding in addition to standard Giemsa staining. Two cases of chromosome anomalies were observed: an intersex presenting 38,XX/38,XY mosaicism or chimerism, and an intersex presenting a break in one chromosome of pair 9, with a karyotype identical to that of the remaining intersex animals, i.e., 38,XX. All parents had normal karyotypes, i.e., 38,XX for females and 38,XY for males. Macro- and microscopic examinations of the genitalia of 26 of the 33 intersex animals revealed that 10 were true hermaphrodites, 14 were male pseudohermaphrodites and two were morphologically normal females except for a slightly hypertrophied clitoris and a "parrot beak"-shaped vulva.

INTRODUCTION

The infertility, subfertility and sex differentiation anomalies of domestic animals are of great economic interest and also provide material for the understanding of the mechanisms related to sex determination and differentiation.

Genetic defects are responsible for the interruption of, or deviation from, the sequential steps of normal sex differentiation. Some faulty steps during the main stages of this differentiation have been well studied, especially in man, and may be

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due to sex chromosome aneuploidies, autosomal or X-linked gene mutations, mosaicism, chimerism, structural alterations of sex chromosomes, or defects of polygenic or multifactorial inheritance.

Since the 1960's, when cytogenetic techniques were developed and refined, several studies have established the association of abnormal conditions of sex determination and differentiation with chromosome alterations. In a review of the literature up to 1964, Biggers and McFeely (1966) had already found 48 reports of cytogenetically studied intersex cases involving swine, cattle, goats, cats, horses and dogs. A large number of reports published after 1964 related the alterations in sex differentiation to chromosome aberrations, and contributed to the understanding of the mechanisms involved in mammalian sex determination (Lodja, 1968; Breeuwsma, 1968; Bruère *et al.*, 1968; Rieck *et al.*, 1969; Basur *et al.*, 1969; Rieck, 1970; Gluhovschi *et al.*, 1970; Bouters and Vandeplassche, 1972; Toyama, 1974; Chandley *et al.*, 1975; Lodja and Havrankova, 1975; Norberg *et al.*, 1976; Dain and Bridge, 1978; Tarocco *et al.*, 1982).

Parallel to the discovery and description of new clinical forms, it has been possible to study the anatomical and histopathological characteristics, as well as to carry out hormonal and mainly cytogenetic investigations of animals presenting distortions in the sex determination and differentiation process.

In addition to genetic factors such as recessive autosomal mutations, chimerism or mosaicism and aneuploidies of sex chromosomes, factors such as autosomal dominant mutations, dominant or recessive X-linked mutations, Y-autosome translocations, Y-X interchange and sex chromosome deletions may occur in swine, contributing to a high frequency of intersex animals. In order to determine the etiology of intersexuality due to the above factors, intersex pigs born on a large swine farm were submitted to cytogenetic analysis.

MATERIAL AND METHODS

The study was conducted on 33 Landrace and Large White pigs born from January to November 1984, that showed intersex characteristics on the basis of clinical examination of the outer genitalia, and on 33 of their ascendants. The animals belonged to a swine farm containing 240 dams, located in the western part of the State of Santa Catarina, Brazil.

Karyotype analysis was performed on metaphases obtained from peripheral blood lymphocyte cultures by the technique of Moorhead *et al.* (1960). The techniques used were G banding (Drets and Shaw, 1971) and C banding (Arrighi and Hsu, 1971), in addition to standard Giemsa staining.

Twenty-six of the 33 phenotypically intersex swine were slaughtered between 150 and 180 days of age for morphological and histological study of the genitalia.

RESULTS AND DISCUSSION

The results of karyotype analysis are presented in Tables I, II, III and IV. Of the 33 intersex pigs studied, 31 (93.93%) presented a normal 38,XX female karyotype, a condition already described by Makino *et al.* (1962) and later confirmed by other investigators (Melander *et al.*, 1971; Sittmann, 1973; Ohno, 1979; Sittmann *et al.*, 1980). Two animals showed karyotypic anomalies. One of them, no. 620, classified as a male pseudohermaphrodite, had a 38,XX karyotype, though with a break in one chromosome of pair number 9 (Figure 1), and the other, no. 420, also classified as a male pseudohermaphrodite, had 38,XX/38,XY chimerism or mosaicism (Figures 2 and 3) with predominance of XY cells (85.71%). Chimerism was first demonstrated by McFee *et al.* (1966), though with predominance of XX cells. Other karyotypic forms involving the sex chromosomes have been described for intersex swine, such as mosaicisms 38,XX/38,XY/37,XO (Lodja, 1968), 39,XXY/40,XXXXY (Harvey, 1968) and 38,XX/39,XXY (Toyama, 1974) and aneuploidies 39,XXY (Breeuwmsma, 1968) and 37,XO (Nes, 1968).

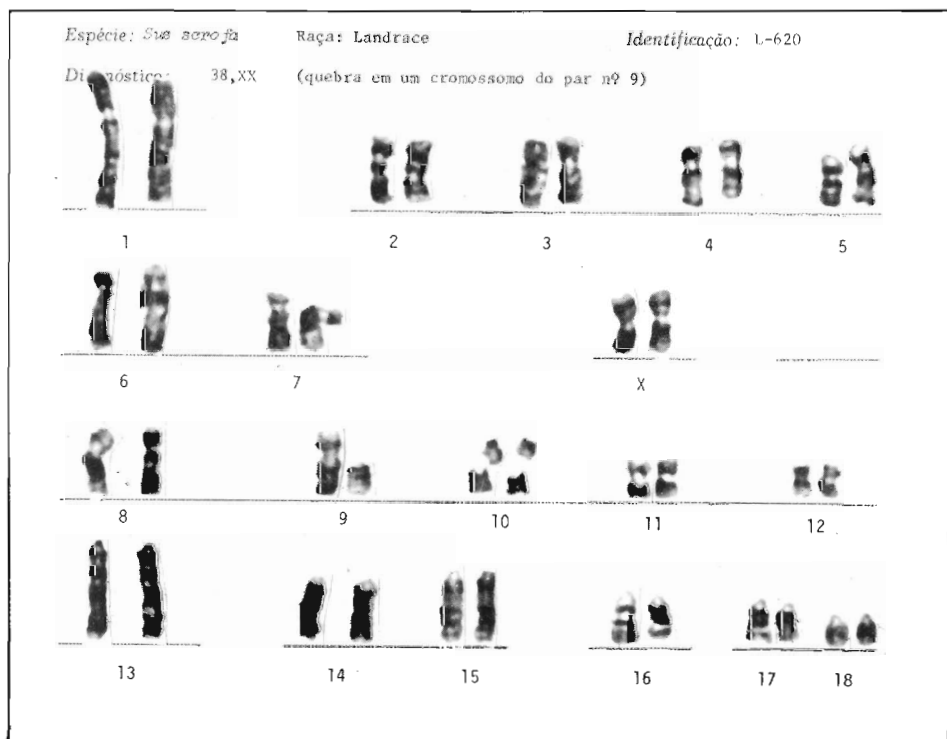


Figure 1 - Karyotype of the intersex no. 620. Landrace. $2n = 38,XX$ with break in a chromosome of pair no. 9. G banding.

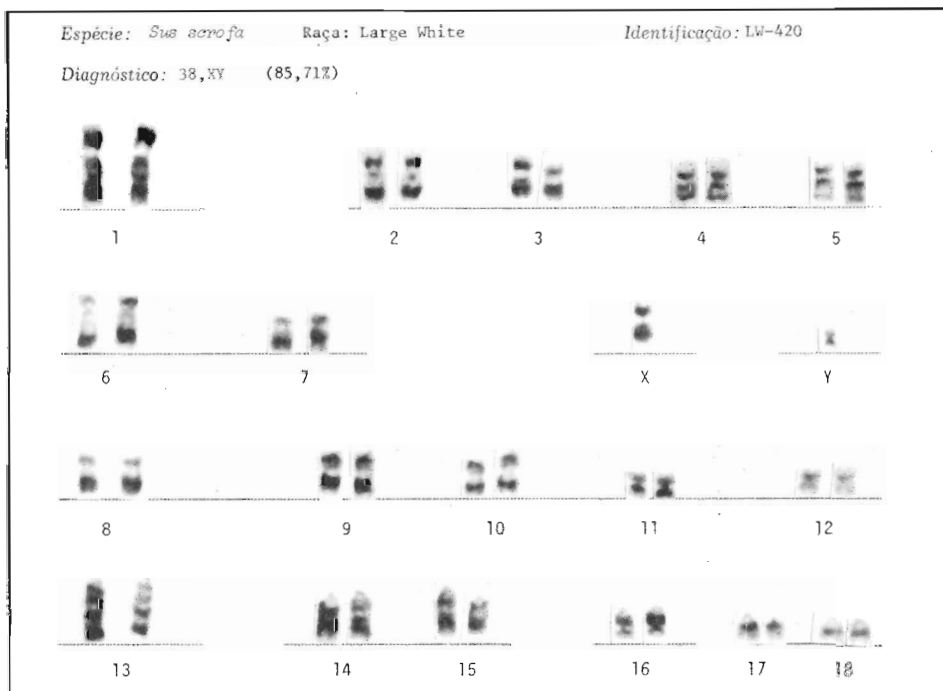


Figure 2 - Karyotype of the intersex no. 420. Large White. $2n = 38,XY$ (85,71%). G banding.

Macro- and microscopic examination of the genitalia of 26 intersex animals in the present study (part of the sample evaluated cytogenetically) permitted an anatomical classification of the animals into male pseudohermaphrodites ($N = 14$, Table I), true hermaphrodites ($N = 10$, Table II) and morphologically normal females except for a slightly hypertrophied clitoris and upturned "parrot beak"-shaped vulva ($N = 2$, Table III). The male pseudohermaphrodites presented male gonads, female outer genitalia with hypertrophied clitoris and female inner genitalia, though usually with some developmental defect (hypoplasia). The true hermaphrodites had a male gonad and a female gonad, or one or both formed by a combination of ovarian and testicular tissue (ootestis); their outer and inner genitalia were similar to those of male pseudohermaphrodites. Detailed results of the macro- and microscopic results will be published in a separate paper.

Karyotypic analysis data for seven animals not submitted to histological or anatomical evaluation are presented in Table IV.

The 33 ascendants of the intersex swine had normal karyotypes, i.e., $38,XX$ and $38,XY$ (Tables I through IV).

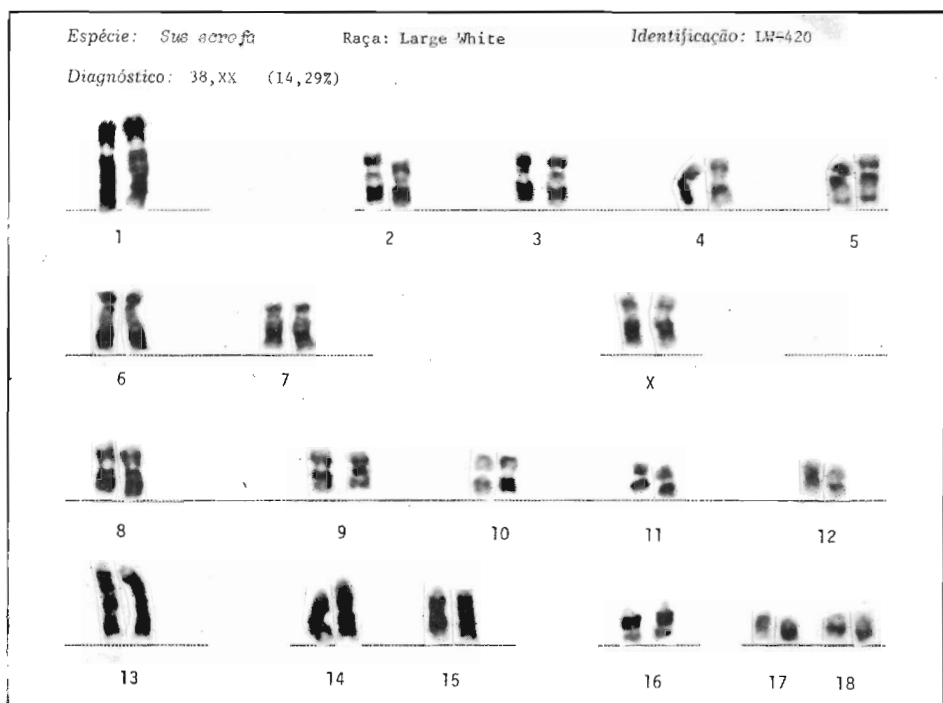


Figure 3 - Karyotype of the intersex no. 420. Large White. $2n = 38,XX$ (14,29%). G banding.

Table I - Karyotypic study of intersex swine classified as male pseudohermaphrodites.

Intersex No.	Breed Karyotype	Karyotype Father no. Mother no	Gonads	Inner genital tract	Outer genital tract
620	Landrace ^a	39 - NE 1127 - 38,XX	2 testes	Female	F*
339	Large White	918 - 38,XY 878 - 38,XX	2 testes	Female	F*
336	Large White	918 - 38,XY 878 - 38,XX	2 testes	Female	F*
420	Large White ^b	1144 - NE 620 - 38, XX	2 testes	c	F*
1446	Large White	588 - 38,XY 697 - 38,XX	2 testes	Female	F*

Continued

Table I - Continued.

Intersex No.	Breed Karyotype	Karyotype		Gonads	Inner genital tract	Outer genital tract
		Father no.	Mother no			
1447	Large White 38,XX	588 - 38,XY 697 - 38,XX		2 testes	Female	F*
1451	Large White 38,XX	588 - 38,XY 697 - 38,XX		2 testes	Female	F*
257	Large White 38,XX	323 - 38,XY 385 - 38,XX		2 testes	Female	F*
536	Large White 38,XX	323 - 38,XY 945 - 38,XX		2 testes	Female	F*
583	Large White 38,XX	41 - 38, XY 1385 - NE		2 testes	Female hypoplastic	F*
785	Large White 38,XX	580 - NE 196 - NE		2 testes	Female	F*
906	Large White 38,XX	186 - 38,XY 49 - NE		2 testes	Female hypoplastic	F*
955	Large White 38,XX	1516 - NE 1592 - 38,XX		2 testes	Female	F*
1065	Large White 38,XX	323 - 38,XY 1565 - 38,XX		2 testes	Female	F*

^a 38,XX, break in a chromosome of pair no.9.

^b 38,XX/38,XY, with a predominance of XY (85,71%).

^c Hypoplastic uterine remnant and vagina.

F*, with a hypertrophied clitoris; NE, not examined.

Table II - Karyotypic study of intersex swine classified as true hermaphrodites.

Intersex No.	Breed Karyotype	Karyotype		Gonads	Inner genital tract	Outer genital tract
		Father no.	Mother no			
512	Landrace 38,XX	217 - 38,XY 854 - 38,XX		1 ootestis 1 testis	Female	F*
1137	Landrace 38,XX	682 - 38,XY 271 - 38,XX		1 testis 1 ovary	Female	F*

Continued

Table II - Continued.

Intersex No.	Breed Karyotype	Karyotype		Gonads	Inner genital tract	Outer genital tract
		Father no.	Mother no			
1540	Large White 38,XX	580 - NE		1 testis	Female	F*
		699 - 38,XX		1 ovary		
201	Large White 38,XX	323 - 38,XY		1 testis	Female	F*
		889 - 38,XX		1 ovary		
242	Large White 38,XX	588 - 38,XY		1 testis	Female	F*
		962 - 38,XX		1 ovary		
538	Large White 38,XX	323 - 38,XY		1 ootestis	Female	F*
		945 - 38,XX		1 ovary		
566	Large White 38,XX	580 - NE		1 ovary	Female	F*
		899 - NE		1 ootestis		
288	Landrace 38,XX	682 - 38,XY		1 ovary	Female	F*
		1242 - NE		1 ootestis		
1042	Large White 38,XX	323 - 38,XY		2 ootestis	Female	F*
		384 - 38,XX				
1152	Large White 38,XX	918 - 38,XY		1 ootestis	Female	F*
		169 - 38,XX		1 testis		

F*, with a hypertrophied clitoris; NE, not examined.

Table III - Karyotypic study of intersex swine with morphologically normal outer genitalia.

Intersex No.	Breed Karyotype	Karyotype		Gonads	Inner genital tract	Outer genital tract
		Father no.	Mother no			
560	Large White 38,XX	580 NE		2 ovaries	Female	F**
		899 - NE				
1064	Large White 38,XX	323 - 38,XY		2 ovaries	Female	F**
		1565 - 38,XX				

F**, Slightly hypertrophied clitoris and upturned "parrot beak"-shaped vulva.

NE, not examined.

Table IV - Karyotypic study of intersex swine not submitted to histological or anatomical evaluation.

Intersex No.	Breed	Karyotype
		Father no. Mother no.
1440	Large White	323 - 38,XY
	38,XX	1509 - 38,XX
255	Large White	323 - 38,XY
	38,XX	385 - 38,XX
113	Landrace	579 - 38,XY
	38,XX	786 - 38,XX
121	Landrace	1283 - 38,XY
	38,XX	1010 - 38,XX
1287	Large White	913 - NE
	38,XX	330 - 38,XX
51	Large White	41 - 38,XY
	38,XX	1549 - 38,XX
139	Large White	186 - 38,XY
	38,XX	852 - 38,XX

NE, not examined.

On the basis of the sample studied, the intersexuality of these animals was mainly due to factors such as autosomal mutation, which have already been identified in swine together with chimerism or mosaicism and aneuploidies of the sex chromosomes. An evaluation of the probable type of inheritance was not possible because of insufficient genealogical data.

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RESUMO

Com o objetivo de esclarecer a etiologia da intersexualidade devido a fatores genéticos, foram examinados 33 suínos intersexuados e 33 de seus ascendentes. O estudo cariotípico foi feito com metáfases obtidas de culturas de linfócitos de sangue periférico e estas foram analisadas utilizando-se bandamento cromossômico (G e C) além de coloração convencional com Giemsa. Observou-se dois casos de anomalias cromossômicas. Um intersexuado apresentou o mosaicismo ou quimerismo 38,XX/38,XY e outro apresentou quebra em um cromossomo do par número 9, com cariótipo igual aos dos demais inter-

sexuados, ou seja, 38,XX. Todos os pais apresentaram os seus cariótipos normais, ou seja, 38,XX (fêmeas) e 38,XY (machos). Exames macro e microscópicos das genitálias foram realizados em 26 dos 33 intersexuados, sendo que 10 foram classificados como hermafroditas verdadeiros, 14 como pseudohermafroditas machos e 2 fêmeas morfológicamente normais exceto para uma leve hipertrofia do clitoris e vulva em "bico de papagaio".

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