DNA polymerase alpha inhibition by aphidicolin and fragile site expression in prometaphase chromosomes of the Italian Mediterranean River Buffalo (*Bubalus bubalis*, 2n=50)

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ABSTRACT: The present study reports on the expression and localization of "fragile sites" (FS) on prometaphase chromosomes of two groups of river buffaloes (*Bubalus bubalis*, 2n=50; Mediterranean Italian breed), reared in two different farms, with the aim to characterize chromosome fragility in this species. Totally, 400 aphidicolin induced breakages were identified and localized on the standardized ideogram of the river buffalo karyotype. Preliminary results can be synthesized as follows: (a) aphidicolin showed a remarkable decondensing effect on chromosome structure, enabling further studies at high resolution level; (b) the chromosomal expression of the breakages was not different in the two groups of animals; (c) the most fragile chromosomes were the inactive-X, chromosomes 9, 8 and active-X, showing 42, 32, 31 and 30 breakages, respectively; (d) the breaks were localized in the RBG-negative bands (corresponding to eterochromatic regions) or at the band-interband regions; (e) the chromosomal distribution of the break sites was not random and only partially related to chromosome length. The study is in progress to determine the relative incidence of the fragile sites at chromosomal band level, in order to construct a 'fragile-site map' of river buffalo, which could be utilized for genetic improvement programs of the species.

Key words: Genetic diversity, *Bubalus bubalis*, Fragile sites, Chromosomes.

INTRODUCTION - Fragile sites are specific loci that show gaps, breaks or rearrangements in metaphase chromosomes when cells are cultured under conditions that inhibit DNA replication. They are late replicating, evolutionarily conserved, 'hot spots' for increased sister chromatid exchanges (SCEs), for translocations and deletions, preferred sites for genetic recombination, viral integration and carcinogenesis. A cospicuous bulk of research has been conducted in humans while domestic animals have received only little attention. Cattle is the most investigated species (Rodriguez *et al.*,2002), followed by pig (Ronne,1995), rabbit (Paulsen and Ronne,1991) and horse (Ronne,1992). In river buffalo, Balakrishnan *et al.*(1988) reported gaps on the X chromosome of anestrous females, suggestive of a fragile site, whereas Pires *et al.*(1998) found fragile sites on the X chromosome in three breeds of river buffalo, but -so far- no detailed information is available on the fragile site expression in the karyotype of this

species. This study was undertaken to provide basic cytogenetic information on the distribution of the break sites within the banded karyotype of river buffalo (*Bubalus bubalis*, 2n=50), which has been quite extensively characterized (Di Berardino *et al.*, 1981; Di Berardino and Innuzzi, 1984) and standardized (Iannuzzi, 1994).

MATERIAL AND METHODS - *Cell cultures*: two groups of four clinically healthy river buffaloes (two males and two females) of the Italian Mediterranean breed , reared in two farms located in the provinces of Naples (group A) and Salerno (group B), were used for the investigation. Conventional lymphocyte cultures were performed; after 48 hours of growth, Aphidicolin (Sigma) was added to the cultures at 0.15 mM (final concentration), as recommended by Rodriguez *et al.*(1990), for other 24 hours; 6 hours before the end, BrdU + H33258 (20 µg/ml, f.c. each) was added to the cultures for labelling late replication regions of the genome. The slides were treated for RBG-banding, stained with Giemsa and examined under bright field optics. Only metaphases with clear RBG-banding and -at least- one clear break site were considered. A total of 400 break sites was scored, 50 for each animal, and localized on the standardized ideogram of the river buffalo (Iannuzzi, 1994). *Statistical analysis*: The break site index (BSI) was established as the ratio between the total number of breaks scored (50 per animal, 200 per group) and the total number of metaphases analyzed. To classify fragile site frequency (low, medium, high) we divided the total number of FS scored (400) for the haploid number of river buffalo chromosomes (26), obtaining a 'mean' value of 15. The statistical analysis was performed using the ANOVA and the Chi-square test for the differences between the two groups of animals. Pearson correlation test was used for evaluating possible relationships between chromosome length and yield of FS.

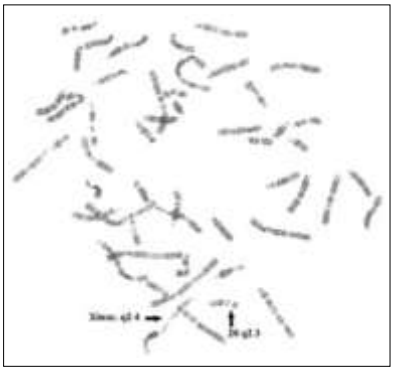
Table 1. Break sites distribution (N, %) on individual chromosomes according to the farm (A-B) and gender.

Chrom.	Group A N(%)	Group B N(%)	Males N(%)	Females N(%)	Total N(%)
1	8(4.0)	18(9.0)	12(6.0)	14(7.0)	26(6.5)
2	6(3.0)	12(6.0)	8(4.0)	10(5.0)	18(4.5)
3	4(2.0)	4(2.0)	7(3.5)	1(0.5)	8(2.0)
4	6(3.0)	3(1.5)	6(3.0)	3(1.5)	9(2.2)
5	4(2.0)	2(1.0)	2(1.0)	4(2.0)	6(1.5)
6	3(1.5)	1(0.5)	4(2.0)	0	4(1.0)
7	10(5.0)	13(6.5)	13(6.5)	10(5.0)	23(5.7)
8	16(8.0)	15(7.5)	18(9.0)	13(6.5)	31(7.7)
9	21(10.5)	11(5.5)	19(9.5)	13(6.5)	32(8.0)
10	12(6.0)	9(4.5)	10(5.0)	11(5.5)	21(5.2)
11	1(0.5)	3(1.5)	2(1.0)	2(1.0)	4(1.0)
12	6(3.0)	7(3.5)	7(3.5)	6(3.0)	13(3.2)
13	12(6.0)	12(6.0)	12(6.0)	12(6.0)	24(6.0)
14	3(1.5)	3(1.5)	4(2.0)	2(1.0)	6(1.5)
15	11(5.5)	5(2.5)	11(5.5)	5(2.5)	16(4.0)
16	2(1.0)	4(2.0)	3(1.5)	3(1.5)	6(1.5)
17	10(5.0)	6(3.0)	10(5.0)	6(3.0)	16(4.0)
18	2(1.0)	4(2.0)	4(2.0)	2(1.0)	6(1.5)
19	9(4.5)	9(4.5)	12(6.0)	6(3.0)	18(4.5)
20	2(1.0)	3(1.5)	1(0.5)	4(2.0)	5(1.2)
21	1(0.5)	4(2.0)	1(0.5)	4(2.0)	5(1.2)
22	2(1.0)	4(2.0)	2(1.0)	4(2.0)	6(1.5)
23	5(2.5)	1(0.5)	4(2.0)	2(1.0)	6(1.5)
24	0	1(0.5)	0	1(0.5)	1(0.2)
autosomes	156(78.0)	154(77.0)	172(86.0)	138(69.0)	310(77.5)
X-	15(7.5)	15(7.5)	10(5.0)	20(10.0)	30(7.5)
X-inactive	21(10.5)	21(10.5)	-	42(21.0)	42(10.5)
Y	8(4.0)	10(5.0)	18(9.0)	-	18(4.5)
gonosomes	44(22.0)	46(23.0)	28(14.0)	62(31.0)	90(22.5)
TOTAL	200	200	200	200	400
N. cells	114	100	111	103	214
BS Index	1.75	2.00	1.80	1.94	1.87

RESULTS AND CONCLUSIONS - Figure 1 shows an RBG-banded pro- metaphase plate with break sites on the inactive X-chromosome (band q24) and on chromosome 20 (band q23).

Chromosomal distribution of breaks among individuals: Table 1 shows the chromosomal distribution of the breaks in the two groups of animals investigated (group A and B) and according to gender (males and females). No statistically significant difference was found in the chromosomal distribution of the breaks 'among' the eight animals investigated, as well as between the two groups A and B. However, when the animals were grouped according to gender, the differences between males and females became significant ($P< 0.01$), mainly because of the sex-chromosomes: in fact, the yield of breaks on the X and Y-chromosomes was 5 and 9 %, respectively, in the males; in the females, the inactive X-showed twice as many breaks compared to the active counterpart (21 vs 10 %, respectively).

Figure 1. RBG banded prometaphase plate with break sites.



Distribution of breaks 'among' river buffalo chromosomes: By assuming 15 as the 'mean' value of breaks in the river buffalo haploid genome (400 breaks /26 chromosomes), three classes (high, medium, low) of chromosomes can be established as follows: high frequency (30 breaks and above): the inactive X-chromosome, chromosomes 9, 8 and the active X; medium frequency (16∏29 breaks): chromosomes 1-13-7-10-2-19-Y-15-17; low frequency (0∏15 breaks): chromosomes 12-4-3-5-14-16-18-22-23-20-21-6-11-24. No breaks were scored on chromosome arms 2p and 3p.The Chi-square test revealed statistically significant differences ($P<0.01$) among individual chromosomes, thus suggesting a 'non-random' distribution of the breaks. This finding was further confirmed by the ANOVA test ($P< 0.01$). When the yield of breaks per chromosome was correlated to the relative length of chromosomes, based on ten GTG-banded metaphases, the Pearson correlation test showed a positive value ($r=0.41$; $P< 0.001$). The study is in progress to construct a 'fragile-site map' of river buffalo which could be utilized for genetic improvement programs of the species and for comparative genotoxicity studies. These preliminary data also indicate the need to extend cytogenetic investigations to other domestic and non-domestic animal species, for a better understanding of the role played by the fragile sites of the genomes in determining phenotypic malformations, infertility, and in the occurrence of chromosomal rearrangements respon-sible for karyotype evolution and speciation (Ruiz Herrera *et al.*, 2006).

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