THE EFFECTS OF ENVIRONMENTAL POLLUTION ON GENETIC MATERIAL INTEGRITY IN BUFFALOES

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Abstract

This paper shows that animals may be used as biological indicators of environmental pollution by using cytogenetic tests, such as those we applied to buffaloes. In the present study we report the preliminary results of our investigation concerning the increases of SCEs found in the buffalo females with chromosome fragility expressed by many gaps, breaks and fragments. In order to give a measure of the biological effect of pollutants on genetic material integrity, the SCEs test has been applied. The cytogenetic investigation carried out on a group of 14 subfertile females presented a higher percentage of abnormal cells (gaps, chromatid breakages, chromosome breaks and fragments) comparative with the normal females. We found significant differences between the normal females and the females with chromosomal fragility. The number of SCEs/cell varied from 11 to 17 and even 21 in some cells. The mean number of SCEs/cell in the 14 subfertile females was higher ($\bar{X}$ =13.9) than those observed in the control group ($\bar{X}$ =7.3). The results of chemical analyses of forages used for animal feeding revealed the presence of aflatoxin at higher doses (100,36ppb) then permitted (≤4,0ppb). Although it is still difficult to establish whether the high chromosomal fragility we found is related to the effect of aflatoxin from forages, we must take into consideration that some of toxicity effects occurs at cellular level and it is well known that aflatoxin causes DNA changes, cell deregulation, cellular changes and death.

Key words: environmental pollution, chromosome fragility, buffaloes

INTRODUCTION

As well as the reproductive performance is the most important characteristic of the domestic animals, during the last five years, a longstanding interest was dedicated to cytogenetic investigations in *river buffalo* females. The presence, localization and frequencies of breakages and achromatic gaps could be used for testing chromosomal instability and for determination of their linkage with physiological and economic traits. The current challenge is to understand the mechanism of this instability and its biological significance. The following report presents the preliminary results of our observations concerning the chromosomal fragility of subfertile buffalo females and the relationship with the increased SCEs level and the chemical analyses of forages used for animal feeding.

MATERIAL AND METHOD

Karyotype analyses were performed on a group of 60 river buffalo females raised in the farm of the Romanian Research and Development Station for Buffalo Breeding Sercaia. 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-bromodeuxiridine (BrdU) during the last two cell cycles for the SCEs 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. Chemical analyses of forages used for animal feeding carried out.

RESULTS AND DISCUSSIONS

The cytogenetic investigation of the 60 buffalo females reavealed normal karyotype, 2n=50,XX for 46 females.

In the group of the 14 buffalo females treated for reproductive disturbances the chromosomal configurations presented a higher percentage of abnormal cells (gaps, chromatid breakages, chromosome breaks and fragments) comparative with the 46 normal females. The subfertile females presented in the examined mitotic cells a chromosomal complement with a wide variety of chromosomal breakages: from mono- and bichromatidic breaks on autosomes and heterosomes till the lost chromosomal fragments (fig. 1). The number of chromosomal breakages varied from 4 to 15/cell. The cytogenetic diagnosis for all 14 females was *chromosomal instability*. The fragility of chromosomes and their relation with chromosome rearrangements were carried out in the main livestock species [1,2,3,4,10]. There are several reports in which chromosomal fragility have been associated with the effect of teratogenic agents [5,6,7,10,12,13]. In order to evaluate the effect of different toxic agents on genetic material integrity the SCEs test has been applied. We found significant differences between the normal females and the 14 females with chromosomal fragility. The number of SCE/cell varied from 11 to 17 and even 21 in some cells (fig. 2). The mean number of SCE/cell in the 14 subfertile females was higher ($\overline{X}$=13.9) than that of those observed in the control group ($\overline{X}$=7.3).

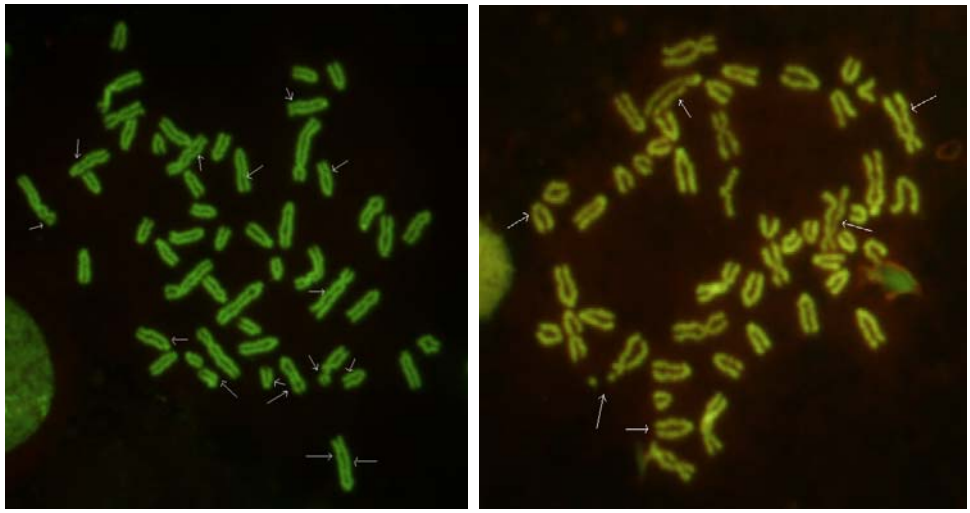

Fig.1 Buffalo female metaphase spreads with many breakages and lost fragments indicated by arrows

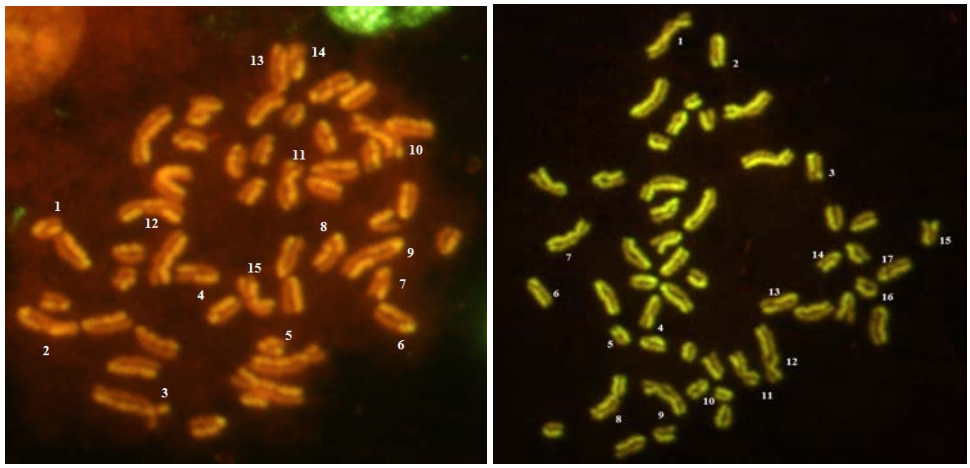

Fig. 2 Buffalo female metaphase spreads (2n=XX) treated with BrdU and stained with acridine orange. SCSs are indicated by numbers

These results suggest that the chromosomal fragility identified at the females with reproductive disturbances are characterized by a high rate of SCEs and could be related with the presence of different environmental toxic agents. For this reason we performed chemical analyses of forages from the animal provenance area. The results(table1) revealed the presence of aflatoxin at higher doses (100,36ppb) then permitted (≤4,0ppb)

Table 1 The results of chemical analyses of forages used for animal feeding

Sample/Element	Pb (ppm)	Cd (ppm)	Se (ppm)	Aflatoxin (ppb)	Ochratoxin (ppb)
Fân de baltă *Marsh hay*	0,57	0,04	**0,01**	6,37	2,01
Rogoz *Sedge*	0,73	0,05	**0,013**	**100,36**	0,163
Mălai furajer *Fodder maize*	0,27	0,03	**0,030**	3,14	1,05
Paie triticale *Triticale strow*	0,77	0,05	**0,020**	**8,36**	1,59
Limita maximă admisă *Maximum permissible limit*	**<10**	**<0,05**	**0,15-0,30**	**<4,0**	**<5,0**

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 still difficult to establish whether the high chromosomal fragility we found is related to the effect of aflatoxin from forages, we must take into consideration that some of toxicity effects occurs at cellular level and it is well known that aflatoxin causes DNA changes, cell deregulation, cellular changes and death.

For this reason we intend to perform chemical analyses of soil 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.

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