

SISTER CHROMATID EXCHANGES IN RIVER BUFFALO FEMALES WITH CHROMOSOMAL FRAGILITY

BENZI SCE LA FEMELE DE BIVOL CU FRAGILITATE CROMOZOMALA

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Sister chromatid exchange (SCE) technique is considered a very important test to check potential damage to the DNA, expressed by chromosomal fragility and associated with high rates of SCEs. In the present study we report the preliminary results of our investigation on chromosomal fragility identified in buffalo chromosomes and the relationship with the increased SCE levels. The karyotype analyses carried out on a group of 16 river buffalo females selected and treated for hormonal stimulation and on a control group of 16 normal river buffalo females revealed that three of the treated females were found to carry a higher number of breakages/cell comparative with the control group. The mean number of SCE/cell in the three treated females was higher ($\bar{X}=11.8$) than that of those observed in the control group ($\bar{X}=7.8$).

Keywords: buffaloes, chromosomes, sister chromatid exchanges

Materials and Methods

Karyotype analyses were performed on a group of 16 river buffalo females selected and treated for hormonal stimulation and on a control group of 16 river buffalo females. Peripheral blood lymphocytes were cultured for about 72 hours at 38,5 ° C in Minimal Essential Medium (Sigma) supplemented with 15 per cent fetal bovine serum (Sigma) and Concanavalin A as mitogen. Two types of cell cultures were performed: without (normal cultures) and with

addition of 5-bromodeoxyuridine (BrdU) during the last two cell cycles for the SCE test. Slides from both cultures were stained with acridine orange. At least 30 metaphase plates per animal were studied under a fluorescence Aristoplan Leitz microscope, captured with a Photometrics Cool Snap camera, transferred on PC and processed by a specific image software. To our knowledge this is the first investigation of SCE in Romanian river buffalo chromosomes.

Results and Discussion

The cytogenetic investigation of the two group of buffalo females revealed normal karyotype, $2n=50,XX$ (fig.1) for 29 females.

In the group of the 16 buffalo females treated for hormonal stimulation were identified three females with abnormal chromosomal configurations expressed by a higher percentage of abnormal cells (gaps, chromatid breaks, chromosome breaks and fragments) than the females from the control group. The female 1 (F1) presented in the examined mitotic figures (fig.2) between 2 and 12 repeated mono- and bichromatidic breaks on the metacentric, acrocentric and sex chromosomes. At female 7 (F7), the number of chromosomal breaks varied from 4 to 15/cell (fig.3). The female 9 (F9), had a chromosomal complement (fig.4) with a wide variety of chromosomal breaks: from mono- and bichromatidic breaks on autosomes and heterosomes till the lost chromosomal fragments (fig.5). The cytogenetic diagnosis for all three females was chromosomal fragility. The fragility of chromosomes and their relation with chromosome rearrangements were carried out in the main livestock species (DiBerardino,1979; DiBerardino,1983, Di Meo,1993, 2000; Ciotola,2005, Peretti, 2006). There are several reports in which chromosomal fragility have been associated with the effect of teratogenic agents (Llambi,1998;Llambi, 1994; Postiglioni,1996; Iannuzzi, 2004, Perucatti,2006; Peretti, 2007; Peretti,2008). In order to evaluate the effect of different toxic agents on genetic material integrity the SCE test has been applied. We found significant differences between the normal females and the three females with chromosomal fragility. A number of 13-15 SCE/cell in female 1 (fig.6), 11-17 SCE/cell (fig.7) in female 7 and 13-15 SCE/cell (fig.8a,b) in female 9 were observed. The mean number of SCE/cell in the three treated females was higher ($\bar{X}=11.8$) than that of those observed in the control group ($\bar{X}=7.8$).

These results suggest that the chromosomal fragility reported in this work are characterized by a high rate of SCEs and could be related with the hormonal treatment or the presence of different environmental toxic agents. For this reason we intend to perform chemical analyses of soil, forages and water from the animal provenance area, the chemical analyses of milk and blood from the investigated animals and also to continue our investigation to a larger samples of animals.

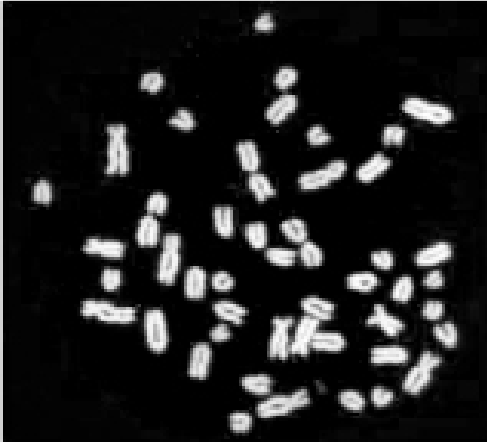


Fig.1 Normal karyotype : $2n=50,XX$

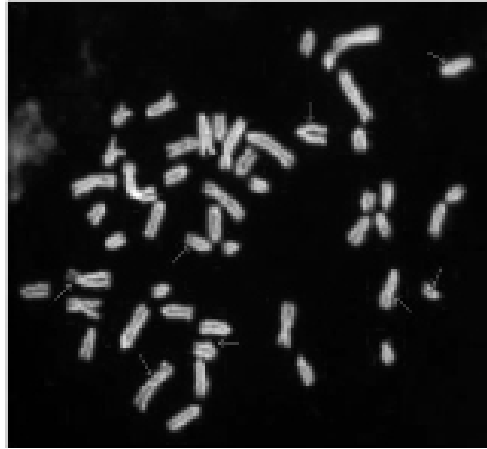


Fig.2 Buffalo female 1 metaphase spread



Fig.3 Buffalo female 7 metaphase spread

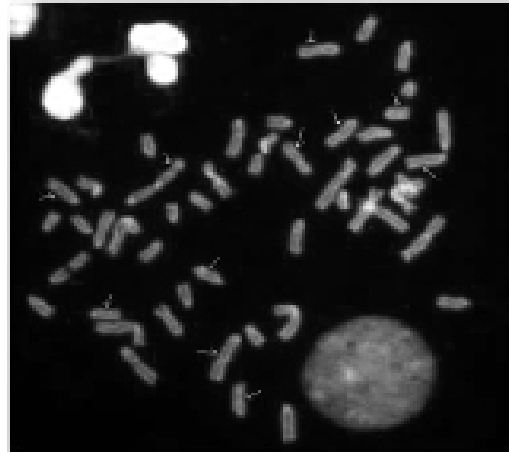


Fig.4 Buffalo female 9 metaphase spread

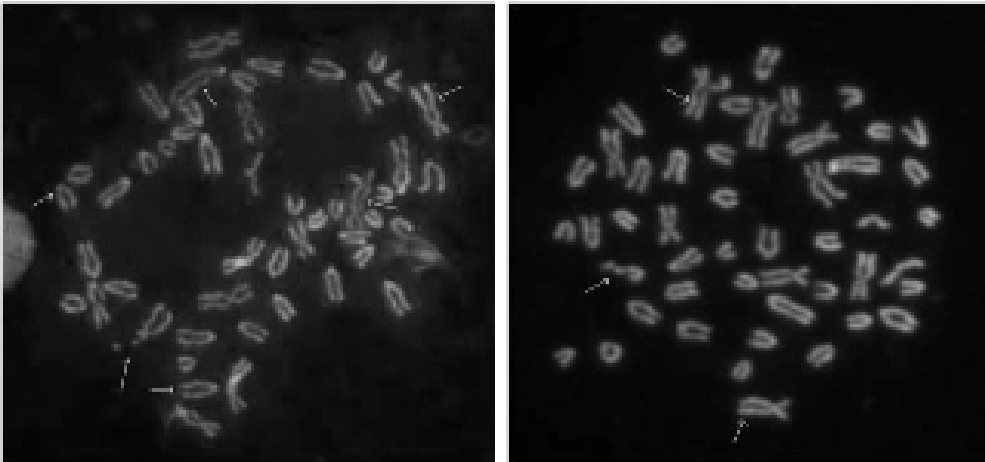


Fig.5 Buffalo female 9 metaphase spreads with lost fragments

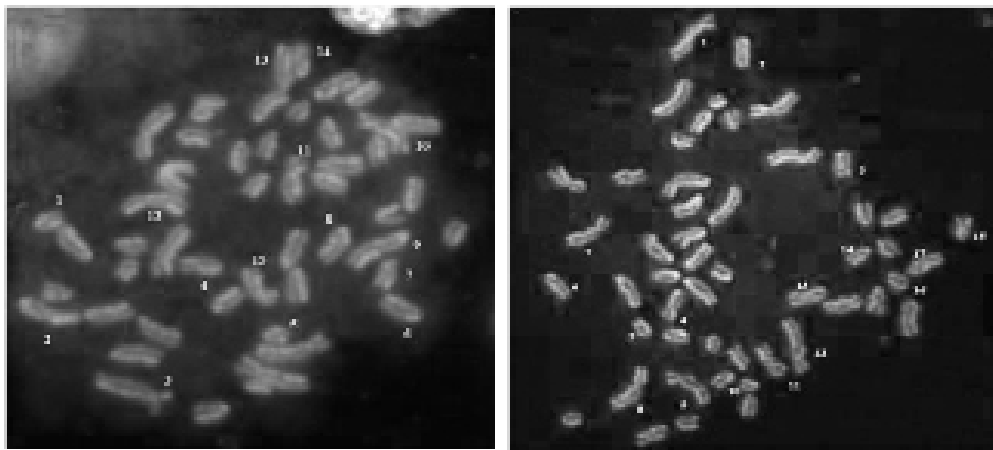


Fig.6 SCEs in metaphase spread of F1

Fig.7 SCEs in metaphase spread of F7

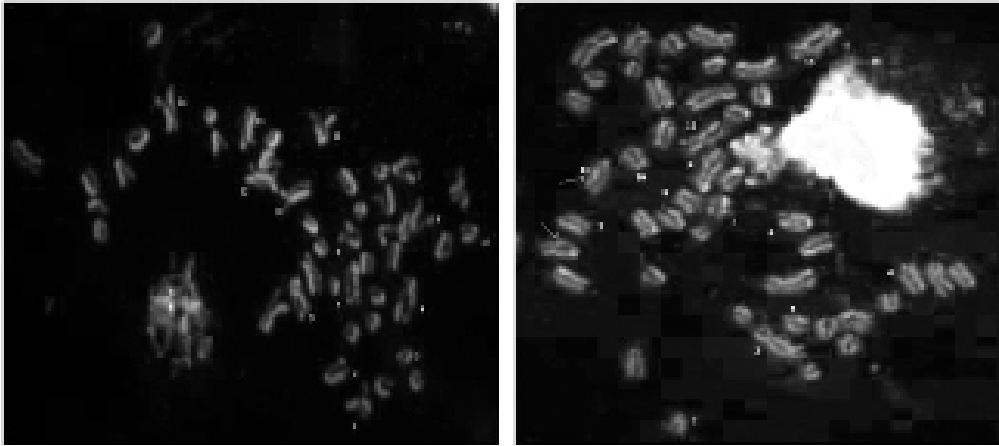


Fig.8 SCEs in metaphase spreads of female 9

Conclusions

In the present study we report the preliminary results of our investigation as significant increases of SCEs found in the females with chromosome fragility expressed by many gaps, breaks and fragments.

Although it is difficult to establish whether the high chromosomal fragility we found is related to the effect of toxic agents, this study shows that animals may be used as biological indicators of environmental pollution by using cytogenetic tests, such as those we applied. This also can give us a measure of the biological effects of the environment pollutants on animals.

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