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Crossbreeding Effect on Genome Stability in Pig (*Sus scrofa scrofa*)

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Aneuploid cell percentages and frequencies of CAs and SCEs were investigated in 10 Calabrian pigs, 10 LW pigs and 19 Calabrian x LW crossbred pigs, in order to compare genome stability between an autochthonous pig breed and a highly selected one and to verify if genome stability of their progeny, as other phenotypic traits, are influenced by heterosis. The mean number of cells per animal with structural aberrations, excluding gaps, was 6.20±2.39, 4.90±2.02 and 4.52±3.34 in Calabrian, LW and crossbred pigs, respectively, while the mean number of total CAs without gaps was 0.14±0.38, 0.11±0.35 and 0.11±0.35, respectively. The mean number of SCEs was 7.30±3.24 in Calabrian pigs, 6.45±2.74 in LW pigs and 6.28±2.90 in the crossbred ones. Percentages of cells with aneuploidy were 7.30, 10.10 and 10.79 in Calabrian, LW and crossbred pigs, respectively. In particular, the Calabrian breed showed higher values compared to LW in each test, however, there were statistically significant differences only in the mean number of SCEs per cell ($P<0.01$). In addition, there is a positive effect of crossbreeding on baseline levels of genome stability in the crossbred group that shows in all tests, excluding gaps, mean values of cellular or chromosome damage similar to the LW group.

Key words: Pig, Calabrian breed, Large White, crossbreeding, sister chromatid exchange, chromosome aberrations.

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Abbreviations: CA – Chromosome Aberration; SCE – Sister Chromatid Exchange; LW – Large White; SD – Standard Deviation; PSS – Porcine Stress Syndrome; BrdU – 5-Bromo-2'-deoxyuridine; FCS – Fetal Calf Serum, F1 – first filial generation, SA – Structural Aberration.

Genome stability ensures accurate transmission of genetic information and a multitude of processes such as control and coordination of cell proliferation, DNA duplication and repair events, preventing the onset of mutations or DNA rearrangements. Despite the control mechanisms regulated by several genes with different functions, such events may occur, and according to their type and incidence, can cause innate genetic variability of a species and of a breed or cell ageing, diseases and predisposition to cancer (AGUILERA & GÓMEZ-GONZÁLEZ 2008).

Several studies report a variable rate of genome stability according to the species analysed, e.g. buffalo (IANNUZZI *et al.* 1988), cattle (CIOTOLA *et al.* 2005; IANNUZZI *et al.* 1991), sheep (DI MEO *et al.* 2000), goat (LOPEZ and ARRUGA 1992; WÓJCIK & SMALEC 2012) and swine (PERETTI *et al.* 2006; RUBES 1987), while only few data are available on variation in genome stability within breeds. In three Italian cattle breeds, Podolian, Friesian and Romagnola, reared under similar conditions, IANNUZZI *et al.* (1991) found different mean values of SCE/cell that were statically significant only between Podolian and Friesian breeds. In addition, WÓJCIK *et al.* (2011) reported statistically significant differences of SCE frequencies in 6 horse breeds (Purebred Arabian, Malopolski horse, Polish noble half-bred, Polish cold-blooded, Hucul and Polish Konik).

The Calabrian pig is an endangered native breed reared in Calabria, a Southern Italian region. This breed is characterized by rusticity, adaptability to pasture and ability to enhance poor foods, converting them into high quality meat for the production of cold cuts. However, low prolificacy and slow growth are the main causes of extinction of this breed (BIGI & ZANON 2008).

As part of a recovery and enhancement project of autochthonous pig breeds of Southern Italy, funded by the Italian Ministry of Agriculture and Forestry (MiPAAF), the genetic combinability of the Calabrian pig with the LW breed, the latter characterized by a high prolificacy rate and an excellent growth index, has been studied in order to obtain a first filial generation (F1) progeny that, thanks to heterosis, has better production traits than the starting breeds.

CA and SCE tests have been used to evaluate genome stability levels in humans (TEKCAN *et al*. 2012; KARAMAN *et al*. 2008; KOMOROWSKI *et al*. 2008) and in major livestock species such as cattle (LIOI *et al*. 2004; CIOTOLA *et al*. 2005; PERETTI *et al*. 2007), river buffalo (PERETTI *et al*. 2008; ALBARELLA *et al*. 2009), goat (DI MEO *et al*. 1993), sheep (PERUCATTI *et al*. 2006) and pig (PERETTI *et al*. 2006; RUBES 1987), either in physiological and pathological (genetic disease and cancer) conditions or after exposition to genotoxic agents.

The aim of this study was to verify if there are differences in genome stability between the autochthonous Calabrian pig breed and a highly selected one such as the Large White (LW) and if genome stability, as other phenotypic traits such as stature, resistance to diseases, fertility and growth index, are influenced by heterosis. In order to evaluate genome stability, cell aneuploidy was calculated and Chromosome Aberration (CA) and Sister Chromatid Exchange (SCE) tests, both expressions of the failure of physiological repair mechanisms for DNA damages that can occur during replication, were applied.

Material and Methods

Genome stability was assessed by aneuploidy, CA and SCE tests in peripheral blood lymphocytes of 10 Calabrian pigs (group 1), 10 LW pigs (group 2) and 19 Calabrian x LW pigs (F1). The animals aged between 8 and 12 months were reared under the same conditions in a farm located in the province of Cosenza and were tested for the absence of the RYR1 g.1843C>T mutation associated with PSS because some autosomal recessive genetic disorders influence genome stability (AL-SWEEDAN *et al*. 2012). The 19 crossbred pigs belonged to 3 different litters, originated by mating three different and unrelated sires of Calabrian breed with three different and unrelated sows of LW breed. Animals were cared for according to guidelines equivalent to those of the Canadian Council on Animal Care.

Two types of cultures were set up for each animal: with and without BrdU for SCE and CA and aneuploidy tests, respectively. Whole blood was added to RPMI 1640 medium enriched with FCS (10%), L-Glutamine (1%) and Lectin (1.5%) and incubated at 37.5°C for 72 h. BrdU (10 μg/ml) was added to the cultures for the SCE test 16 h before harvesting. Colcemid was added to all cultures for 1 h, then the cells were subjected to a hypotonic treatment (KCl 0.5%) and to three fixations in methanol-acetic acid (3:1), the final one overnight. Three drops of cell suspension were air dried on cleaned and wet slides which were stained a day later with acridine orange (0.01% in a phosphate buffer, 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 being stained, or later (1 week). At least 100, 50 and 35 metaphase plates for each animal were observed for aneuploidy, CA (gaps, chromatid and chromosome breaks) and SCE tests, respectively (Fig. 1), under a fluorescence microscope, captured with a digital camera Nikon DS U1, transferred onto a PC and later processed with the software UTHSCSA ImageTool.

Aneuploidy was evaluated as the percentage of cells with $2n \neq 38$; the heterosis effect on aneuploidy, CAs and SCEs was calculated as H= F1 – (group 1 + group 2)/2.

The three groups and the three litters forming F1 were compared with the following statistical tests: (i) average difference evaluation with Student's *t*-test of gaps, chromatid and chromosome breaks, total CAs with and without gaps, aneuploidy, SCEs; (ii) evaluation of group variance difference using the F test; (iii) evaluation of intra-group variance using the $\chi 2$ test. The aims were to verify if the three litters were homogeneous and if the average differences among groups 1, 2 and F1 were statistically significant and related to the breed (groups 1 and 2) or to the heterosis effect (F1).

Results

According to the statistical analysis the three litters can be considered as belonging to the same population.

Cell aneuploidy percentage was higher in F1 (10.79%) than in group 1 (7.30%) and group 2 (10.10%), although the differences among the three groups are not statistically significant; the H value was 2.09.

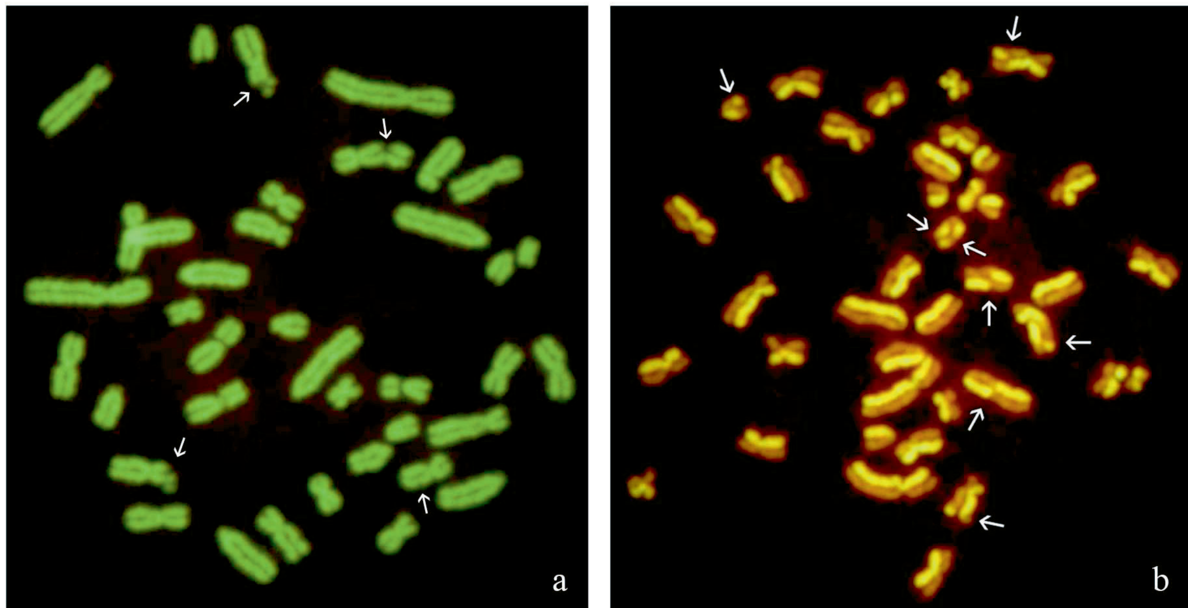

Fig. 1. Metaphase plates of a crossbreed (F1). a – Arrows show SAs. b – Arrows show SCEs.

The mean number of total chromosome aberrations per cell was similar between group 1 (2.86±2.11) and group 2 (2.78±2.00) and higher in F1 (3.54±2.17); however after excluding gaps the mean number of structural aberrations per cell was higher in group 1 (0.14±0.38) than in group 2 (0.11±0.35) and F1 (0.11±0.35) (Table 1). The H value for structural aberrations per cell excluding gaps was -0.015. The mean number of cells with at least one structural aberration, excluding gaps, per animal was higher in group 1 (6.20±2.39) and group 2 (4.90±2.02) than in F1 (4.52±3.34); the H value was – 1.03.

A comparison of the differences in the mean number of chromosome aberrations per cell among the three groups was not statistically significant.

The mean number of SCEs was 7.30±3.24 per cell and 0.19 per chromosome in group 1; 6.45±2.74 per cell and 0.17 per chromosome in group 2; 6.28±2.90 per cell and 0.17 per chromosome in F1. The H value was -0.6.

The mean number and SD of chromosomes per cell without SCEs is higher in F1 than in groups 1 and 2. The mean number and SD of chromosomes per cell with one, two, three and four SCEs is lower in F1 than in groups 1 and 2, although these differences were not statistically significant in comparisons between group 1/group 2; group 1/F1 and group 2/F1. The trend of the distribution of single, double, triple and quadruple SCEs in all groups is equal (Fig. 2). For group 1 the mean number of SCEs per cell was significantly higher than in group 2 and F1 ($P<0.01$), while a comparison of the mean numbers of SCEs per cell between group 2 and F1 did not demonstrate statistically significant differences.

Table 1

Mean and standard deviation of cells with structural aberrations excluding gaps per animal, total aberrations/cell with and without gaps, chromatid breaks, chromosome breaks and gaps in Calabrian (group 1), LW (group 2) and crossbred (F1)

Group	Cells examined *n*	Total aberrations/cell with gaps	Total aberrations/cell without gaps	Chromatid breaks	Chromosome breaks	Gaps	Number of Cells /animal with at least one SA excluding gaps
		Mean/cell ± SD	Mean/cell ± SD	Mean/cell ± SD	Mean/cell ± SD	Mean/cell ± SD	Mean/animal ± SD
1 (10)	500	2.86 ± 2.11	0.14 ± 0.38	0.11 ± 0.34	0.03 ± 0.17	2.72 ± 2.04	6.20 ± 2.39
2 (10)	500	2.78 ± 2.00	0.11 ± 0.35	0.09 ± 0.31	0.01 ± 0.13	2.67 ± 1.90	4.90 ± 2.02
F1 (19)	950	3.54 ± 2.17	0.11 ± 0.35	0.08 ± 0.30	0.02 ± 0.17	3.44 ± 2.10	4.52 ± 3.34

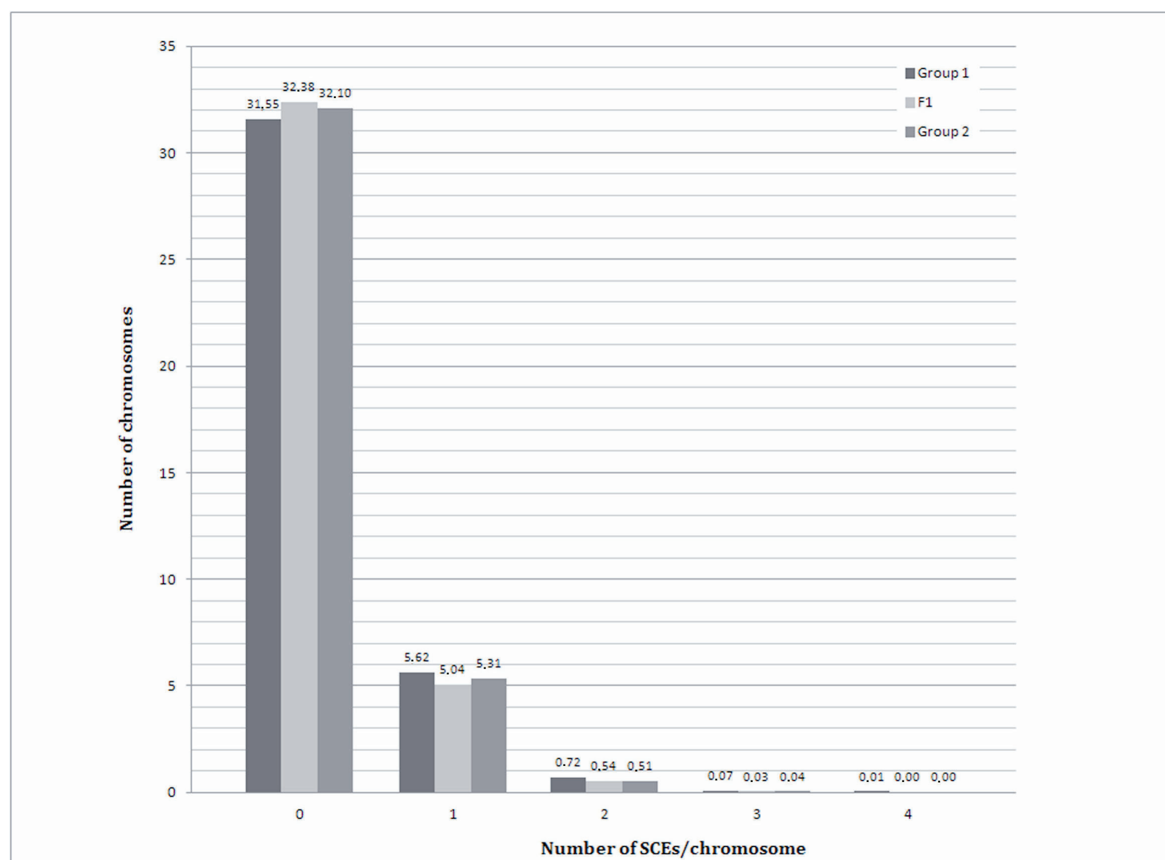

Fig. 2. Trend of the distributions of single, double, triple, quadruple and total SCEs in Calabrian (group 1), crossbreed (F1) and LW (group 2).

Table 2

Distribution of SCEs in Calabrian (group 1), LW (group 2) and crossbred (F1)

Group	N. cells examined	N. chromosome	Mean number and SD of chromosomes/cell					Mean rate and SD of SCEs	
			without SCEs	with SCEs				/cell	/chromosome
				1	2	3	4		
1 (10)	350	13,288	31.55±2.65	5.62±2.39	0.72±0.83	0.07±0.28	0.01±0.09	7.30±3.24^{z}	0.19
2 (10)	350	13,288	32.10±2.40	5.31±2.27	0.51±0.68	0.04±0.20	0	6.45±2.74^{y}	0.17
F1 (19)	665	25,251	32.38±2.63	5.02±2.42	0.54±0.72	0.03±0.20	0	6.28±2.90^{x}	0.17

$^{z.y;\ z.x}$ P<0.01

Discussion

Genome stability can be considered as a complex, polygenic and multifactorial trait. In fact, it is related to the coordinate action of several genes that set DNA duplication and cell proliferation control mechanisms and it is influenced by several factors such as age, nutrition and environment. In particular, several studies have highlighted that this complex trait shows a variability of baseline levels of genome stability among different species either in physiological or pathological (congenital and acquired diseases) conditions. However, few studies have pointed out variability among animal breeds belonging to the same species and none have examined the effect of crossbreeding on genome stability. Crossbreeding is a husbandry technique widely used in swine breeding to improve the production of F1 progeny by mating two individuals of different breeds. This is the first study in which genome stability of two genetically different breeds (a native breed and a highly selected

one) is compared and the influence of heterosis was investigated. By the use of aneuploidy, CA and SCE tests, the Calabrian breed was shown to have higher values than LW and these differences were statistically significant only when the average number of SCEs per cell was compared.

These results confirm that also in swine there is variability in genome stability due to breed differences and it is not possible to attribute such differences only to selective pressure.

Data reported in the literature confirm that the breed significantly affects SCE incidence (RUBES 1987; CATALAN *et al*. 1995; IANNUZZI *et al*. 1991; CIOTOLA *et al*. 2005; WÓJCIK & SMALEC 2011). In the native Casertana breed, PERETTI *et al*. (2006) found an average SCE number per cell of 5.94±2.84 in animals younger than one year and 6.68±2.95 in animals older than one year; RUBES (1987) in pigs belonging to the highly selected breed Landrace, aged between 2 and 3 years, found an average SCE number per cell of 7.73±0.86. Variability in the mean number of SCEs has also been observed in bovines, in the native Podolian – 7.95±3.41 (IANNUZZI *et al*. 1991) and Agerolese – 5.39±2.58 (CIOTOLA *et al*. 2005) and the highly selected breeds Friesian – 7.11±3.35 (IANNUZZI *et al*. 1991) and Romagnola – 7.32±3.18 (IANNUZZI *et al*. 1991).

A positive effect of heterosis, even if only at a low level, was found in CA (excluding gaps) and SCE tests. H values and the statistical analysis suggest the existence of a positive effect of crossbreeding on baseline levels of genome stability in pig. In fact, in all tests the crossbred pigs showed (excluding gaps and aneuploidy) mean values of cell or chromosome damage more similar to the breed with a high rate of genome stability i.e. the LW. This is also confirmed by the fact that in F1 the mean number of cells per animal with SAs (excluding gaps) was lower than in groups 1 and 2 (Table 1). It is interesting to note that the trend of the distribution of single, double, triple and quadruple SCEs in all groups is equal despite the differences of mean number of SCEs per cell, meaning that in swine in physiological conditions even if the number of SCEs increases, their distribution on chromosomes remains unchanged.

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References

AGUILERA A., GÓMEZ-GONZÁLEZ B. 2008. Genome instability: a mechanistic view of its causes and consequences. Nat. Rev. Genet. **9**: 204-217.

ALBARELLA S., CIOTOLA F., DARIO C., IANNUZZI L., BARBIERI V., PERETTI V. 2009. Chromosome instability in Mediterranean Italian buffaloes affected by limb malformation (transversal hemimelia). Mutagenesis **24**: 471-474.

AL-SWEEDAN S.A., KHABOUR O., ISAM R. 2012. Genotoxicity assessment in patients with thalassemia minor. Mutat. Res. **744**: 167-171.

BIGI D., ZANON A. 2008. *Apulo-Calabrese*. (In: Atlante delle razze autoctone. Ed. Edagricole – Edizioni Agricole de Il Sole 24 ORE Business Media s.r.l., Milano): 427-428.

CATALAN J., MORENO C., ARRUGA M.V. 1995. Sister chromatid exchanges in cattle: breed, sex, and BrdU dose effect. Mutat. Res. **331**: 205-211.

CIOTOLA F., PERETTI V., DI MEO G.P., PERUCATTI A., IANNUZZI L., BARBIERI V. 2005. Sister chromatid exchanges (SCE) in the Agerolese cattle population. Vet. Res. Commun. **29**: 359-361.

DI MEO G.P., IANNUZZI L., PERUCATTI A., FERRARA L., PIZZILLO M., RUBINO R. 1993. Sister chromatid exchange in the goat (*Capra hircus*). Hereditas **118**: 35-38.

DI MEO G.P., PERUCATTI A., FORNATARO D., INCARNATO D., FERRARA L., MATASSINO D., IANNUZZI L. 2000. Sister chromatid exchange in chromosomes of sheep (*Ovis aries*). Cytobios **101**: 71-78.

IANNUZZI L., PERUCATTI A., DI MEO G.P., FERRARA L. 1988. Sister chromatid exchange in chromosomes of river buffalo (*Bubalus bubalis* L.). Caryologia **41**: 237-244.

IANNUZZI L., DI MEO G.P., PERUCATTI A., FERRARA L., GUSTAVSSON I. 1991. Sister chromatid exchange in chromosomes of cattle from three different breeds reared under similar conditions. Hereditas **114**: 201-215.

KARAMAN A., BINICI D.N., KABALAR M.E., DURSUN H., KURT A. 2008. Alteration of sister chromatid exchange frequencies in gastric cancer and chronic atrophic gastritis patients with and without *H. pylori* infection. World J. Gastroenterol. **14**: 2534-2539.

KOMOROWSKI J.R., GREENBERG D., JUTURU V. 2008. Chromium picolinate does not produce chromosome damage. Toxicol. In Vitro **22**: 819-826.

LIOI M.B., BARBIERI R., BORZACCHIELLO G., DEZZI S., ROPERTO S., SANTORO A., RUSSO V., ROPERTO F. 2004. Chromosome aberration in cattle with chronic enzootic haematuria. J. Comp. Path. **113**: 233-236.

LOPEZ N.L., ARRUGA M.V. 1992. Sister chromatid exchanges (SCEs) analysis in goats. Caryologia **45**: 135-144.

PERETTI V., CIOTOLA F., DARIO C., ALBARELLA S., DI MEO G.P., PERUCATTI A., BARBIERI V., IANNUZZI L. 2006. Sister chromatid exchange (SCE) for the first time in Casertana pig breed. Hereditas **143**: 113-116.

PERETTI V., CIOTOLA F., ALBARELLA S., RUSSO V., DI MEO G.P., IANNUZZI L., ROPERTO F., BARBIERI V. 2007. Chromosome fragility in cattle with chronic enzootic haematuria. Mutagenesis **22**: 317-320.

PERETTI V., CIOTOLA F., ALBARELLA S., RESTUCCI B., MEOMARTINO L., FERRETTI L., BARBIERI V., IANNUZZI L. 2008. Increased SCE levels in Mediterranean Italian buffaloes affected by limb malformation (transversal hemimelia). Cytogenet. Genome Res. **120**: 183-187.

PERUCATTI A., DI MEO G.P., ALBARELLA S., CIOTOLA F., INCARNATO D., JAMBRENGHI A.C., PERETTI V., VONGHIA G., IANNUZZI L. 2006. Increased frequencies of both chromosome abnormalities and SCEs in two sheep flocks exposed to high dioxin levels during pasturage. Mutagenesis **21**: 67-75.

Rubes J. 1987. Chromosomal aberrations and sister-chromatid exchanges in swine. Mutat. Res. **191**: 105-109.

Tekcan A., Elbistan M., Ulusoy A.N. 2012. Sister chromatid exchanges in breast cancer patients who underwent chemotherapy. J. Toxicol. Sci. **37**: 235-243.

Wójcik E., Smalec E., Danielewicz A. 2011. Sister chromatid exchange in selected horse breeds (*Equus caballus*). Arch. Tierz. **54**: 107-114.

Wójcik E., Smalec E. 2012. Sister Chromatid Exchange in Polish White Improved Goats (*Capra hircus*). Folia Biol. (Kraków) **60**: 141-146.