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# Analysis of chromosome damage by sister chromatid exchange (SCE) and redox homeostasis characterization on sheep flocks from Sardinian pasturelands

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## HIGHLIGHTS

- SCE test was used to test the genome damage of Sardinian sheep for the first time.
- Significant SCE means were found in three groups of polluted areas.
- Plasma levels of Asc, Toc and Ret were significantly lower in the exposed sheep.
- N-Tyr, PC, and LPO levels were lower in the control than in the exposed groups.

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## ABSTRACT

Over the last decades, an increase of pollutants of diverse origin (industrial, military, mining, etc.) was recorded in several areas of Sardinia Island. We report the results of a multidisciplinary and complementary study based on cytogenetic and physiological analyses. The data obtained show the effects of the environmental impact on six sheep flocks (Sardinian breed) grazing on natural pasturelands next to possible polluted areas and compared to three herds grazing in different areas far from those potentially contaminated and used as control. Sister chromatid exchange (SCE) test was used as cytogenetic test to analyze chromosomal damages and it was performed on peripheral blood samples collected from 129 adult sheep (age > 4 years) randomly selected from polluted (92 animals) and control (37 animals) areas. Two types of cell cultures were performed: without (normal cultures) and with the addition of 5-BrdU. SCE-mean values estimated over 35 cells counted for each animal were 8.65 ± 3.40, 8.10 ± 3.50, 8.05 ± 3.08, 7.42 ± 3.34, 9.28 ± 3.56 and 8.38 ± 3.29 in the exposed areas, whereas the average values were 7.86 ± 3.31 in the control group. Significant increases ($P < 0.01$) of SCEs were found in three investigated areas of Southern Sardinia. Furthermore, sheep of the same flocks were characterized for blood redox homeostasis in order to define the potential targets of oxidative damage and to identify biomarkers of the extent of animal exposure to environmental contaminants. The plasma levels of Asc, Toc and Ret were found to be significantly lower ($P < 0.001$) in exposed sheep (I, II, IV and V) than in the control group. TAC as well as GPx and SOD activities were higher in control than in the exposed groups ($P < 0.001$). Finally, plasma levels of N-Tyr, PC, and LPO were significantly lower ($P < 0.001$) in the control group than in the exposed groups.


## 1. Introduction

Sardinia is the second largest island in the Mediterranean Sea, well known for its natural landscapes and for its endemic species. The island is considered as a major center of plant diversity. Despite that, various areas of this region are at environmental risk due to the contamination and/or pollution generated by civil and industrial activities.

*Abbreviations:* SCE, sister chromatid exchange; 5-BrdU, 5-bromodeoxyuridine; ROS, reactive oxygen species; Ret, Retinol; Toc, alpha-Tocopherol; Asc, Ascorbic Acid; N-Tyr, nitrotyrosine; PC, protein-bound carbonyl; LPO, lipid hydroperoxide; SOD, superoxide dismutase; GPx, glutathione peroxidase; TAC, total antioxidant capacity; ELISA, enzyme linked immunosorbent assay; HPLC, high performance liquid chromatography.

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The most polluted areas are localized in the southwest of the Island (Boni et al., 1999; Sanna et al., 2003; Beccaloni et al., 2013) where high concentrations of volatile organic compounds (benzene, formaldehyde, xylene), polycyclic aromatic hydrocarbons and heavy metals have been found as a result of metallurgic industrial and mining activities (Peluso et al., 2013; Madeddu et al., 2013; Varrica et al., 2014; Cidu et al., 2014). In the same areas, residues of chemical emission due to military tests have been also found (Cristaldi et al., 2013).

A total of 18 areas assessed as industrial, mining, military and urban zones have been considered at environmental risk in this Island (Biggeri et al., 2006).

In this context, it is worth mentioning that noxious pollutants, such as sulfur oxides, nitrogen oxides, hydrocarbons, carbon monoxide, and transition metals, may induce DNA damage and genome mutations exerting carcinogenic effects (Goulart et al., 2005; Mateuca et al., 2005; Cavallo et al., 2008). These pollutants also promote highly reactive oxygen species (ROS) production (Matés et al., 2010), and depression of several ROS quenching systems (Ishida et al., 2009), with subsequent accumulation of toxic compounds in blood and tissues (Knerr et al., 2006). In physiological conditions, the antioxidant defense system, provided by enzymes and antioxidants, scavenges ROS thus limiting or preventing oxidative damage (Halliwell, 2012). The imbalance between ROS production and neutralizing capacity of antioxidant mechanisms may lead to oxidative stress (Cadenas and Davies, 2000; Halliwell and Gutteridge, 2000), which is associated with modifications of physiological and metabolic functions (Halliwell and Gutteridge, 2000).

In the last decades, the epidemiological data on the incidence of human pathologies analyzed in Sardinia have showed a general increasing trend, as well as rising mortality rates have been reported for the most prevalent types of cancer in both sexes (Budroni et al., 2013).

A recent investigation of the National Association of Italian Veterinarians (FNOVI) reported a correlation between the incidence of cancers in sheep farmers and the emergence of genetic malformations in newborn lambs located in potentially polluted areas (Mellis and Lorrai, 2013). In this respect, the farm animals represent good environmental sentinels (especially those naturally pastured) to facilitate the assessment of human exposure to environmental contaminants. For instance, they can be used as a monitoring system to reveal early environmental contamination, to monitor contamination of the food web, and to investigate the presence of contaminants in environmental media (van der Schalie et al., 1999).

Among farm animals, sheep are particularly suitable to be used as environmental sentinels. The reason for that lies in their feeding characteristics. In fact, differently from the other ruminants, sheep are raised on pasture and about 12% of their daily feed is represented by soil. Pollutants are accumulated mostly in soil and their permitted values are a far times higher than those admitted in plants (i.e. dioxin's values are 0.75 ng/kg and 10 ng/kg in plants and soil, respectively).

Cytogenetic tests represent direct and sensitive methods that have been used for detecting DNA damages in chromosomes for the bio-monitoring of different species (Penders et al., 2012; Wójcik and Smalec, 2013; Yang et al., 2014). In particular, SCE is a short-term test for the detection of reciprocal exchanges of DNA between two sister chromatids, involving DNA breakage and subsequent re-junction. The increased frequencies of SCE due to negative conditions, like pollutant exposition, lead to DNA single strand breaks as reported in several studies on livestock populations (Rubes et al., 1997; Di Meo et al., 2000, 2011; Iannuzzi et al., 2004; Perucatti et al., 2006; Genualdo et al., 2012; Wójcik and Smalec, 2013). The goal of this study is to evaluate the impact of environmental pollutants in some areas of Sardinia Island on the genome of sheep flocks living in this region by using the SCE test. Furthermore, as the analysis of blood redox homeostasis has become an important complementary tool for the evaluation of health and metabolic status of dairy cows (Bernabucci et al., 2005; Castillo et al., 2003, 2005, 2006), and feedlot calves (Castillo et al., 2012), we also characterized blood redox homeostasis of sheep in order to define the potential targets of oxidative damage in blood, and to describe physiological changes associated with exposure to pollutants.

## 2. Materials and methods

### 2.1. Farm selection

The different areas were selected according to the chemical emissions of different activities as reported in former investigations (Table 1).

The cytogenetic study was performed in Sardinia on 129 adult sheep (age > 4 years) of Sardinian breed, 92 grazing on natural pasturelands near possible polluted areas selected as follows: two herds (~12 sheep for each herd) located in the Northern area (industrial and military zones) and four flocks (~15 sheep for each flock) located in the Southern area (military, industrial and mine zones) of the island. For comparison, 37 sheep, reared in three different herds, were randomly selected in areas far from possible polluted zones and used as control (Fig. 1).

Furthermore, physiological investigations were performed on 80 exposed sheep (20 per each group) and 20 sheep used as a control group belonging to the same farms.

### 2.2. Cell cultures

Whole blood samples were collected from the jugular vein using sterile Vacutainer tubes containing sodium heparin as anticoagulant. About 1 ml of whole blood sample was added to the culture mix composed of 7 ml of RPMI medium, enriched with fetal calf serum (20%), L-glutamine (0.25%), antibiotic–antimycotic mixture (0.5%) and concanavalin A (20 μg/ml) as mitogen. Culture flasks were incubated at 37.8 °C for 72 h. Cell cultures from the investigated animals were

**Table 1**
Chemical emissions due to the various activities of selected areas of Sardinia Island.

| Areas | Activity | Emissions | References |
|---|---|---|---|
| Control | Agriculture | n.a. | |
| Exposed I | Mining | Ag, Ba, Cd, Cr, Ni, Pb, Rb, Sb, U, V, Zn | Pirastu et al. (2011)<br>Safronova et al. (2012)<br>Madeddu et al. (2013)<br>Varrica et al. (2014) |
| Exposed II | Military | Former nuclear submarine base | Aumento et al. (2005)<br>Hernandez et al. (2011) |
| Exposed III | Industrial | Hg, Cd, Pb, Zn | Schintu and Degetto (1999)<br>Sanna et al. (2003) |
| Exposed IV | Industrial | IPA | De Luca et al. (2004)<br>Pirastu et al. (2011) |
| Exposed V | Military | Rb, Tl, W, Ti and Al, Cd, Pb | Cristaldi et al. (2013)<br>Gatti et al. (2013) |
| Exposed VI | Military/industrial | PCDD, PCDF, PCB | Storelli et al. (2012) |