Fertility in two cats with X-chromosome mosaicism and unilateral ovarian dysgenesis

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Summary. Two pregnant cats showed unilateral ovarian dysgenesis at surgery. Cytogenetic examination revealed a 37,X/39,XXX karyotype in Cat 1, a 37,X/38,XX karyotype in Cat 2 and a marked difference in frequency of mosaicism between fibroblasts and lymphocytes in both cats. Histologically the ovarian morphology ranged from normal to complete dysgenesis in both cats. Three fetuses examined showed a normal feline karyotype.

Introduction

X-chromosome monosomy is well recognized in man (Ford *et al.*, 1959) as well as in several other species including the domestic cat (Norby *et al.*, 1974; Long & Berepubo, 1980; Johnston *et al.*, 1983). The anomaly is associated with infertility due to ovarian dysgenesis in the species examined, except the mouse (Russell *et al.*, 1959) and some other species with a short generation interval. In mice with X-chromosome monosomy, reduced fertility is caused by germ-cell deficiency during fetal (Burgoyne & Baker, 1981) and postnatal (Lyon & Hawker, 1973; Burgoyne & Baker, 1985) life.

X/XX, X/XXX and X/XX/XXX mosaics are well known and mostly fertile in man (Grumbach & Conte, 1985). Such X-chromosome mosaicism has been reported in domestic animals for the horse (Chandley *et al.*, 1975) and the sheep (Baylis *et al.*, 1984). All reported cases were referred for cytogenetic investigation due to reduced fertility and the animals showed gonadal dysgenesis.

No case of X-chromosome mosaicism has been reported for the cat.

Materials and Methods

Animals. Two pregnant stray cats were presented to the veterinary clinic of an animal shelter for ovariohysterectomy. Both cats appeared to be young (i.e. less than 6–7 years) by the degree of wear of the teeth and general appearance. Radiography of Cat 1 showed closed epiphyseal lines indicating an age of more than 3 years. At pre-surgical physical examination both cats were found to be normal.

The coat colour of Cat 1 was predominantly orange–white, but 6 small tabby spots were present at the base of the tail. Cat 2 was tabby and white.

Lymphocyte cultures were processed by standard methods (Christensen & Pedersen, 1982). At 7 h before harvest 200 µg 5-bromo-2'-deoxyuridine/ml (Sigma Chemical Co., P.O. Box 14508, St Louis, MO, U.S.A.) and R-banding was carried out according to Dutrillaux *et al.* (1973). The karyotyping was done according to the recommendations of the Reading conference (Ford *et al.*, 1980).

Tissue culture. Primary culture of muscular and connective tissue was established from both pregnant cats and 3 fetuses from Cat 2 and processed by standard procedures (Freshney, 1983) before karyotype studies.

Histology. Samples were taken from the ovaries and uteri. The tissue was fixed in 4% phosphate-buffered formaldehyde and processed for histology. Sections of 5 µm were stained with haematoxylin–phloxin and eosin.

Results

Cat 1

Ovariohysterectomy revealed a uterus with a total of 4 fetuses, 2 in each uterine horn. The average crown–rump length of the fetuses was 50 mm, which corresponds to a stage of pregnancy of about 36 days (Christiansen, 1984). At the left ovarian ligament no ovarian tissue could be recognized macroscopically. Palpation of the ovarian ligament, however, revealed the presence of a small nodule, approximately 1 mm in diameter, at the ovarian site. The genital system was otherwise found to be normal. The right ovary was of normal size and corpora lutea were visible on the surface. In the abdominal cavity no abnormalities were found.

Table 1. Chromosome analysis of 2 fertile cats

Animal	Material	Chromosome number					Total cells counted	Diagnosis
		< 37	37	38	39	> 39		
Cat 1	Lymphocytes	0	100	0	0	0	100	37,X /39,XXX
	Fibroblasts	4	92	0	4	0	100	
Cat 2	Lymphocytes	0	6	44	0	0	50	37,X /38,XX
	Fibroblasts	0	0	25	0	0	25	
Fetus a	Fibroblasts	0	0	20	0	0	20	38,XX
Fetus b	Fibroblasts	0	0	20	0	0	20	38,XY
Fetus d	Fibroblasts	0	0	10	1	0	11	38,XY

The fetuses showed no congenital abnormalities. The fetal gonads were macroscopically normal. The results of cytogenetic analysis are presented in Table 1. In fibroblasts, 2 cell lines were found, the major line containing 37 chromosomes. R-banding consistently showed the missing chromosome to be an X (Fig. 1). All lymphocytes had 37 chromosomes and the missing chromosome was again shown to be an X. The minor cell line of the fibroblasts containing 39 chromosomes showed a triple X chromosome constitution (Fig. 2).

The right ovary contained corpora lutea which appeared histologically normal. Multiple follicles of different sizes were present. Almost all antral follicles were degenerating with a thin granulosa layer with pycnotic nuclei. Primordial follicles were not observed in the sections available.

Histological evaluation of the small nodule present at the left ovarian ligament revealed no ovarian structures. The nodule consisted of a network of small cell cords with 3–10 cells in cross sections embedded in connective tissue.

Cat 2

At ovariohysterectomy a 2 × 1 mm nodule of ovarian-like tissue was found at the right ovarian ligament whereas the left gonad was of normal size and appearance. The uterus contained 5 fetuses, 2 in each uterine horn and 1 in the uterine body. Three fetuses were males, two were females. They had an average crown–rump length of 90 mm and hair and claws were developed corresponding to a stage of pregnancy of about 47 days.

One fetus showed palatoschisis, the other 4 were normal. Gonads of the fetuses were macroscopically normal. The results of cytogenetic analysis of the mother and 3 fetuses are presented in

Fig. 1. R-banded karyotype of Cat 1 showing a 37,X chromosome complement. $\times 2700$.

Table 1. Two cell lines were present in lymphocytes of the mother, the major one had a normal 38,XX karyotype and the minor had the karyotype 37,X.

In fibroblasts, only 25 cells could be karyotyped, but all had a 38,XX chromosome constitution. All metaphase spreads of fibroblasts of fetuses examined showed a normal feline karyotype.

The left ovary appeared to be normal with many follicles and several corpora lutea. Most of the small antral follicles appeared to be healthy, whereas the larger ones were atretic with many pycnotic granulosa cells. Numerous primordial follicles occupied the cortical area.

The nodule present in the right ligament contained ovarian tissue. Several small antral and preantral healthy follicles were noticed, predominantly containing 2 oocytes of different sizes. No large follicles were present. Remnants of atretic follicles were scattered in the fibrous stroma, which often also contained hyalinized zonae pellucidae. There was no sign of corpora lutea or luteinized interstitial tissue. A few primordial follicles were observed.

Fig. 2. Giemsa stained karyotype of Cat 1 showing a 39,XXX chromosome complement. $\times 1300$.

Discussion

Whether the nodule present in the left ovarian ligament of Cat 1 is in fact the remnant of the ovary is uncertain. However, the network of small cell cords have some resemblance to cell cords possibly of mesonephric origin which may develop in ageing ovaries devoid of germ cells (Crume yrolle-Arias *et al.*, 1976). Follicles of the dysgenic ovary of Cat 2 were apparently not able to ovulate as neither large follicles nor any trace of luteinized tissue were present.

In addition to the findings already described Cat 1 appeared somewhat 'compressed' in stature. The mental capacity of this cat might also be impaired as the owner claims it to be unable to adapt properly to the cats of the household.

A cat with the karyotype 37,X and a tortoiseshell coat colour pattern strongly suggests the presence of a second cell line of different genetic constitution (Lyon, 1961). Such a cell line was shown in fibroblasts of Cat 1 which furthermore demonstrated that X-chromosome mosaicism may result in a lower frequency of certain colour patches than would be expected for the random process of X-chromosome inactivation generating the tortoiseshell coat colour pattern. This phenomenon may be helpful in detecting X-chromosome mosaicism of cat populations.

Otherwise, the unilateral ovarian dysgenesis and fertility of the two present cats with 37,X mosaicism are comparable to the findings in man including the need pointed out by Morishima & Grumbach (1968) to include more than one sort of tissue in cytogenetic investigations of cases of infertility. In previous studies domestic animals with X-chromosome mosaicism have always been infertile but this may simply reflect the fact that infertility is the main reason for cytogenetic investigation of these animals.

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