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## **Cytogenetic Analysis in Domestic Cats**

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### **Abstract**

The present study comprised of 25 blood samples from domestic cats were cultured in duplicates for comparing efficacy of two mitogens i.e. Concanvalin A and Lectin. Concanvalin A was found to be significantly superior to Lectin. GTG- banding was used for preparation of domestic cat karyograms. Diploid number of chromosomes in domestic cats were found to  $2n = 38$  consisting of 18 pairs of autosomes and 1 pair of sex chromosomes. The 18 pairs of autosomes were classified into 5 groups as A,B,C,D,E, and F consisting of autosome pairs as Large submetacentric 3 pairs, Large subtolocentrics 4 pairs, Large metacentrics 2 pairs, Small submetacentric and subtolocentrics 4 pairs, Small metacentrics 2 pairs and Telocentric or acrocentric 2 pairs, respectively. Whereas 'X' chromosome was found to be medium sized subtelomeric and 'Y' chromosome was small areocentric. No major significant abnormalities were reported in the present investigation.

**Keywords:** Cat; Chromosomes; GTG banding; Cytogenetics; mitogen efficacy.

### **1. Introduction**

In India, application of cytogenetics, especially in pets like cats and dogs is uncommon, unlike developed countries.

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In metropolitan cities like Mumbai, dog and cat population as a companion is increasing day by day and their breeding is also carried out as a commercial enterprise, where purebred males are kept for breeding. Cytogenetic investigations in cat breeding will help to detect abnormalities at an earlier stage, so that a particular tom or queen can be prevented from breeding to control genetic disorders. Though cytogenetic research in domestic cat is attempted worldwide, no literature has been found encountered from Indian domestic cats. Hence, in the present investigation of 25 domestic cats comprised of 20 Non-descript, one Persian, one Bobtail and three Bombay cats were carried out.

## **2. Material and Methods**

Total 25 blood samples were collected from various private pet clinics from Mumbai. Four ml of whole blood was collected aseptically from femoral vein of 25 domestic cats into standard vacutainers with net content 170 I.U. of Lithium heparin as blood anti-coagulate and silicon lubricated stopper. All the domestic cats used for experiment were apparently healthy and normal. The basic short term lymphocyte culture technique [9] with few modifications was adopted for lymphocyte culture of domestic cats. The cultures were set up in duplicates. About 7.5 ml of medium (RPMI 1640) was transferred aseptically to each pre-sterile centrifuge tubes of 15 ml capacity. About 2 ml (20 per cent of culture) of foetal bovine serum (FBS, GIBCO) was added to provide some essential nutrients. Two mitogens (15 -20 per cent of culture) were used to study efficacy of mitogens for cat chromosomes. 80 µl of Concanavalin A (SIGMA) and 80 µl of Lectin (SIGMA) as a mitogenes were added. Addition of mitogen helps to stimulate mitosis process. Aseptically 80 µl of Penicillin and Streptomycin combination solution (SIGMA) and 80 µl of Heparine (anticoagulant) was added. About 0.5 ml of blood was added to each centrifuge tube. The contents of the tubes were mixed thoroughly and caps of these tubes were made tight. The cultures were then incubated at 37°C for a period of 72 hours. The cultures were gently agitated twice a day during the incubation. Two hours prior to harvesting of cultures, 50 µl of metaphase arresting agent Colchicine (0.2 µg / ml) (SIGMA) was added in each centrifuge tube. Then the cultures were again kept for incubation at 37°C for remaining two hours. The centrifuge tubes were removed from the incubator after two hours of incubation, mixed and centrifuged at 1200 G for eight minutes. The supernatant was discarded carefully and 8 ml of pre-warmed hypotonic solution (0.56 per cent or 0.075 M KCl) was added in each tube and these tubes were kept in water bath for 20 minutes at 37°C. Hypotonic treatment was terminated by adding 2 ml of freshly prepared chilled fixative (Methanol : Glacial acetic acid 3:1) solution for pre-fixative treatment. Tubes were mixed and centrifuged at 1000 G for ten minutes. The supernatant was discarded and cells were re-suspended in 8 ml chilled fixative, again and centrifuged at 1000 G for 10 minutes and supernatant was again discarded carefully. Two such chilled fixative washes were given to obtain milky white cell pellets and then the tubes were stored overnight in the refrigerator at 4°C. Before preparation of slides fresh fixative wash was given to all the cell pellets. Two – three drops of cell suspension was dropped on clean, chilled slides held at an angle of 30° from the height of two feet. The labeling of slides was done. After proper drying, conventional Giemsa staining was done.

### **2.1 GTG banding**

Freshly dropped slides were allowed to age for five days at room temperature. After ageing, slides were

immersed in 0.025 percent fresh trypsin (1:250, GIBCO) for 40 seconds and quickly rinsed with PBS (pH 6.8). Finally slides were stained for 9 minutes in 10 per cent Giemsa stain in PBS. The slides were rinsed thoroughly in distilled water thrice and kept for air drying and finally mounted with DPX.

### 2.2 Screening and analysis

Stained slides were screened and analysed under Olympus BX 61 Photomicroscope using Cytovision software. Modal chromosome number and mitotic drives and mitotic indices were calculated from each slide. Karyotypes were prepared on the basis of “Standard karyotype of cat as recommended in the first international conference for standardization of banded karyotypes of domestic animals” (Ford and his colleagues 1980).

## 3. Results and Discussion

### 3.1 Comparison of the efficiency of different mitogens

The objective of the technique was to obtain good quality metaphase spreads and to evaluate the mitogen based on yield of mitotic figures. Mitotic drives and Mitotic indices were calculated and subjected to student’s t-test. The two mitogens differed significantly ( $P < 0.05$ ) for both, mitotic drives and mitotic indices for domestic cat lymphocyte culture. Concanvalin A was found to be significantly superior to Lectin.

### 3.2 Modal Chromosome Number and Morphology

The modal chromosome number was found to be  $2n = 38$  in both the sexes. The number and morphology were same in male and female cats except that of the sex chromosomes. There were 18 pairs of autosomes and one pair of sex chromosome. The cat chromosomes were classified into six groups of autosomes and one group of sex chromosome as per standard classification [6]. ‘X’ chromosome was found to be medium sized subtelomeric where as ‘Y’ chromosome was small areocentric.

**Table No.1:** Classification of cat autosomes

Group	Morphology	Number of pairs
Group A	Large submetacentrics	3 pairs
Group B	Large subtelocentrics	4 pairs
Group C	Large metacentrics	2 pairs
Group D	Small submetacentric and subtelocentrics	4 pairs
Group E	Small metacentrics.	3 pairs
Group F	Telocentric or acrocentric	2 pairs

### 3.3 GTG Banding

Best GTG banded fields were captured and karyotypes were prepared manually by using Cytovision software .



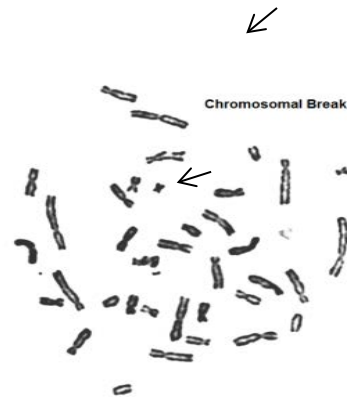
**Figure 1:** GTG-banded karyotype of domestic male cat



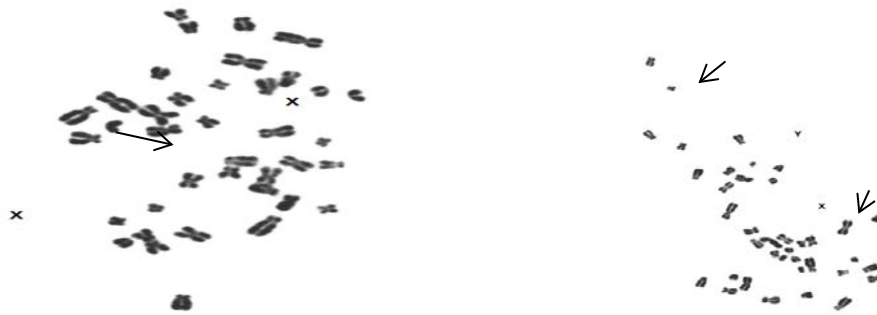
**Figure 2:** GTG-banded karyotype of domestic female cat

### ***3.4 Chromosomal abnormalities***

No major significant chromosomal abnormalities were encountered in domestic cats studied under the present investigation. Sporadic metaphases of three domestic female cats showed chromosomal breaks. All these cats were phenotypically normal and healthy. Chromosome analysis from peripheral blood of one tri-coloured cat revealed the presence of 38, XX / 38, XY; chromosomes complement with predominance of female cells. The mixoploid cell population ratio XX : XY in percentages was found to be 64 : 36. This cat was phenotypically normal. However, the cat had been neutered prior to cytogenetic investigations hence no information was available on her reproductive status.



**Figure 3:** Chromosomal break



**Figure 4:** Metaphase of Chimeric tri-colored cat showing 38, XX and 38 XY

#### 4. Conclusions

X' chromosome was found to be medium sized subteleric and 'Y' chromosome was small areocentric. No major significant abnormalities were reported in the present investigation.

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