

Sister chromatid exchange (SCE) for the first time in Casertana pig breed

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Lymphocyte cell cultures from 30 Casertana pigs (13 males and 17 females), reared in southern Italy, underwent the sister chromatid exchange (SCE) test. The Casertana pig is an endangered native breed from the region of Campania, raised chiefly half-wild. In the 1500 cells we studied, the mean SCE was 6.32 ± 2.92 and SCE frequency did not follow a Poisson distribution. A higher mean value of SCE cell⁻¹ was found in the older group (SCE cell⁻¹ = 6.68 ± 2.95) compared with the younger (SCE cell⁻¹ = 5.94 ± 2.84), the difference being statistically significant ($P < 0.01$). To our knowledge, this is the first investigation in a representative sample of Italian pig breed using the SCE test. Furthermore, this is the first report where the differences found in the mean SCE values were related to age in domestic species.

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The sister chromatid exchange (SCE) test has been used to detect genome stability in humans (CHAGANTI et al. 1974) and the main livestock species (DI BERARDINO AND SHOFFNER 1979; DI MEO et al. 1993, 2000; CIOTOLA et al. 2005), and to discover DNA damage caused by a variety of natural and artificial chemical compounds (IANNUZZI et al. 1990, 2004; PERUCATTI et al. 2006). SCEs can be seen after two cell-cycle-replications in the presence of the thymine analogue 5-bromodeoxyuridine (BrdU). While the sister chromatid with a native polynucleotide chain (with thymine) is stained, the other sister chromatid, with BrdU in both polynucleotide chains, is not stained. This allows the presence of SCEs to be easily visualized, although spontaneous rates of SCE were detected in lymphocytes of bovids (DI BERARDINO et al. 1997). A high SCE number means higher genome instability and occurrence of possible mutations compared to similar species-groups showing lower SCE mean values. The SCE test has also been used to determine the frequency of exchanges on the active and inactive X chromosome in bovids (IANNUZZI et al. 1990), to compare genome stability of three different cattle breeds reared under similar conditions (significant differences in SCE mean values were found between Friesians-with lower of SCE numbers-and Podolians, IANNUZZI et al. 1991), and

to compare the SCE-mean values of animals with normal karyotype and those (with significant higher SCE/cell mean values) carrying rob (1;29) (RANGEL-FIGUEIREDO et al. 1995). The only previous report using SCE-test in a representative sample of pig was that reported by RUBES (1987), along the comparison among three different pig herds (breeding sows and fattening pigs) of conventional breeds (e.g. landrace breed) kept in closed large scale production farms with hundreds of other pigs using both SCE-test and chromosomal abnormalities screening.

To our knowledge this is the first investigation in a representative sample of Italian pigs using the SCE test and where differences in the SCE-levels were related to age in domestic animals. In addition, this test was applied in Casertana pig, an endangered breed which is native to Campania (southern Italy), chiefly raised half-wild.

MATERIAL AND METHODS

Thirty pigs from the Casertana breed (13 males and 17 females), from five months to three years of age, were used for this study. The pigs were listed in the birth register administered by the National Association of Pig Breeders (ANAS), and came from farms in the provinces of Caserta, Benevento and Naples.

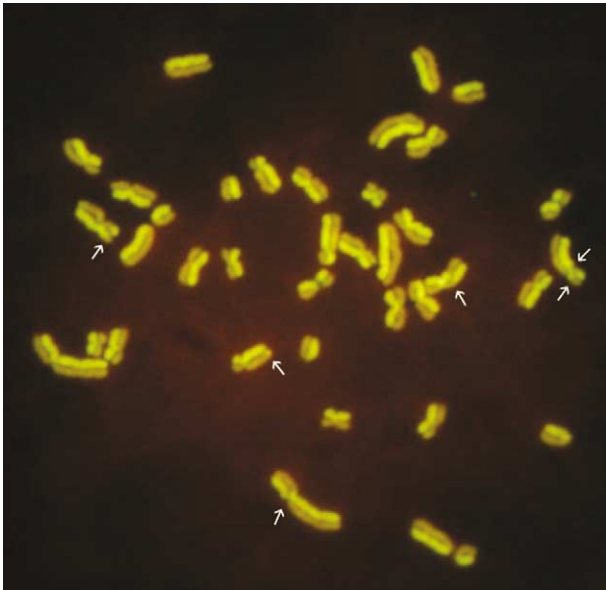


Fig. 1. Pig female metaphase plate ($2n=38$, XX) from Casertana breed treated for the SCE test and stained with acridine orange. Arrows indicate SCEs.

Peripheral blood (0.8 ml) was cultured at 37.8°C in RPMI medium, enriched with FCS (10%), L-glutamine (1%) and lectin (1.5%) for about 48 h. BrdU ($10\ \mu\text{g ml}^{-1}$) and Colcemid were added 16 h and 1 h before harvesting, respectively. This was followed by hypotonic treatment (KCl 0.5%) and three fixations in methanol-acetic acid (3:1), the last overnight. Three drops of cell suspension were air dried on cleaned and wet slides which were stained a day later with acridine orange (0.1% in a phosphate buffer, $\text{pH}=7.0$) for 10 min, washed in tap and distilled water and mounted in the same phosphate buffer. Slides were observed about 24 h after staining or later (one week). At least 50 metaphase plates per animal were observed under a fluorescence Nikon Eclipse 80i microscope, captured with a digital camera Nikon

Sight DS-5M (Fig. 1), transferred to PC and later processed.

RESULTS AND DISCUSSION

Figure 1 shows a pig early-metaphase cell with SCEs. Of 1500 cells and 57 000 chromosomes studied, the mean SCE was 6.32 ± 2.92 (Table 1). Mean SCE was higher in female cells (6.44 ± 2.89) than in male (6.15 ± 2.97) (Table 2), but the difference was not statistically significant. This is in contrast with previous studies in humans (MARGOLIN AND SHELBY 1985), while it agrees with the SCE values reported in some domestic species, namely goat (DI MEO et al. 1993), sheep (DI MEO et al. 2000), cattle (DI BERARDINO AND SHOFFNER 1979) and Agerolese cattle (CIOTOLA et al. 2005).

Table 3 shows distribution of SCEs on cells and chromosomes of Casertana pig in two distinct groups: below and over one year of age. A higher mean value of SCE cell^{-1} was found in the older group ($\text{SCE cell}^{-1} = 6.68 \pm 2.95$) compared with the younger ($\text{SCE cell}^{-1} = 5.94 \pm 2.84$), the difference being statistically significant ($P < 0.01$). To our knowledge, this is the first time that differences in SCE mean values were related to age in domestic species. These results agree with those reported in humans (WAKSVIK et al. 1981).

The SCE frequency distribution in the total cell population (Fig. 2) did not follow a Poisson distribution. The same was observed in cattle (IANNUZZI et al. 1991), goat (DI MEO et al. 1993) and sheep (DI MEO et al. 2000). By contrast, DI BERARDINO AND SHOFFNER (1979) reported a Poisson distribution in American Friesian cattle.

Our data show an SCE mean value (6.32 ± 2.92) which is lower than those (7.73 ± 0.86 , 7.06 ± 1.47 and 6.51 ± 0.89) found in three pig herds earlier investigated by RUBES (1987), although these pigs (breeding

Table 1. Distribution of SCEs on cells and chromosomes in Casertana pig.

Breed	Number of animals	Number of cells	Number of chromosomes	Number of SCE cell^{-1}	
				Mean \pm sd	Range
Casertana	30	1500	57 000	6.32 ± 2.92	0–17

Table 2. Distribution of SCEs on cells and chromosomes in male and female Casertana pig.

Source	Number of animals	Number of cells	Number of chromosomes	Number of SCE cell^{-1}	
				Mean \pm sd	Range
Males	13	650	24 700	6.15 ± 2.97	0–17
Females	17	850	32 300	6.44 ± 2.89	0–17

Table 3. Distribution of SCEs on cells and chromosomes in Casertana pig by age.

Source	Number of animals	Number of cells	Number of chromosomes	Number of SCE cell ⁻¹	
				Mean ±sd	Range
Up to 1 year	15	750	28 500	5.94 ±2.84	0–17
Over 1 year	15	750	28 500	6.68 ±2.95*	0–17

*P < 0.01.

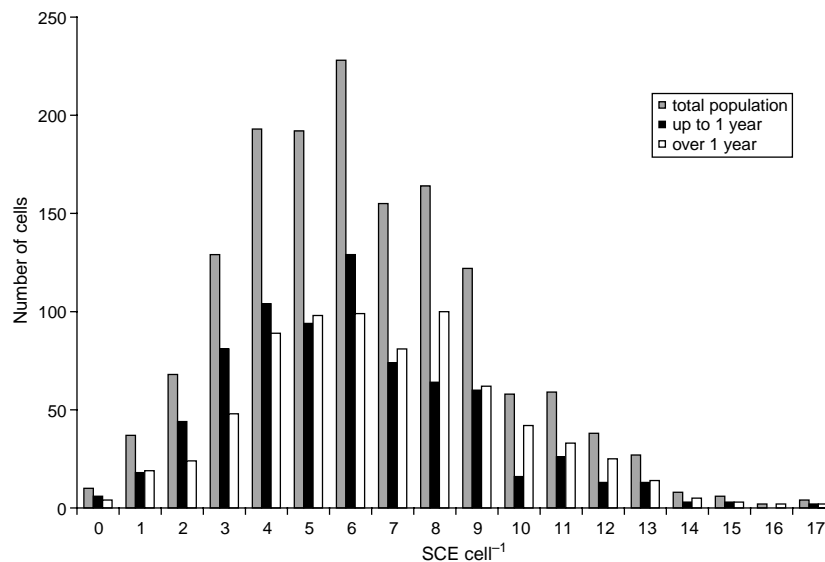


Fig. 2. SCE frequency distribution in Casertana pig cell population, total, up to 1 year and over 1 year.

sows and fattening pigs of conventional breeds, e.g. landrace) were kept in closed large scale production farms with hundreds of other pigs, while the Casertana pig breed is raised chiefly half-wild.

The mean SCE-value found in Casertana pig breed was also lower than those observed in other animal species, namely Italian cattle breeds such as the Friesian (7.11 ± 3.35), the Podolian (7.95 ± 3.41) and the Romagna (7.32 ± 3.18) (IANNUZZI et al. 1991), Italian goats (6.60 ± 3.00) (DI MEIO et al. 1993) and river buffalo (8.30 ± 3.40) (IANNUZZI et al. 1988).

These observations seem to show that the Casertana pig, endangered rare breed, has a more stable genome than those of other Italian species and breeds. This may be related to the different food chain, higher number of generations (pig), genetic improvement, breeding management and environmental factors.

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