Aneuploidy and genome organization in sperm nuclei of river buffalo and cattle detected by fluorescence *in situ* hybridization (FISH) and chromosome microdissection

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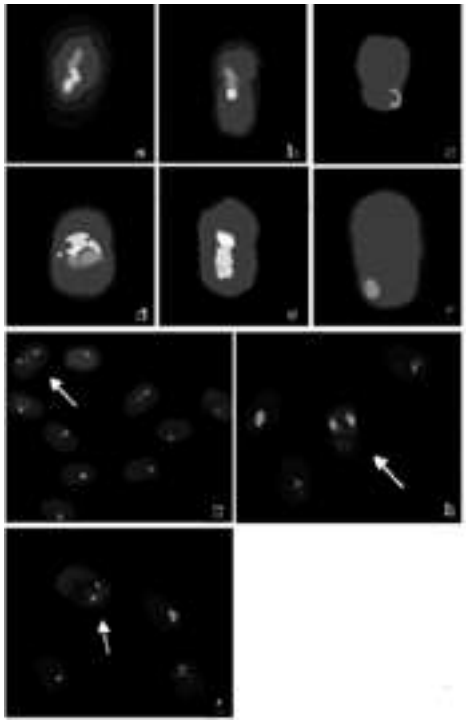
RIASSUNTO – Studio delle aneuploidie e organizzazione del genoma in spermatozoi di bovino e bufalo mediante microdissezione cromosomica e ibridazione fluorescente *in situ* (FISH). *Scopo della ricerca è stato la definizione della frequenza di base delle aneuploidie dei cromosomi X-Y- e 5p in spermatozoi della specie bufalina, quale presupposto indispensabile per investigare possibili relazioni tra aneuploidia e efficienza riproduttiva e per ulteriori studi di mutagenesi ambientale. Vengono, inoltre, riportati alcuni risultati preliminari sull'organizzazione territoriale di detti cromosomi nel nucleo degli spermatozoi; difetti di tale organizzazione vengono classificati "non compensabili", in grado di alterare le prestazioni riproduttive dei tori.*

KEY WORDS: chromosome, aneuploidy, sperm architecture, FISH.

INTRODUCTION – New technologies in molecular cytogenetics have remarkably facilitated the visualization of whole chromosomes or subchromosomal regions in interphase nuclei. Consequently, both somatic and sperm nuclei have been under investigation, in order to study the territorial organization of genetically 'active' versus 'inactive' genomes. Purpose of the present study was to investigate whether in river buffalo sperm nuclei, chromosomes X-, Y- and 5p (corresponding to chromosome 29 in cattle) maintain or change their relative position compared to cattle. Furthermore, since chromosome organization in the nucleus could affect meiotic segregation, we decided to study X-Y chromosome segregation in river buffalo sperm and developed a dual colour FISH method for evaluating the baseline level of aneuploidy in spermatozoa of this species, that could be used as reference for further investigations on the genotoxic effects of environmental mutagens and bio-hazards directly on the germ-cell line.

MATERIAL AND METHODS – Chromosome microdissection, amplification of chromosomal DNA, labelling of the probe, decondensation of sperm and *in situ* hybridization were performed as described earlier (Di Berardino *et al.*, 2004). For studying genome organization the following probes were prepared: Y, labelled with Spectrum Orange (SO); Yt (telomeric region), labelled with FITC, Xp (SO); Xq, labelled with Spectrum Green (SG); 29 (5p in river buffalo)(SO). For detecting aneuploidy the following probes were used: Xcen (SO) and Y (SG).

Figure 1. Main position of chr. X, Y and 29 in cattle (a-b-c). Main position of chr. X-Y-5p in river buffalo (d-e-f). Abnormal sperm in river buffalo: disomic X (g), disomic Y (h), diploid XX (i).



RESULTS AND CONCLUSIONS – *Genome organization*. Even though the data are not conclusive yet, and results will be exposed elsewhere, some broad considerations can be drawn. The X- and Y-chromosomes seem to occupy the central region of the sperm nucleus and the median position, both in cattle and in river buffalo. Also chromosome 29 tends to occupy the same territories in both species, being proximally located and lateral in position (Figure 1).

By considering that this chromosome is free in cattle whereas it is fused to chromosome 16 (centric fusion) in the river buffalo karyotype, it would be interesting to verify whether also chromosome 16 maintains the same position in both species. This study indicated that it is possible to investigate by FISH the genome organization inside the sperm nucleus. Under this point of view, river buffalo and cattle are two interesting models to study because in river buffalo there are five different sets of centric fusions, involving homologous chromosomes which in cattle are free (Iannuzzi, 1994). This study also demonstrated that, despite structural rearrangements, chromosome position inside the sperm nucleus seems to be conserved among different related species, confirming that spatial compartmentalization and packaging of individual chromosomes inside the nucleus would play important role in DNA replication and regulation of gene expression above the level of single genes (Cremer *et al.*, 1993; Marshall *et al.*, 1997; Visser *et al.*, 1998). *Aneuploidy detection*. Preliminary results on five river buffalo breeding bulls scoring are summarized in Table 1.

Totally, more than 50.000 sperm cells were scored (range 10.145-10.308 per bull). The average frequencies of the XX, YY and XY disomies were 0.198% (range 0.156-0.286), 0.023% (range 0.013-0.039) and 0.051% (range 0.029-0.079), respectively, with an overall mean of 0.272%. So far, no significant interindividual differences were found, but subject number 1 showed a higher disomic incidence compared to other animals. It is possible that the number of sperm examined is still not enough to detect significant differences. The disomic sperm (Figure 1) cells could be distinguished by their smaller size from the diploid XX , YY and XY spermatozoa

Table 1. Frequency of X- and Y-bearing sperm, disomic and diploid sperm in river buffalo.

Subject	X	Y	Disomic			Diploid			Sperm with signal	Sperm w/o signal	Total sperm
			X	XY	Y	X	XY	Y			
1	4919 (48.46)	5062 (49.86)	29 (0.29)	8 (0.08)	2 (0.02)	36 (0.35)	0 (0)	4 (0.04)	10060 (99.10)	91 (0.89)	10151
2	5090 (50.28)	4914 (48.54)	18 (0.18)	4 (0.04)	4 (0.04)	4 (0.04)	2 (0.02)	0 (0)	10034 (99.14)	87 (0.86)	10123
3	5108 (49.84)	5004 (48.82)	16 (0.16)	3 (0.03)	1 (0.01)	3 (0.03)	14 (0.14)	4 (0.04)	10153 (99.06)	96 (0.94)	10249
4	5042 (48.91)	5148 (49.94)	18 (0.17)	5 (0.05)	3 (0.03)	4 (0.04)	8 (0.09)	2 (0.02)	10230 (99.24)	78 (0.76)	10308
5	5029 (49.57)	4980 (49.09)	20 (0.20)	6 (0.06)	2 (0.02)	6 (0.06)	18 (0.18)	0 (0)	10061 (99.17)	84 (0.83)	10145
Total	25188 (49.41)	25108 (49.25)	101 (0.20)	26 (0.05)	12 (0.02)	53 (0.10)	42 (0.08)	10 (0.02)	50540 (99.14)	436 (0.85)	50976

which occurred with a frequency of 0.104% (range 0.029-0.355), 0.020% (range 0-0.039) and 0.082% (range 0-0.177), respectively, with an overall mean of 0.206%. The overall X-Y aneuploidy rate (disomic plus diploid) in river buffalo sperm was found to be 0.478%.

These results can be used as reference for further investigations. More bulls should be analyzed, in order to verify the possible relationship between sperm aneuploidy and fertility levels. Furhermore, this type of analysis could be used to study the genotoxic effects of enviromental mutagens and bio-hazards directly into the germ-cell line.

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