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Cytogenetic Monitoring of Domestic Species

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Abstract: Toxic substances exposure of human alimentation-destined animals can be an important risk factor for human health. Besides the detection of the concentrations of these substances in animal products, the cytogenetic tests represent a good method for the evaluation of the effects of xenobiotics. Sister chromatid exchanges, chromosome aberrations, comet assay and micronucleus test are relatively easy methods that allow detecting the damage produced by one or more substances not yet identified.

Key words: Food production, public health, biomonitoring, genotoxicity

The increased attention paid to food safety has produced an augment of the technical and legislative instrument to control animal production. This shows risks of different kind depending on the type of the stock farm organization. For products obtained by animals bred in intensive stock farms, besides infective pathologies, the major risk is represented by the possible presence of drugs, whereas for those coming from the outdoors, the risk derives above all from a possible environmental contamination. Therefore, control of contaminants in animals destined to food production is of primary role for public health, but in some cases it may also be useful for environmental monitoring. Many authors agree that, among domestic mammals, herbivorous and Bovines especially, are the most suitable environmental bioindicators (Parada and Jasczak, 1993; Rubes et al., 1997). In fact, they are very sensitive to many environmental pollutants and accumulate xenobiotics in their body (Garcia-Repetto et al., 1997).

In many countries, the legislation provides for control of the concentrations of drugs, metals and other xenobiotics through casual sampling. These may be compared to what in toxicology is called dose indicator, that is to say the concentrations of some substances in the living animal (often detected in blood). Besides the dose indicators, effect indicators exist. These are biomarkers that reveal possible alterations of biological parameters. While the use of dose indicators implies knowledge of what has to be searched, the use of effect indicators allows to detect the damage produced by a substance not yet detected. Between these biomarkers, cytogenetic assays are particularly useful, as they allow detecting damages produced by several substances with probable carcinogenic properties (Tucker and Preston, 1996).

Sister Chromatid Exchanges (SCE) are recognized as exchanges of chromosomal fragments between two

chromatids of the same chromosome during replication of damaged DNA (Latt et al., 1981), while Chromosome Aberrations (CA) can be analyzed in cells as structural chromatid- or chromosome-type aberrations, like gaps and breaks within a chromosome or rearrangement within or between chromosomes (Carrano and Natarajan, 1988). A correlation between nearness to factories and chromosomal aberration frequency in swine lymphocytes was demonstrated, confirming that this assay can be used as a good environmental marker (Rubes, 1987). More recently, high levels of both chromosome abnormalities (gap, chromosome and chromatid breaks) and sister chromatid exchanges were found in sheep herds exposed to high dioxin levels during pasturage (Perucatti et al., 2006).

Another useful assay is the alkaline single cell gel electrophoresis, also known as Comet test. This is a technique for measuring DNA strand breaks and thereby DNA damage. The assay involves detection, under alkaline conditions, of cell DNA fragments which, on electrophoresis, migrate from the nuclear core, resulting in the formation of the comet tail (Singh *et al.*, 1988).

By the way, one of the most popular cytogenetic assays is the micronucleus test. Micronuclei are nuclear remnants, produced during mitosis when a chromosome fragment or a whole chromosome does not migrate with one of the two daughter nuclei formed. These inclusions may be found in any kind of cell, both somatic and germinal. The micronucleus test is a cytogenetic assay consisting in the detection of the variations of micronucleated cells frequencies. The most popular methodology of this test consists in its application either on lymphocytes or erythrocytes.

Rubes *et al.* (1992) compared genotoxic effects induced in some domestic mammals, such as cows, horses, pigs and deer, by the exposure to different levels of industrial pollution and demonstrated a biological

impact on animal bred in the most industrialised areas. In particular, in lymphocytes sampled from cows, horses and deer a micronuclei frequency higher than in pigs was observed. This may be due to differences in their diet.

Since mammalian erythrocytes are anucleated, this test is particularly suitable on red blood cells of mammals because micronuclei can be easily detected (Schmid, 1975). Erythrocytes can be sampled both from peripheral blood and bone marrow. However, sampling cells from bone marrow is a very invasive method and not very suitable for biomonitoring. Actually, it can be performed just in abattoirs (Cristaldi et al., 2004). While micronucleated erythrocytes from the hemopoietical organ (bone marrow) reflect a genotoxic damage which occurred during a time equivalent to the cell cycle, those from the peripheral circulation reflect events that occurred in a time equal to the lifespan of the circulating erythrocytes (Schlegel and MacGregor, 1982). Therefore, the application of the micronucleus test on peripheral blood samples is particularly indicated for conditions of chronic exposure. However, it should be added that not every species are suitable for the application of the micronucleus test on erythrocytes from peripheral blood (Cristaldi et al., 2004). In fact, in some species the spleen selectively removes micronuclei from the circulation. Among domestic species, bovines, ovines, rabbits and dogs own this kind of spleen (Udroiu, 2006). On the other hand, the micronucleus test can be carried out on equine blood samples. An increase in micronuclei (known in haematology as Howell-Jolly bodies) has been found in horses fed with lead-contaminated hay (Burrows and Borchard, 1982; Bianu and Orășanu, 2004).

In conclusion, cytogenetic assays may be used as a helpful and easy instrument to monitor animal production and can give a measure of the biological effects of pollutants or drugs before overt disease develops.

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