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Cytogenetic Diagnosis of a British Shorthair Tomcat with a 37,X/38,XY/39,XY+der(Y) Karyotype

Wiktoria KOWAL, Barbara KIJ-MITKA, Kamila BEDNAREK, Halina CERNOHORSKA, Svatava KUBICKOVA, and Monika BUGNO-PONIEWIERSKA

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Short communication

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In this study, the determination of three different cell lines in a two-year-old British shorthair tomcat, using fluorescence *in situ* hybridisation (FISH), is described. The FISH technique was instrumental in the identification of this chromosomal aberration, which had not been previously described in cats. The mosaic karyotype with three cell lines (37,X; 38,XY; and 39,XY+der(Y)), detected using X-, Y- and autosomal B1-whole chromosome painting probes, were all visible in the metaphase and interphase nuclei. The ratios of the three cell lines were 3.6%, 85.6% and 10.8%, respectively. In addition, at the time of this examination, the cat had a temperamental disposition that had persisted beyond castration.

Key words: cat, FISH, whole chromosome painting probes.

Wiktoria KOWAL, Barbara KIJ-MITKA, Kamila BEDNAREK, Monika BUGNO-PONIEWIERSKA, Department of Animal Reproduction, Anatomy and Genomics, University of Agriculture, Mickiewicza 24/28, 30-059, Kraków, Poland.*
E-mail: wiktoria.kowal@student.urk.edu.pl
Halina CERNOHORSKA, Svatava KUBICKOVA, Veterinary Research Institute, Hudcova 296/70, 621 00, Brno, Czech Republic.

Chromosomal abnormalities are classified as structural or numerical, with the latter divided into autosomal chromosome aneuploidy and sex chromosome aneuploidy, both of which are caused by abnormalities occurring in the course of cell division. Chromosomal aberration can result in mosaicism or chimerism, where an individual has two or more cell lines in the body (FORD 1969).

The most commonly described sex chromosome abnormalities in the cat include 39,XXY trisomy and mosaic variants such as XY/XXY and XY/XYY (MEYERS-WALLEN 2012). In the currently available literature on feline sex chromosomal aberrations, the most common cases involve tortoiseshell and calico males (SZCZERBAL *et al.* 2018). At present, most cases of chromosomal abnormalities are diagnosed with the use of fluorescence *in situ* hybridisation (FISH), in which fluorescently labelled probes are used to enable chromosome identification via the detection of specific chromosomal signals. The FISH technique is precise and allows for the identification of chromosomal aberrations, even in poor-quality metaphase spreads or interphase nuclei. In addition, FISH provides the ability to analyse a large number of metaphase spreads (BUGNO *et al.* 2003), meaning that the chromosome painting technique is an effective tool for a chromosomal analysis (BUGNO and SLOTA 2007).

A range of aberrations have been detected using FISH, including: testicular 38,XY (SRY-positive) disorder; 37,X monosomy; 38,XX (SRY-positive) disorder; 37,X/38,XY mosaicism; testicular 38,XX disorder; 37,X/38,X,r(Y) mosaicism; leucocyte 38,XX/XY chimerism; 39,XXY trisomy; and 38,XY/38,XY true chimerism (NOWACKA-WOSZUK *et al.* 2014; SZCZERBAL *et al.* 2015a; SZCZERBAL *et al.* 2015b; BALOGH *et al.* 2016; DE LORENZI *et al.* 2017; SZCZERBAL *et al.* 2017; SZCZERBAL *et al.* 2018; BUGNO-PONIEWIERSKA *et al.* 2020).

The aim of the present study was to perform a cytogenetic diagnosis of a British shorthair tomcat using

molecular painting probes for the X, Y and autosomal B1 chromosomes.

Materials and Methods

Ethical approval is not required in the case of work carried out on material obtained by a veterinarian for the purpose of routine blood tests, and as medical waste after a blood sample collection.

A two-year-old British shorthair tomcat was brought for a cytogenetic diagnosis at the owner's request. A blood sample for a routine lymphocyte culture was taken from the cephalic vein of the forearm by a veterinarian, and collected into a lithium heparin tube. The cat was already castrated at the time of the blood collection, but was still characterised by temperamental behaviour as well as a withdrawn and lonely disposition. No abnormalities were observed during the castration by the veterinarian. The male's reproductive organs were properly developed. His weight of about 6 kg was in the average range (4-9 kg).

The lymphocyte culture was prepared for a cytogenetic analysis using the method described by ARAKAKI and SPARKES (1963). A volume of 1 ml of peripheral blood was added to the culture medium (8 ml of RPMI 1640 solution with 1.5 ml of foetal bovine serum, penicillin – 100 IU/ml of culture medium and streptomycin – 0.1 mg/ml of culture medium), and 0.25 ml of pokeweed mitogen (Sigma Aldrich, Germany) was then added in a 10% concentration to the lymphocytes stimulation. The cells were incubated in an incubator at 38°C for 72 hours. Two hours before the end of the incubation, a colchicine solution (0.4 µg/ml of culture medium) was added. After 72 hours of incubation, the cells were centrifuged at 100 g for 10 min, then hypotonised in 0.05 mol/l of potassium chloride and incubated at 38°C for 20 minutes. The cells were subsequently centrifuged at 100 g for 10 min, and the supernatant was withdrawn. The suspension was fixed by washing the precipitate three times with 8 ml of methyl alcohol and glacial acetic acid (3:1), and was then centrifuged at 100 g for 10 min. The suspension was spotted on slides and dried at room temperature (RT). Slides were prepared for the metaphase spreads and to perform Giemsa-trypsin banding (GTG) with FISH on the same slide. The Giemsa-trypsin banding technique was used for the chromosome identification and validation of the molecular painting probes.

The cat chromosome X-, Y-, and B1-specific painting probes were prepared by laser microdissection (PALM Microlaser system, Carl Zeiss MicroImaging GmbH, Munich, Germany). The amplification of the microdissected DNA was performed using the GenomePlex® Whole Genome Amplification Kit (Sigma-Aldrich, St. Louis, MO, USA). Labelling of the cat-specific X chromosome probe with spectrum orange fluorochrome (Abbot, IL, USA), and of the B1-specific probe with spectrum red fluorochrome (Abbot, IL, USA) was performed using the GenomePlex® WGA Reamplification Kit (Sigma-Aldrich). Labelling of the Y-specific probe with the fluorochrome spectrum green (Abbot, IL, USA) was performed using a BioPrime™ Array CGH Genomic Labelling System (Invitrogen, USA), all performed according to the manufacturer's instructions.

Fluorescence *in situ* hybridisation (FISH)

FISH was performed as described by BUGNO *et al.* (2009). The prepared slides were processed by pepsin digestion for 10 minutes at 37°C, then washed in PBS (pH = 7.4) twice for 5 min at room temperature (RT), and washed in PBS with magnesium chloride for 5 min at RT. The spreads were then dehydrated by a passage through a sequential series of 70%, 80% and 90% alcohol, each for 3 min, at RT. The slides were dried at 80°C for 15 min, before a denaturation in 70% formamide at 70°C for 2.5 min, followed by a passage through a sequential series of 70%, 80% and 90% alcohol, each for 3 min, at -20°C. Simultaneously, the molecular painting probes were denatured for 10 min at 70°C and were then placed on ice. The slides were subsequently dried through the chilled alcohol series, and the X, Y and B1 painting probes were applied. The slides were hybridised overnight in a moist chamber at 37°C. On the next day, the slides were washed three times in 50% formamide (Sigma Aldrich, Germany) at 42°C for 3 min each, then three times in 2x SSC (Sigma Aldrich, Germany) at 42°C for 5 min each, and the coverslip was detached. Each slide was then washed in an equilibration buffer (400 ml H_2O, 100 ml 20x SSC and 250 µl Tween20) for 10 min at RT, followed by the application of DAPI (Cambio Biomibo, Warsaw, Poland) onto the wet slide, which was then covered with a coverslip.

Hybridisation signals were examined using an Axio Imager fluorescence microscope (Zeiss, Germany) equipped with a digital camera, driven by the ZenPro software. The filters used in the images were: DAPI, spectrum green, spectrum orange, and spectrum red with the pseudo colour violet.

Results

A cytogenetic analysis revealed the presence of 37, 38 and 39 chromosomes in the 139 metaphase spreads that were examined (Fig. 1). In the prevailing majority of the metaphase spreads (119), one signal of the FCAY and one signal of the FCAX were observed. Two signals of the FCAY (i.e. one for FCAY and one for der(Y)) were observed in 15 metaphase spreads (Fig. 3) and one signal of the FCAX was detected in 5 metaphase spreads (Fig. 2). The ratios of the three cell lines were 85.6%, 10.8% and 3.6%, respectively. A control sample showing the correctness of the molecular probes is presented in Figure 4.

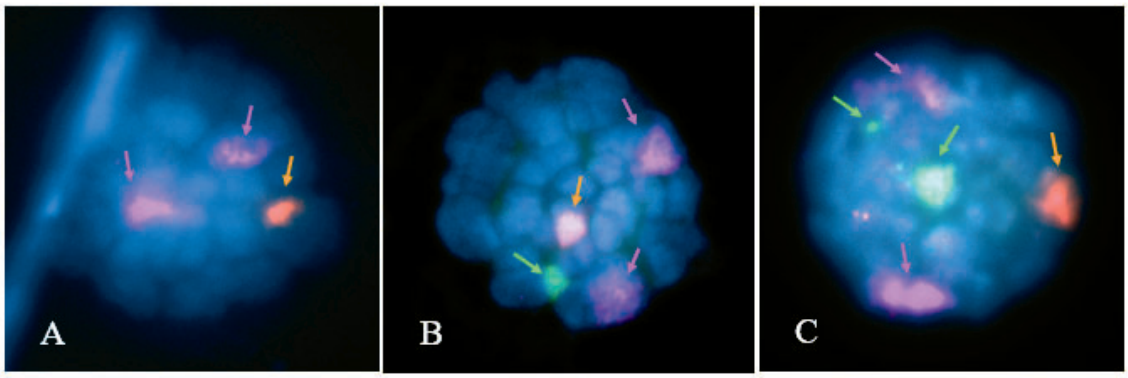

Fig. 1. FISH results using X (orange), Y (green) and B1 (violet) whole chromosome painting probes on the tomcat metaphase spreads: A – 37,X; B – 38,XY; C – 39,XY+der(Y).

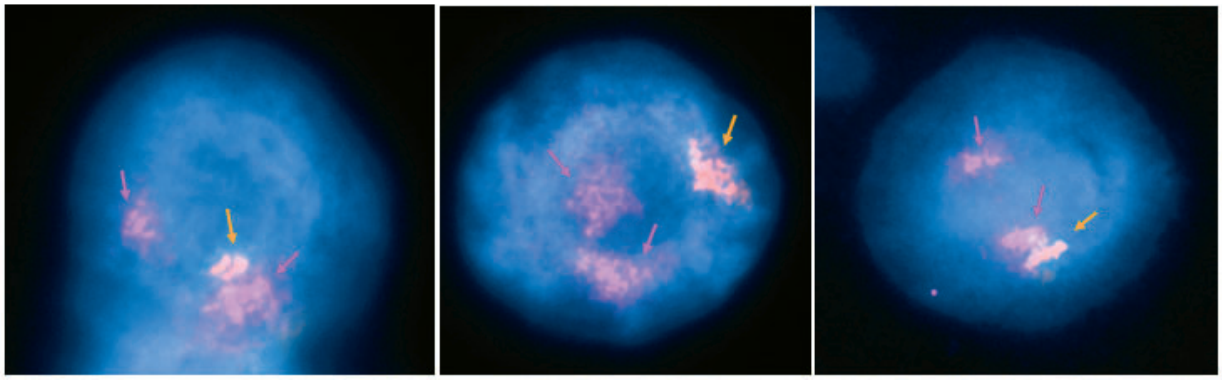

Fig. 2. FISH results on the tomcat 37,X cell line using X (orange), Y (green) and B1 (violet) whole chromosome painting probes showing two signals of the B1- and one signal of the X-specific probes, while none of the Y-specific probe (green) was detected.

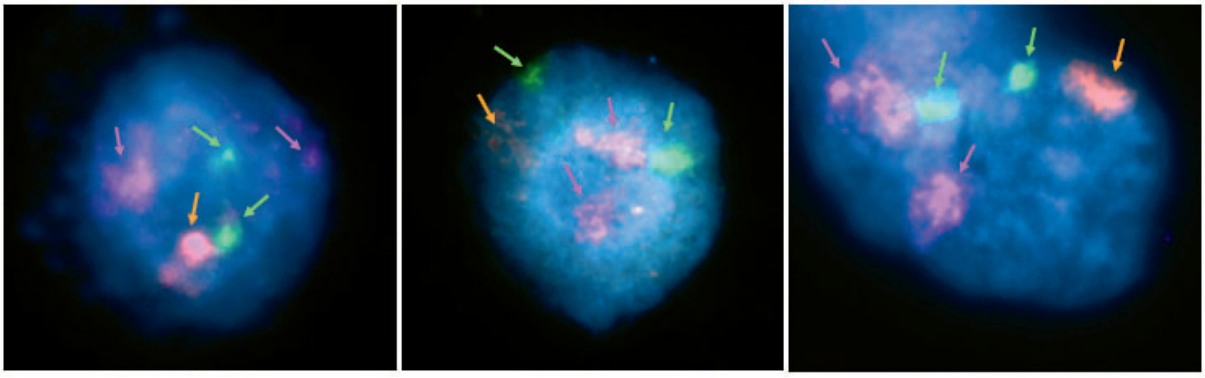

Fig. 3. FISH results on the tomcat 39,XY+der(Y) cell line using X (orange), Y (green) and B1 (violet) whole chromosome painting probes, showing two signals of the Y-, one signal of the X- and two signals of the B1-specific probes.

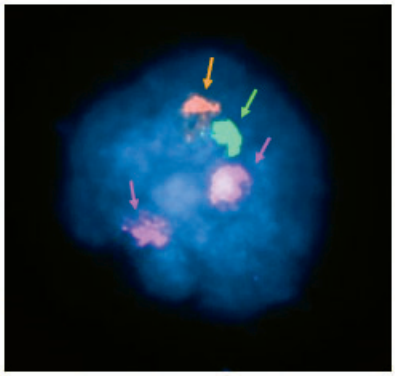

Fig. 4. FISH results on the tomcat with a normal 38,XY cell line using X (orange), Y (green) and B1 (violet) whole chromosome painting probes as an experiment control.

Discussion

Chromosomal abnormalities related to disorders of sex development (DSD), especially sex chromosome aneuploidy, can lead to infertility, behavioural changes or an increased risk of gonadal carcinogenesis (RAUDSEPP and CHOWDHARY 2016). Furthermore, some individuals with DSD have been reported to exhibit unusual behaviour (SZCZERBAL and ŚWITOŃSKI 2020). A few other abnormalities, as described below, have shown a similar correlation between chromosomal and physical changes.

In a study of four 38,XY cats with rudimentary penises and testicular disorder (NOWACKA-WOSZUK *et al*. 2014), testes with normal spermatogenesis were reported in two cases, testes without spermatozoa in one case, and testes with only Sertoli cells in the seminiferous tubules in the fourth.

LEAMAN (1999) described the cytogenetic analysis of 11 tortoiseshell cats, of which six had a 38,XX/38,XY complement of chromosomes, identified as blood chimeras. Some of these chimeras had testes with spermatogenesis in some areas and empty seminiferous tubules in others (as confirmed by a histological analysis), while another animal had one gonad as an ovo-testis, and yet another had seminiferous tubules without spermatogenesis. Two of the eleven tortoiseshell cats had a 39,XXY complement of chromosomes identified as Klinefelter syndrome with the typical histological characteristics, while the remaining three had a normal chromosome complement.

Another true chimerism 38,XY/38,XY in a tomcat was discovered by BUGNO-PONIEWIERSKA *et al.* (2020) and was confirmed by genetic analyses. In this case, the cat had properly developed male reproductive organs, was fertile and the tortoiseshell coat colour was predominantly orange with only a few small patches of black. In our study, the male also had properly developed reproductive organs. No effects of a chromosomal aberration were observed.

A similar case was reported by PEDERSEN *et al*. (2014), in which a tortoiseshell tomcat with a 39,XXY karyotype was found to have a normal body size, but testes of a seemingly smaller size than usual for its age. However, the phenotypical characteristics of a tortoiseshell cat can be highly variable.

Cases of feline mosaicism have also been previously noted. BALOGH *et al.* (2016) described the case of a Bengal tomcat with a 37,X/38,XY karyotype and tubular structures, which were identified as Müllerian duct remnants, as well as cryptorchidism. The tomcat also had behavioural problems, which were similar to the unusual behaviour observed in our study.

A similar case involving a European shorthair cat with a 37,X/38,X,r(Y) karyotype showed the presence of two cell lines, with 90% of the cells having 37,X chromosomes and 10% with 38,X chromosomes and an abnormally small Y-derived chromosome (SZCZERBAL *et al.* 2017).

In our study, we identified an aberration that had not been previously described in cats, although a similar karyotype, 63,X/64,XX/65,XX,del(Y)(q?), was described in a stallion that was diagnosed with testicles in the abdominal cavity (BUGNO *et al.* 2008).

The tomcat described in this study was previously castrated. Based on an interview with the cat's owner, the impetus for requesting diagnostic tests was related to the cat's temperamental behaviour, and no changes in the cat's sexual organs had been observed.

Conclusions

The use of the FISH technique allowed for the diagnosis of a Y chromosome aberration that had not been previously described in cats. By providing the ability to quantify the number of copies of fluorescent signals, even in the interphase nuclei, this technique supported the analysis of a large number of cells.

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Author Contributions

Research concept and design: B.K.-M., M.B.-P.; Collection and/or assembly of data: W.K., B.K.-M., K.B., H.C., S.K.; Data analysis and interpretation: W.K., B.K.-M., K.B., M.B.-P.; Writing the article: W.K., B.K.-M., H.C., S.K., M.B.-P.; Critical revision of the article: W.K., B.K.-M., H.C., S.K., M.B.-P.; Final approval of article: M.B.-P.

Conflict of Interest

The authors declare no conflict of interest.

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