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Classical and molecular cytogenetic studies in some cattle breeds

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RIASSUNTO – Studi di citogenetica classica e molecolare in alcune razze bovine. *Sono riportati gli studi intrapresi per caratterizzare citogeneticamente alcune anomalie cromosomiche legate a sterilità o a ipofertilità in alcuni bovini di razza Chianina, Marchigiana, Romagnola, Maremmana Grigio Alpina e Podolica. Sono state impiegate sia tecniche di citogenetica classica (bandeggio cromosomico) che molecolare (tecnica FISH) con specifici marker molecolari per indagare le seguenti anomalie cromosomiche: fusioni centriche, traslocazioni reciproche e inversioni pericentriche. Studi con il marker molecolare INRA143, che mappa nella regione pericentromerica del cromosoma 29, hanno evidenziato chiari segnali di ibridazione nella parte prossimale del braccio q di tale traslocazione, supportando l'ipotesi che questa traslocazione si sia formata per fusione centrica dei cromosomi 1 e 29 e per inversione pericentromerica del cromosoma 29. Sono stati condotti studi su alcuni animali giovani, alcuni dei quali risultati sterili o ipofertili e portatori di traslocazioni reciproche e di un inversione pericentrica al cromosoma Y*

KEY WORDS: cytogenetics, chromosomal abnormality, FISH, banding, hypofertility.

INTRODUCTION – Numerical autosome aberrations have a few importance in the animal breeding since the carriers show generally abnormal body conformation. For this reason, these abnormalities are systematically eliminated from the animal population by the breeders during the animal breeding. Numerical sex chromosome abnormalities are more tolerate by species, since genes are present in single copy (one of two X-chromosome is genetically inactive), and the carriers show usually normal body conformation. For this reason these abnormalities escape the normal breeding selection. They are often correlated with sterility (Gustavsson, 1980; Iannuzzi *et al.*, 2001, 2002, 2004). Structural (and balanced) chromosome abnormalities are also very important in the animal breeding because the carriers show normal body conformation but have a reduced reproductive value due to the formation of unbalanced gametes which give rise to unbalanced zygotes and embryos which die in the early embryonic life (Gustavsson, 1969, 1980). The female returns in oestrus but with delay, comparing to the normal delay due to erroneous services. This increases the interval between two following births and, at the same time, decreases the number of calves produced during the reproductive life. This means a remarkable reduction of the animal productions. The most known and frequent chromosomal abnormality in cattle is the rob(1;29). This abnormality has been found in more than 50 different breeds (mainly meat breeds) in all the world (Popescu and Pech, 1991), with different frequencies, being very high (70%) in some breed (Rangel-Figueiredo and Iannuzzi, 1993). Although this abnormality has been studying for about 40 years, the mechanism originating this famous chromosomal polymorphism still remains unclear. We know that this

fusion is monocentric (a single constitutive heterochromatin block) and for this reason stable, opposite to all other centric fusion translocations found in cattle which were all dicentric (and unstable). In addition to the centric fusions, also reciprocal translocations and pericentric inversions can be found in cattle, even if they are very rare. A possible explanation for that may be due to the fact that it is quite difficult to show the reciprocal translocations in cattle (all acrocentric autosomes). Only when smaller or taller chromosomes than normal ones (chromosome 29 among the smallest and chromosome 1 among the tallest) are found, these abnormalities can easily be detected and studied. So, it is possible that many of these abnormalities escape the cytogenetic analysis, especially when no banding techniques are applied. The same situation can be found in the paracentric inversion. In this study we summarize the most important results we obtained to characterize these chromosome abnormalities by combining classical banding techniques and the fluorescence *in situ* hybridisation (FISH) technique with specific molecular markers.

MATERIAL AND METHODS – Peripheral blood samples from animals carrying chromosome abnormalities of Chianina, Marchigiana, Romagnola, Maremmana, Grigio Alpina and Podolica cattle breeds were cultured for about 72 h in RPMI medium enriched with FBS (10%), Penicellin-Streptomicin (1%) and Concanavalin A (15 µg/ml) as mitogen. Two types of cell cultures were performed: without (normal cultures) and with addition of the base analogue 5-Bromodeoxyuridine (BrdU) for R-banding technique. In the latter, thymidine (300 µg/ml) was added, after about 48 h, for 17 h to synchronize cells in the S-phase. Cell block was removed by washing cells twice with Puck's saline solution and recovering cells in fresh medium containing both 5-BrdU (15 µg/ml) and Hoechst 33258 (30 µg/ml) to obtain enhanced R-banding patterns. Slides obtained from normal cultures were treated for CBA-banding (C-banding by acridine orange staining), while those from BrdU-treated cells were used for both R-banding and FISH-techniques (Iannuzzi, 2003). As probes, both bovine and caprine BAC-clones were used. Chromosome identification and banding followed the latest international chromosome nomenclature (ISCNDB2000, 2001).

RESULTS AND CONCLUSIONS – Upon 13 heterozygous carriers of rob(1;29) we studied, all cells showed a single C-band block at the proximal regions of the translocated chromosome q-arms (Figure 1), confirming the monocentric nature of this translocation, and possible loss of C-band material from chromosome 29. However, the intensity of the fluorescence C-band block was less intense in the translocated chromosome than those present at the centromeric regions of the remaining acrocentric chromosomes (Figure 1). This can be explained by the lack in the rob(1;29) of some satellite DNA which are present in all acrocentric chromosome (Chaves *et al.*, 2003). The R-banding technique and the use of FISH-technique with the official markers of BTA1 (SOD1) and BTA29 (IGF2) (not shown) confirmed that the two chromosomes involved in this famous translocation are 1 and 29. Figure 2 shows a cattle metaphase plate of an heterozygous carrier of rob(1;29) treated for FISH-technique with the marker INRA143 mapping to the pericentromeric region of BTA29 and proximal region of rob(1;29) q-arms. To check if this phenomenon was common to all carriers of rob(1;29), we studied carriers from all cited Italian breeds and found the same pattern in all carriers. Studies we are performing in rob(1;29) carriers of some Portuguese cattle breed seem to confirm the pattern we found in the Italian cattle. In conclusion, our data strongly support the hypothesis that rob(1;29) originated by two chromosome rearrangements: centric fusion and pericentric inversion with possible loss of constitutive heterochromatin (and some satellite DNA). The use of both G- and R-banding techniques, as well as of the FISH-technique with specific markers were also very useful to identify the chromosomes involved in the second most common centric fusion found in cattle: rob(26;29) found in Grigio Alpina breed. Indeed, upon 322 investigated animals of this breed, 36 (11.5%) were found carriers of this centric fusion translocation which was dicentric (two HC-blocks). It is interesting to note that also this translocation involves the same chromosome present in the famous rob(1;29). Two reciprocal translocations were found: rcp(1;5) in Grigio Alpina breed and rcp(9;Y) in Chianina breed. The former was found in a both young bull (eliminated) and its dam which was fertile but with reduced fertility. Indeed, the dam required 2.5 services for calf comparing to the average of the breed (1.2 service for calf). The rcp(9;Y) was

found in a young bull of Chianina breed. This bull was sterile due to a pronounced azoospermia, as revealed by the microscope observation of the sperm. A pericentric inversion on the Y-chromosome was found in 11 young males from Podolian breed but only one carriers was sterile with atrophic penis, absence of testis and showing also some female traits (head and horns). It is possible that mutations in the genes involved in the sex differentiation occurred only in this animal during the pericentric inversion originating the abnormal Y-chromosome. All these abnormalities were studied by both banding and FISH-mapping technique with specific markers.

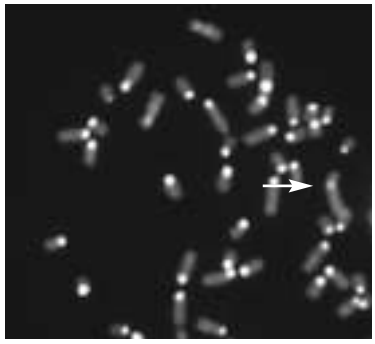

Figure 1. Details of cattle female cell from an heterozygous carrier of rob(1;29) and treated for CBA-banding. Note the single HC-block in the proximal q-arms of the translocated chromosome (arrow).

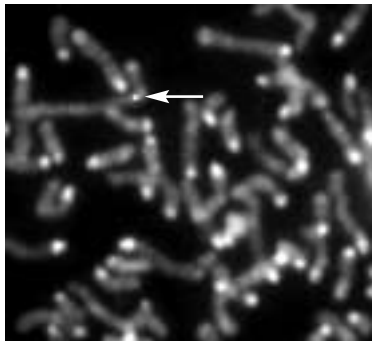

Figure 2. Details of female cattle cell treated for FISH-technique with a BAC-clone containing INRA143 mapping on the pericentromeric region of chromosome 29 (small arrow) and proximal rob(1;29) q-arms (large arrow).

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