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consdabi@consdabi.org

Study of genome instability in Ancient Autochthonous Genetic Type (AAGT) 'Casertana' pig by using Micronucleus Test

Matassino, D.[1,2], Castellano, N.[1], Falasca, D.[1], Varricchio, G.[1], Fornataro, D.[1]

[1]ConSDABI. NFP.I.-FAO- Centro di Scienza Omica per la Qualità e per l'Eccellenza Nutrizionali, Località Piano Cappelle-82100 Benevento, Italy,
[2]Dipartimento di Scienze biologiche e ambientali - Università degli Studi del Sannio, Via Port'Arsa,11-82100 Benevento, Italy

1. INTRODUCTION

The clastogenic and/or mutagenic effects of physical and/or chemical and/or environmental agents on genome express principally as oxidative DNA damage, which may be detected, among the other things, by means of '*Comet test*', frequency of '*sister chromatid exchanges*' (SCE), number of '*nucleolar organizer regions*' (NORs) and '*micronuclei*' (MN). The latter is particularly used to detect a DNA oxidative damage on genome induced by physical and/or chemical and/or environmental agents.
In the most of cell systems, the absolute or relative frequency of primary aberrations varies in relation to cell cycle phase. Of consequence, the generation of acentric fragments 'excluded' from nucleus (hence of micronuclei) may be temporally fluctuating.
Several Authors evidenced, in the human field, a positive relation between MN frequency and age ($P< 0.001$). It is known that one of the explanations for human aging is the temporal accumulation of DNA errors which may verify during DNA and other macromolecules synthesis; this synthesis may undergo to the effect of mutagenic and clastogenic agents present in the bioterritory in which he lives; furthermore, the metabolic processes (thermoregulation, nutrition, etc.) may be contribute to error accumulation (Hando *et al*., 1994).

2. AIM

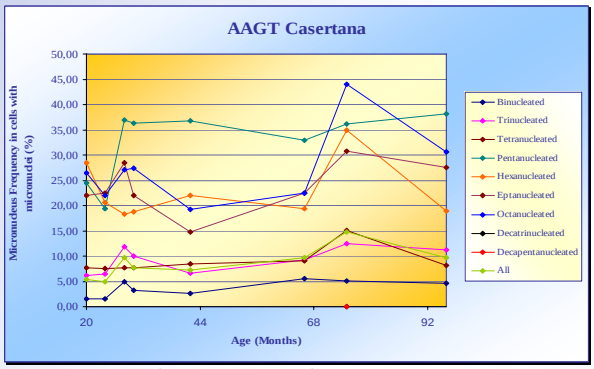
It is reported in Matassino et al., 'Preliminary results on the genome instability evaluated by Micronucleus Test in four Italian pigs Ancient Autochtonous Genetic Types (AAGT), *57th Annual EAAP Meeting*, Antalya September 17 ÷ 20, 2006.

3. MATERIALS AND METHODS

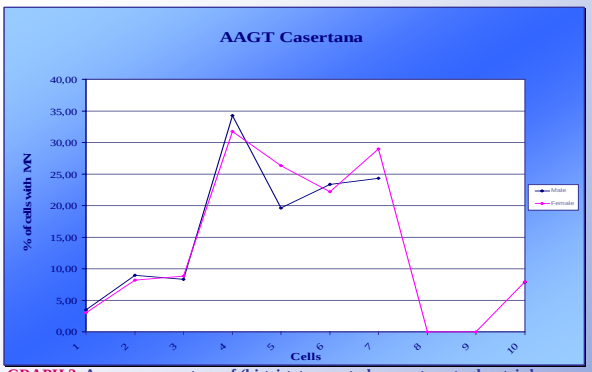
The research was carried out on individuals samples (20 males and 13 females) of 'Casertana' pig ancient autochthonous genetic type (AAGT), mostly present in Campania (Italy) and reared at ConSDABI experimental farm. The age of examined subjects ranged from 20 to 96 months. The micronuclei individuation was performed using the technique reported by Matassino *et al*. (1994) appropriately modified. Peripheral blood was collected from femoral vein using heparinized vacutainers. Cell cultures were prepared with 8 ml of RPMI (Gibco), 1ml blood, 15 % inactivated *Foetal Calf Serum* (FCS), 10 ml/ml L-glutammine and 10 ml/ml Pokeweed. After 44 hours of incubation, cytochalisin B was added to give final concentration of 6 mg/ml (*Scarfì et al*., 1993). After 72 h of growth at 37 °C, cell suspension was treated with eritrocyte lysis buffer. After washing in RPMI 1640 (Gibco) supplemented with 2% FCS, cell suspension has undergone to hypotonic solution for 15'. Slides were examined using a Leitz Diaplan microscope at 200 X magnification. The statistical analysis was performed by chi square test.

4. RESULTS AND DISCUSSION

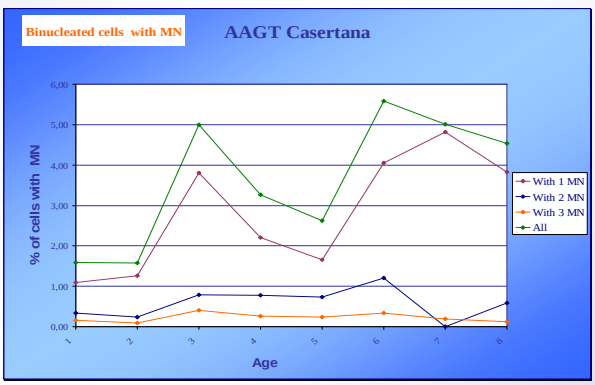
The results evidenced: *(i)* presence of polynucleated cells, with one, two, three, four, five, six, seven, eight nuclei and one cell with thirteen nuclei and another cell with fifteen nucleus (graph 1); *(ii)* MN number ranges from a minimum value of 1 MN to a maximum value of 8 MN; *(iii)* percentage of cells with micronuclei depends on cell nuclear number, independently on the sex (graph 2); *(iv)* the percentage of cells with MN shows a positive correlation with the age according to a prevalently linear function ($P<0.07$) (graph3). This trend confirms, in the limits of the observation field, what observed for human. Further research is necessary to deepen the trend of this phenomenon in the autochthonous genetic types (AGTs) and/or ancient autochthonous genetic types (AAGTs); *(v)* the percentage of cells with MN shows a linear positive correlation with number of nuclei per cell ($P<0.06$).



GRAPH 1. Variation of average percentage of bi-tri-tetra-penta-hexa-epta-octa-decatri-deca penta and all) with MN in relation to the age.



GRAPH 2. Average percentage of (bi-tri-tetra-penta-hexa-epta-octa-decatri-deca penta and all) cells with MN within the sex (n = 20 males; n = 13 females).



GRAPH 3. Variation of average binucleated percentage distinctly with 1, 2, 3 MN as well as of all binucleated cells.

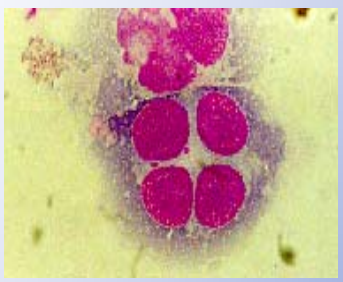


FIG. 1. Tetranucleated cell with 2 MN.

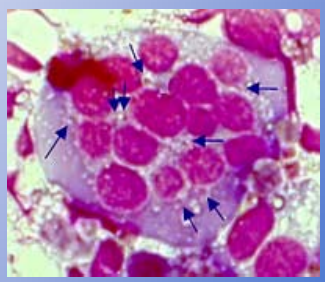


FIG. 2. Pentanucleated cell with 8 MN.

5. CONCLUSIONS

The values of mean percentage of cells with spontaneous micronuclei, being in the normal range, could be a valid indicator of animal welfare state.