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Analysis of the segregation profiles in several cows heterozygous for rob(1;29) using OPU techniques and FISH with 1-29 painting probes

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The 1/29 Robertsonian translocation is the most widespread translocation in cattle breeds. Since its discovery, several studies have shown the negative effect of this translocation on the fertility of heterozygous carriers. A recent study using the sperm-FISH technique revealed that, on average, 97.1 % of spermatozoa were balanced in the semen of rob(1;29) carrier bulls. Data from the literature support the hypothesis that female meiosis is more prone to errors than male meiosis. Moreover some data suggest that the percentage of unbalanced gametes is higher in females than in males heterozygous for the rob(1;29). In this context, we initiated a meiotic segregation study on heterozygous carrier cows: (i) to determine the proportion of unbalanced gametes and (ii) to compare these values with those obtained in bulls. Oocytes were obtained from four heterozygous carrier cows by the ovum pick-up technique and then matured *in vitro*. Dual colour *in situ* hybridization using commercial painting probes of chromosomes BTA1 and BTA29 was carried out on metaphases spreads of oocytes II to assess their chromosomal status (balanced or unbalanced).

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Non-random expression and localization of aphidicolin-induced fragile sites in prometaphase chromosomes of mediterranean river buffalo (*Bubalus bubalis*, 2n=50)

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Common fragile sites (FS) 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), translocations and deletions, preferred sites of recombination, viral integration and carcinogenesis. Fragile sites are, therefore, ideal candidates for studying and characterizing the degree of stability/instability of the genome of domestic animals. In this study we report on the expression of aphidicolin-induced fragile sites in a group of four clinically healthy and unrelated river buffaloes, two males and two females, of the Mediterranean breed, reared in the province of Naples. Conventional lymphocyte cultures were performed in RPMI 1640 medium enriched with 10 % FBS, L-glutamine, antibiotic and antimicotic, and stimulated with Concanavalin A. After 48 hours of growth, Aphidicolin (Sigma) was added to the cultures at 0.15 mM (final concentration) for other 24 hours. 5 hours before the end, BrdU (20 µg/ml, f.c.) was added to the cultures for labelling late replication regions of the genome. Colcemide (0.040 µg/ml, f.c.) was added 40 minutes before the end of the cultures. Hypotonic and fixation treatments were performed in the usual manner. The slides were treated for RBG-banding, stained with Giemsa and examined under bright field optics. A total of 200 fragile sites were scored, 50 for each animal, and localized according to the standardized karyotype of river buffalo. The preliminary results show that the expression of fragile sites is not random, some chromosomes carrying more FS than expected,

others less. 21 FS out of 200 (10,5 %) were found on chromosome 9 , followed by chromosome 8 with 16 FS (8 %), 10 and 13 with 12 FS, and so on; other chromosomes revealed less FS than expected, namely chromosomes 11 and 21 with 1 FS, chromosomes 16-18-20 and 22 with 2 FS, and so on. When the fragile sites were localized on individual RBG-banded chromosomes, 90.5 % of the FS were localized on band q213 of chromosome 9, 90 % on band q21 of chromosome 17, 81.8 % on band q23 of chromosome 15, 77.7 % on band q21 of chromosome 19. The inactive X chromosome of the females showed twice as many FS compared to its active counterpart, thus confirming that FS are late replicating.