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The Effect of Bromodeoxyuridine on Spontaneous Sister Chromatid Exchange Frequency in Rabbit (*Oryctolagus cuniculus*) and Coypu (*Myocastor coypu*) Chromosomes

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The sister chromatid exchange test is regarded as a highly sensitive cytogenetic assay. It measures chromosome sensitivity to particular damage factors and provides information on control and repair mechanism performance. It is instrumental in the early identification of the effects of noxious factors present in the habitat. This investigation was aimed at identifying sister chromatid exchange sites in coypu and rabbit chromosomes, as well as determining the spontaneity of the process by applying different BrdU doses. The chromosomes were obtained from an *in vitro* culture of blood lymphocytes, supplemented with 4 different BrdU doses: 0.25/0.5/1.0/2.5 µg/ml in order to identify spontaneous sister chromatid exchanges in both animal species. The chromosomes were stained according to the FPG method. Spontaneous SCEs were observed in coypu at a concentration of 1.0 µg/ml, and in rabbits at 0.5 µg/ml. The mean SCE/cell incidence was 1.41±1.15 in coypu, and 2.69±2.14 in rabbits. Differences in SCE incidence were identified between the analysed animal species and the applied BrdU doses.

Key words: coypu; rabbit; mitotic chromosome; sister chromatid exchange.

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Recent decades have seen cytogenetic studies of the influence of environmental factors, xenobiotics and other pollutants entering animal organisms along with consumed food and inhaled air. Such substances negatively affect the genetic material (BILBAN & JACOBIN-BILBAN 2005; WÓJCIK & SMALEC 2013). The determiners of the consequences of organism exposure to the influence of exogenous factors include both cytogenetic and molecular biological markers. Cytogenetic markers are considered to be important tools in disease diagnostics. Biomonitoring tests can be used for screening to enable the detection of the risk of disease development prior to the appearance of typical clinical symptoms, i.e. at a stage which allows appropriate preventive action (BONASII 1999). They consist in the determination of the degree of toxicity of environmental factors by means of the above assays, based on the reactions of the living organism. The analysis involves various tissues used to assess the intensity of the exogenous factors and their role in inducing changes in DNA, chromatin and chromosome structures. Exogenous substances can exert influence on the cell either directly or indirectly, as metabolites, causing interactions between the noxious factor and the cell.

An important diagnostic tool for chromosome instability is the sister chromatid exchange test (SCE). It can help identify the earliest effects of the action of environmental genotoxic factors (WÓJCIK & SMALEC 2013). It is considered to be one of the less labour-consuming, and highly sensitive, cytogenetic assays. The degree of chromosome dam-

age in the animal organism is measured by the frequency of exchanges between sister chromatids. The frequency is a measurable biomarker of sensitivity to exogenous genotoxic, mutagenic and carcinogenic factors and of the innate or acquired capacity of the organism to respond to exposure to a specific noxious factor. The detection of the instabilities at an early stage make it possible to introduce possible changes in habitat conditions, thus enabling potential reversion of certain types of damage, since the reversal of SCE changes is much quicker than that of chromosome aberrations (MIELŻYŃSKA 2007). The mechanism of the exchanges has not been fully explored. SCE consists of mutual swapping of homologous chromatid fragments in the same chromosome, in the aftermath of single-strand or double-strand DNA breaks occurring during DNA replication (WILSON & THOMPSON 2007). Detected unrepaired or incorrectly repaired instances of damage can be observed as discontinuities or asymmetries in the chromatid staining pattern in the chromosome (GERMAN & ALHADEFF 2001). The essence of the SCE tests consists of using the process of DNA base analogue incorporation during replication. SCEs can spontaneously occur during the cell cycle or be additionally induced by various mutagenic factors. Studies using the SCE test conducted to date in man and selected animal species have shown that SCEs are characterised by considerable species-related conservatism (IANNUZZI *et al.* 1991b; DI MEO *et al.* 1993, 2000; WÓJCIK *et al.* 2011, 2012). This principally refers to SCE location in the chromosomes and the concentrations of the base analogues, e.g. BrdU, applied in the SCE test (DI BERARDINO *et al.* 1996).

Coypu (*Myocastor coypu*) and rabbit (*Oryctolagus cuniculus*) are herbivorous mammals. Coypus originate from the southern part of South America (GOSLING & BERKER 1991). Coypu farming in European countries started only in the 1920s (CARTER & LEONARD 2002). Coypu breeding went into significant decline in Poland in the 1990s. Despite the diminished interest in the breeding of the species, numerous studies have been conducted, e.g. to analyse meat quality, determine the genetic diversity of populations, and investigate coypu genetics and cytogenetics (CALLAHAN *et al.* 2005; JUNG & JO 2012). The coypu has been insufficiently explored in cytogenetic terms. Its chromosome number is 2n=42. All the autosomes have two arms: 32 of them metacentric and 8 submetacentric. The X sex chromosome is metacentric, and Y – the smallest of all – is acrocentric (FREDGA 1966, TSIGALIDOU 1966; HSU & BENIRSCHKE 1971; ILIKER *et al.* 2009). The banding pattern standard for coypu chromosomes has not been published, although several reports have offered preliminary distributions of C bands in the chromosomes (PIEŃKOWSKA *et al.* 1994; ILIKER *et al.* 2009; KUCHTA *et al.* 2009). Moreover cytogenetic analysis of nucleolar organizer regions (NORs) revealed the presence of 2 active NORs in the coypu karyotype (PIEŃKOWSKA & ŚWITOŃSKI 1993).The use of the G-banding method has produced a more detailed characterization of the karyotype (LUNGEANU *et al*. 1980).

Initially, rabbits were indigenous to the Iberian Peninsula, the south of France and North Africa. Later, they spread across Europe and Asia. In Poland, rabbits appeared in 1860. Unfortunately, there has been reduced interest in the animals since the 1980s. As a result, their population considerably diminished (PINKOWSKI 1994).

Cytogenetically, rabbits have been more thoroughly investigated than coypu. The diploid chromosome number of this species is 2n=44. The karyotype chromosomes are divided into three morphological groups: metacentric (34 autosomes), acrocentric (8 autosomes) and submetacentric (sex chromosomes) (ROBINSON *et al.* 2002). The rabbit karyotype pattern of G, Q, R, C and NOR banded chromosomes has been determined (HAGELTRON & GUSTAVSSON 1979; POULSEN *et al.* 1988; MARTIN-DELEON *et al.* 1978; ŚWITOŃSKI *et al.* 1982; YERLE *et al.* 1987; RØNNE 1995; KUCHTA *et al.* 2009) and banding patterns have also been established. The rabbit karyotype has been used for comparisons with the human karyotype (DUTRILLAUX 1980). Comparisons of genomes of different species are feasible due to karyotypic genetic conservatism including chromosome banding patterns, nucleotide sequences and conjugated gene groups. Genetic conservatism is often analysed in species belonging to the same family (IANNUZZI & DI MEO 1995; ANSARI *et al.* 1999).

The present work was aimed at identifying the sites of sister chromatid exchanges in the coypu and rabbit chromosomes, as well as determining the spontaneity of the process by applying different BrdU doses.

Material and Methods

The research was carried out on a group of 10 coypu (*Myocastor coypu*) and 10 domestic rabbit (*Oryctolaguc cuniculus*) males. All the animals were aged 7-8 months. The research material was constituted by full peripheral blood taken from *V. marginal ear* (rabbits) and *V. cephalica antebrachi* (coypu). All the animals came from the same type of environment (rural). The cytogenetic analyses were performed on chromosomes obtained from an *in vitro* lymphocyte culture main-

tained for 72 hours at 37°C. At the 36th hour of the culture, BrdU was added at different concentrations: 0.25 µg/ml, 0.5 µg/ml, 1.0 µg/ml and 2.5 µg/ml according to applicable procedure (DI BERARDINO *et al.* 1995, 1996). Only a set index of BrdU was applied in the case of the other animals.

A harlequin structure of chromosomes was revealed on the basis of the fluorescence plus Giemsa (FPG) technique according to KIHLMAN and KRONBORG (1975). The procedure of FPG staining consisted of two stages: preliminary digestion and Giemsa staining. In the first stage, the chromosomes were digested with a RNAse solution (10 µg/ml) for 1h; incubated in Hoechst's solution 33258 (0.5 µg/ml) for 1h; and exposed to UV radiation for 1h. The next incubation was carried out at 4°C in darkness for 24 h. On the following day, the procedure of exposure to UV radiation for 0.5 h was repeated; the preparations were incubated in 0.5xSSC (0.75M Sodium Chloride + 0.075 Sodium Citrate, pH=7.0) for 2h, at 58°C degrees; and, finally, the preparations were stained with a 3% Giemsa solution (pH=6.8) for 45 min.

The preparations were analysed using a NIKON Eclipse 80i microscope, and the estimation of SCEs was conducted using the NIS Elements F2.30 program for computer-based analysis of images. 50 metaphases were analysed for each animal. SCEs were identified in all the chromosomes in the karyotype. The total SCE number was determined as the sum of three different forms of SCEs (interstitial, terminal and centromeric). 500 metaphases were analysed for each species. The SCEs identified in the chromosomes of the animals were profiled by means of the arithmetic mean and standard deviation. The statistical assessment was based on the chi-square test.

Results

The mean SCE/cell incidence was 1.41±1.15 in the coypu, and 2.69±2.14 in the rabbits. The four different BrdU doses applied in the coypu and rabbit chromosome cultures permitted the determination of the BrdU portion at which spontaneous sister chromatid exchange occurs in the cell. The lowest BrdU concentration (0.25 µg/ml) applied in the chromosome cultures, in the case of both the coypu and rabbits, was too small to obtain a stained chromosome structure showing sister chromatid exchanges. The concentration of 0.5 µg/ml in the coypu revealed a very poorly stained chromosome structure with sister chromatid exchanges. SCE identification was highly impeded. The concentration of 0.5 µg/ml was found to be too low to carry out a viable test for the coypu. This concentration proved, in turn, to be sufficient to identify spontaneous SCEs in the rabbit cells. The BrdU concentration of 1 µg/ml was assumed as the dose at which spontaneous SCEs are observed in coypu chromosomes. On the other hand, the BrdU concentration of 1ìg/ml turned out to induce SCEs in the rabbits. The highest BrdU concentration of 2.5 µg/ml applied in the analyses was determined to induce the exchanges (Fig. 1 and Fig. 2). The different exchange frequencies within a species observed with the different BrdU doses are presented in Table 1. The differences were confirmed statistically (P=0.00000). We also identified differences in SCE frequency and between the applied BrdU concentrations in these species (apart from the concentration of 0.25 µg/ml) (P=0.000000).

A detailed chromosome analysis revealed which part of the chromosome underwent the most numerous exchanges. This was the distal chromosome region in the coypu, at all three applied BrdU concentrations. At the lowest BrdU concentration (0.5 µg/1 ml of culture), no sister chromatid exchanges were observed in the interstitial and proximal q chromosome arms. Sporadic SCEs were identified in the distal and proximal region of the p arm. The least frequent exchanges at the concentrations of 1.0 and 2.5 µg BrdU/1 ml of culture took place in the interstitial area. In the rabbits, the most frequent swaps occurred in the proximal parts of the chromosomes. The same as in the case of the coypu, more SCEs were observed in the distal regions of the rabbit chromosomes at the concentra-

Table 1

Sister chromatid exchanges in coypu and rabbit chromosomes, depending on the applied BrdU concentration in the *in vitro* culture

Species	BrdU concentration (µg/ml of culture medium)				
	0.25	0.5	1.0	2.5	Total
Coypu	0	*0.08±0.14	1.39±0.32	2.78±0.16	1.41±1.15
Rabbit	0	1.99±1.02	2.60±2.08	3.49±2.47	2.69±2.14

*Mean±SD

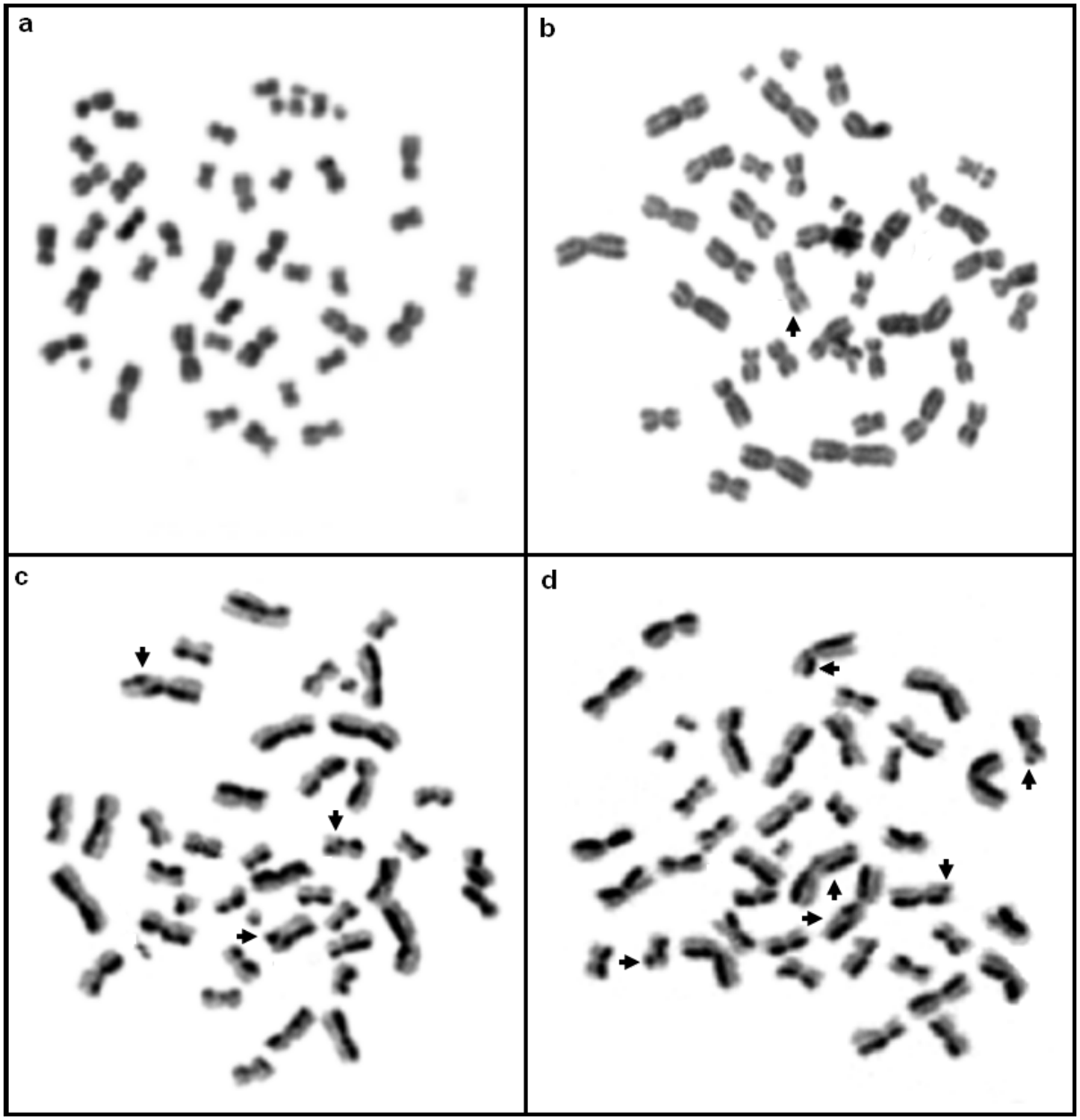

Fig. 1. The effect of BrdU concentration on the numbers of sister chromatid exchanges in the Coypu karyotype: a) 0.25µg/ml, b) 0.5µg/ml, c) 1.0µg/ml, d) 2.5µg/ml. Arrows indicate SCEs.

tion of 0.5 µg/ml. At the other concentrations, SCE incidence varied in the particular chromosome regions. The most numerous exchanges were proximal and distal, with the fewest in the interstitial area. Fig. 3 shows the frequency distribution of sister chromatid exchanges (%) in the particular chromosome regions in both species. Moreover, a tendency was observed in the rabbits for more numerous chromatid cracks and regroupings to appear in the p arm (54 %) than in the q arm (46 %). A similar regularity was also observed in the coypus. More sister chromatid exchanges were identified in the p arm (72 %) than in the q arm (28 %) in this species.

Discussion

The sister chromatid exchange test enables the detection of the mutual exchange of DNA replication products between chromatids within the same chromosome. The observation of differentially

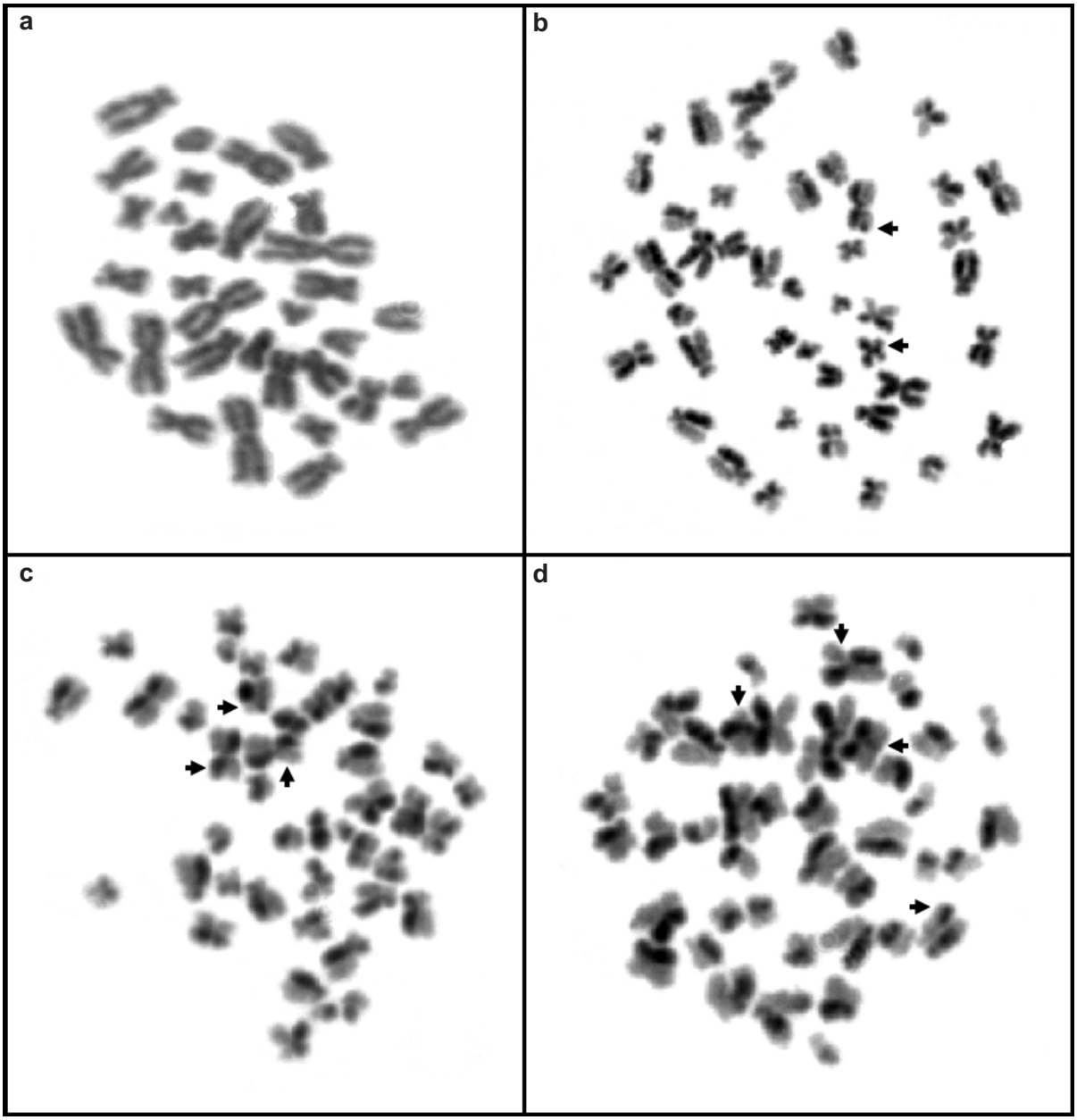

Fig. 2. The effect of BrdU concentration on the numbers of sister chromatid exchanges in the rabbit karyotype: a) 0.25µg/ml, b) 0.5µg/ml, c) 1.0µg/ml, d) 2.5µg/ml. Arrows indicate SCEs.

stained chromatids is possible through the incorporation of bromodeoxyuridine in the DNA chain. BrdU is a thymidine analogue which helps visualise spontaneous SCEs. However, at excessive concentrations it induces additional exchanges. The BrdU levels must be high enough to enable the observation of the exchanges without causing additional ones due to dosing excess. The selection of an appropriate BrdU dose is a very important step in the standardisation of the methodology for a given species. Four different BrdU concentrations were used in the present study to observe spontaneously occurring exchanges. We also tested the size of the BrdU dose that causes SCE induction. The lowest dose that reveals spontaneous SCEs in coypu cells was found to be 1.0 µg/ml, and 0.5 µg/ml in rabbits. Higher BrdU concentrations induced additional damage. Most researchers consider the standard BrdU dose to be 10 µg/ml of culture (NICOLAE *et al.* 2009; WÓJCIK *et al.* 2011; WÓJCIK & SMALEC 2012a, b). Scientists applied various BrdU doses in this type of research from

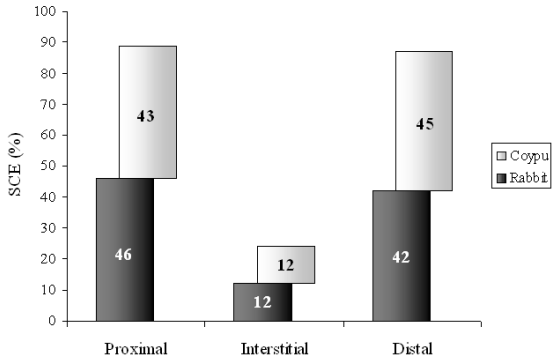

Fig. 3. Percentage distribution of SCEs in the coypu and rabbit in the particular chromosome regions (proximal, interstitial and distal region).

0.1 µg/ml of culture to 120 µg/ml (LEIBENGUHT & THIEL 1986; VIJH *et al*. 1992; DI BERARDINO *et al.* 1996, 1997; SZELESZCZUK *et al.* 2014). According to WILSON and THOMPSON (2007), the level of the analogue added to chromosome culture should be very low, approaching 0, since only then is it possible to observe the spontaneous phenomenon during the cell cycle. BrdU added to chromosome culture during DNA incorporation leads to a rise in the level of single DNA strand damage. The higher the analogue dose, the higher the DNA damage level.

Apart from detectability with BrdU, sister chromatid exchanges are characterised by substantial species-related conservatism relating to the frequency of their occurrence in cells. We determined spontaneously occurring SCEs in the coypu and rabbit chromosomes at 1.39 and 1.99, respectively. Exchange incidence has different profiles in different species: 2.3-6.2 in the Chinese hamster (WÓJCIK *et al.* 2003), 2.48 in cattle (DI BERARDINO *et al.* 1995), 3.28 in goats (DI MEO *et al.* 1993; DI BERARDINO *et al.* 1996; WÓJCIK & SMALEC 2012a), 3.6-6.6 in horses (WÓJCIK *et al.* 2011), 3.4 in cats (SZELESZCZUK *et al.* 2014), 7.8 in chickens (WÓJCIK *et al.* 2012) and 6.04 in quail (WÓJCIK *et al.* 2014). Different frequencies observed within a species can stem from the specific breeds of the animals, as well as individual differences such as age and sex. These factors significantly affect SCE incidence (CATALAN *et al.* 1995; IANNUZZI *et al.* 1991a; CIOTOLA *et al.* 2005; PERETTI *et al.* 2006).

Another factor that can induce SCEs is the habitat. Some physical, chemical and biological factors have mutagenic effects on DNA and contribute to chromatin stability disruption (WÓJCIK & SMALEC 2013; GENAUALDO *et al.* 2015). In order to eliminate the influence of additional factors, such as breed, age and sex, two animal species, representing the same breed within a species, of the same sex (males only) and the same age were analysed in this study. The results of instability identification in such a uniform group of animals not only provide species profiles but can also be taken advantage of to assess individual genetic resistance. Despite similar exposure of the analysed animals to environmental conditions, different SCE frequencies were obtained, which can reflect innate or acquired resistance to specific noxious effects of the environment. Varied response of the organism is a specific sensitivity biomarker, enabling the detection of changes preceding the appearance of a disease so that appropriate prophylactic action can be taken.

The sister chromatid exchange site in a chromosome is not accidental. In man and cattle, SCEs have been predominantly detected in the G bands and interstitial bands (LATT 1974; IANNUZZI *et al.* 1991a). In goats and horses, in turn, they have been found at the point of contact between heterochromatin and euchromatin (WÓJCIK & SMALEC 2012a, 2013). In the Chinese hamster, rats and mice, SCEs predominantly occurred in the proximal region. This area is characterised by a high level of DNA repetitions, vulnerability to recombinational chromosome cracks, and thus SCE induction (MARTIN & PRESCOTT 1964; GIBSON & PRESCOTT 1972; LIN & ALFI 1976). In chickens the exchanges mainly took place in the proximal region of the chromosomes, where constitutive heterochromatin segments are located (WÓJCIK *et al.* 2013). In the present work, high SCE incidence was observed in the proximal and distal regions of the coypu and rabbit chromosomes. In the coypu, these regions are largely formed by constitutive heterochromatin (ILIKER *et al.* 2009). Heterochromatin is principally located in the centromere region in rabbits as well. However, it has also been identified in telomeres (ŚWITOŃSKI *et al.* 1982; KUCHTA *et al.* 2009).

We observed a very interesting regularity in our study. Namely, the applied dose of 0.5 µg/ml BrdU induced SCEs chiefly in the distal chromosome area both in rabbits and coypu. Higher BrdU concentrations induced exchanges in the distal, proximal and interstitial regions. Such sensitivity of the distal chromosome area can correspond with high susceptibility of the chromosome to damage even at the lowest mutagene concentrations. The distal region contains telomeric and subtelomeric sequences which are vulnerable to damage caused by oxidative stress exerted by free oxygen radicals (KAWANISHI & OIKAWA 2004; CHEN *et al.* 2007; BAIRD 2008). UV radiation can also contribute to DNA damage and SCE induction (WÓJCIK *et al.* 2003; JIN & IKUSHIMA 2004). High SCE incidence in the distal chromosome region has been

observed by WÓJCIK and SMALEC (2013) in horses (33%), and SZELESZCZUK *et al.* (2014) in cats (65%). According to BLAGOEV *et al*. (2010), telomeres are hot spots of sister chromatid exchange. The SCE incidence result in this region can be regarded as an index of the ageing process. Accumulated and unrepaired or incorrectly repaired damage, mutations and replication errors give rise to high SCE incidence and survival of cells containing damaged genetic material which contributes to neoplastic transformations (AMOR-GUERET 2006).

Applied for the assessment of chromosome instability in standardised conditions, the sister chromatid exchange test can help diagnose damage resulting from mutagenic, genotoxic and carcinogenic factors. It is a measure of chromosome sensitivity to a specific impairing factor and provides information on the degree of control and repair mechanism performance. It can also serve as a reliable and highly sensitive tool for the assessment of the genetic resistance of an animal, as well as lending itself to comparisons of two different animal species.

References

AMOR-GUERET M. 2006. Bloom syndrom, genomic instability and cancer: the SOS-like hypothesis. Cancer Lett. **236**: 1-12.

ANSARI H.A., BOSMA A.A., BROAD T.E., BUNCH T.D., LONG S.E., MAHER D.W., PEARCE P.D., POPESCU C.P. 1999. Standard G-, Q- and R-banded ideogrames of the domestic sheep (*Ovis aries*): homology with cattle (*Bos Taurus*). Report of the committee for the standardization of the sheep karyotype. Cytogenet. Cell Genet. **85**: 317-324.

BAIRD D.M. 2008. Mechanisms of telomeric instability. Cytogenet. Genome Res. **122**: 308-314.

BILBAN M., JACOBIN BILBAN C. 2005. Incidence of cytogenetic damage in leadzinc mine workers exposed to radon. Mutagenesis **20**: 187-191.

BLAGOEV K.B., GOODWIN E.H., BAILEY S.M. 2010. Telomere sister chromatid exchange and the process of aging. Aging-US **2**: 727-730.

BONASII S. 1999. Combining environment al exposure and genetic effect measurements In health outcome assessment. Mutat. Res. **428**: 177-185.

CALLAHAN C., HENDERSON A., EACKLES M., KING T. 2005. Microsatellite DNA markers for the study of population structure and dynamics in nutria (*Myocastor coypus*). Mol. Ecol. Notes **5**: 124-126.

CARTER J., LEONARD B.P. 2002. A review of the literature on the worldwide distribution, spread of, and efforts to eradicate the coypu (*Myocastor coypus*). Wildl. Soc. Bul. **30**: 162-175.

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

CHEN J.H., OZANNE S.E., HALES C.N. 2007. Methods of cellular senescence induction using oxidative stress. Methods Mol. Biol. **371**: 179-189.

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 BERARDINO D., LIOI B.M., SCARFI M.R., JOVINO V., MARIGLIANO P. 1995. Spontaneous sister chromatid exchanges in mitotic chromosomes of cattle (*Bos taurus*). Genet. Sel. Evol. **27**: 385-393.

DI BERARDINO D., JOVINO V., LIOI B.M., SCARFI M.R., BURGUETE I. 1996. Spontaneous rate of sister chromatid exchange (SCEs) and BrdU dose – response in mitotic chromosomes of goat (*Capra hircus*). Hereditas **124**: 137-143.

DI BERNARDINO D., LIOLI M.B., CRASTO A., JOVINO V., SCARFI M.R., BURGUETE I. 1997. Differential sister chromatid exchange response in phtohemagglutinin and pokeweed stimulated lymphocytes of goat (*Capra hircus* L.). Genet. Sel. Evol. **29**: 185-192.

DI MEO G.P., IANUZZI L., PERUCATTI A., FERRARA L., PIZILLO 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.

DUTRILLAUX B.R. 1980. Chromosomes banding analogies between a prosimians (*Microcebus murinus*), a Platyrrhini (*Cebus capucinus*) and man. Amer. J. Phys. Anthropol. **52**: 127-133.

FREDGA K. 1966. Chromosomes studies in five species of South American rodents (Suborder: *Histricomorpha*). Mamm. Chrom. Newsletter **20**: 45.

GENUALDO V., PERUCATTI A., PAUCIULLO A., IANNUZZI A., INCARNATO D., SPAGNUOLO M. S., SOLINAS N., BULLITTA S., IANNUZZI L. 2015. Analysis of chromosome damage by sister chromatid exchange (SCE) and redox homeostasis characterization on sheep flocks from Sardinian pasturelands. Sci. Total. Environ. **15**: 393-400.

GERMAN J., ALHADEFF B. 2001. Analysis of sister chromatid exchange. Curr. Protoc. Hum. Genet. **8**: Unit 8.6.

GIBSON D.A., PRESCOTT D.M. 1972. Introduction of sister chromatid exchanges in chromosomes of rat kangaroo cells by tritium incorporated into DNA. Exp. Cell Res. **74**: 397-402.

GOSLING L.M., BAKER S.J. 1991. *Family Myocastoridae*. (In: Handbook of British Mammals. Blackwell Scientific, London): 267-275.

HAGELTORN M., GUSTAVSSON I. 1979. Identification by banding techniques of the chromosomes of the domestic rabbit (*Oryctolagus cuniculus* L.). Hereditas **90**: 269-279.

HSU T.C, BENIRSCHKE K. 1967. *Rodentia*. (In: Atlas of mammalian chromosomes. T.C. Hsu and K. Benirschke eds Springer-Verlag, New York): 213-227.

IANNUZZI L., DI MEO G.P., PERUCATTI A., FERRARA L., GUSTAVSSON I. 1991a. Sister chromatid exchange in cattle marker chromosomes. Caryologia **44**: 145-152.

IANNUZZI L., DI MEO G.P., PERUCATTI A., FERRARA L., GUSTAVSSON I. 1991b. Sister chromatid exchange in chromosomes of cattle from three different Breeds reared under similar conditions. Hereditas **114**: 201-205.

IANNUZZI L., DI MEO G.P. 1995. Chromosomal evolution in bovids: a comparison of cattle, sheep and goat G- and R-banded chromosomes and cytogenetic divergences among cattle, goat and river buffalo sex chromosomes. Chromosome Res. **3**: 291-299.

ILIKER A., ARSLAN A., PAMUKOĞLU N., ALBAYRAK I. 2009. C-banded karyotype of *Myocastor coypus* (Molina, 1782) from Turkey (Mammalia: Rodentia). Folia Biol. (Kraków) **57**: 33-36.

JIN G., IKUSHIMA T. 2004. Frequent occurrence of UVB-induced sister chromatid exchanges in telomere regions and its implication to telomere maintenance. Cytogenet. Genome Res. **104**: 310-314.

JUNG J., JO Y.S. 2012. A preliminary study of genetic structure and relatedness analysis of Nutria (*Myocastor coypus* in Upo Wetland). J. Species Res. **1**: 100-103.

KAWANISHI S., OIKAWA S. 2004. Mechanism of telomere shortening by oxidative stress. Ann. Acad. Sci. **1019**: 278-284.

KIHLMAN B.A., KRONBORG D. 1975. Sister chromatid exchanges in *Vicia faba*. I. Demonstration by a modified fluorescent plus Giemsa (FPG) technique. Chromosoma **51**: 1-10.

KUCHTA M., SZLESZCZUK O., NIEDBAŁA P., ONDERKA A. 2009. Comparative analysis of heterochromatin distribution in the coypu (*Myocastor coypus*) and rabbit (*Oryctolagus cuniculus*) sex chromosomes. Acta Sci. Biol. Sciences **9**: 37-43.

LATT S.A. 1974. Localization of sister chromatid exchanges in human chromosomes. Science **185**: 74-76.

LEINBENGUTH F., THIEL G. 1986. BrdU and EMS dependent sister chromatid exchange and chromosomal breaks in cattle. Arch. Zoot. **35**: 1-8.

LIN M.S., ALFI O.S. 1976. Detection of sister chromatid exchanges by 4-6-diamidino-2-phenylindole fluorescence. Chromosoma (Berl) **57**: 219-225.

LUNGEANU A., VIOCULESCU J., AVRAM N. 1980. Cytogenetic investigation in farm fur-bearing animals. Proc. 4th European Colloquium on Cytogenetics of Domestic Animals, Uppsala, Sweden: 412-418.

MARTIN G., PRESCOTT D.M. 1964. The frequency of sister chromatid exchanges following exposure to varying doses of H3 thymidyne or X-rays. J. Cell Biol. **21**: 159-167.

MARTIN-DELEON P., PETROSK D.L., FLEMING M.E. 1978. Nucleolar organizer regions in the rabbit oryctolagus cuniculus as shown by silver staining. *C*an. J. Genet. Cytol. **20**: 377-382.

MIELŻYŃSKA D. 2007. Exposure to carcinogenic substances – exposure biomarkers. http://www.srodowiskoazdrowie.pl

NICOLAE I., ENCULESCU M., VIDMICHI D., PARASCHIVESCU M., BOTA A., HARCEAGA L. 2009. Sister chromatid exchange in River Buffalo females with chromosome al fragility. Zootehnie şi Biotehnologii **42**: 64-69.

OSSOWSKA A., BOGDZIŃSKA M., KAMIŃSKI P. 2008. Environmental pollution and changes within of the chromosomes. Prace Komisji Nauk Rolniczych i Biologicznych BTN, B, 64. (In Polish).

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.

PIEŃKOWSKA A., ŚWITOŃSKI M. 1993. Nucleolar organizer regions (NORs) in mammals: an pccurrence and size polymorphism. Post. Biol. Kom. **20**: 135-141.

PIEŃKOWSKA A., ŚWITOŃSKI M., RZEPNY J. 1994. Occurrence of constitutive heterochromatin in the karyotype of the Coypu (Myocastor Corpus). Genetica Polonica **3**: 205-210.

PINKOWSKI M. 1994. The wild rabbit – attempts at reintroduction. Łowiec Polski **7**: 8. (In Polish).

POULSEN B.S., SHIBASAKI Y., RONNE M. 1988. The high resolution R-bamded karyotype of *Oryctolagus cuniculus L*. Hereditas **109**: 57-60.

ROBINSON T.J., YANG F., HARRISON W.R. 2002. Chromosome painting refines the history of genome evolution in hares and rabbits (order Lagomorpha). Cytogenet. Genome Res. **96**: 223-227.

RŘNNE M. 1995. The high resolution RBG-banded karyotype of *Oryctolagus cuniculus*. Comparison between different classifications of landmarks and bands. In Vivo **9**: 239-245.

SZELESZCZUK O., KUCHTA-GŁADYSZ M., WÓJCIk E., OTWINOWSKA-MINDUR A., WOJNAR T., GODYNIA B., NIEDBAŁA P. 2014. Sister chromatid exchange analysis in cats (*Felis catus*). Arch. Tierz. **57**: 1-10.

ŚWITOŃSKI M., STRANZINGER G., FRIES R. 1982. C-band polymorphism in the karyotype of the rabbit (*Orychtolagus cuniculus*). J. Anim. Breed. Genet. **100**: 390-400.

TSIGALIDOU V., SIMOTAS A.G., FASCULAS A. 1966. Chromosome of the coypu. Nature **211**: 994-995.

VIJH R.K., SAHAI R., SHARMA A., GUPTA I.D. 1992. Sister chromatid exchange frequency in Sahiwal cattle (*Bos indicus*). Indian J. Anim. Sci. **62**: 473-476.

WILSON D.M., THOMPSON L.H. 2007. Molecular mechanisms of sister chromatid exchange. Mutat. Res. **616**: 11-23.

WÓJCIK A., SONNTAG C., OBE G. 2003. A pplication of the biotin-dUTP chromosome labelling technique to study the role of 5-bromo-29-deoxyuridine in the formation of UV-induced sister chromatid exchanges in CHO cells. J. Photochem. Photobiol. B, Biol. **69**: 139-144.

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

WÓJCIK E., ANDRASZEK K., GRYZIŃSKA M., WITKOWSKI A., PAŁYSZKA M., SMALEC E. 2012. Sister chromatid exchange in Greenleg Partridge and Polbar hens covered by the gene-pool protection program for farm animals in Poland. Poult. Sci. **91**: 2424-2430.

WÓJCIK E., SMALEC E. 2012a. Sister chromatid exchange in polish white improved goats (*Capra hircus*). Folia Biol. (Kraków) **3-4**: 141-146.

WÓJCIK E., SMALEC E. 2012b. Assessment of chromosome instability in geese (*Anser anser*). Can. J. Anim. Sci. **92**: 49-57.

WÓJCIK E., ANDRASZEK K., CISZEWSKA M., SMALEC E. 2013. Sister chromatid exchange as an index of chromosome instability in chondrodystrophic chickens (*Gallus domesticus*). Poult. Sci. **92**: 84-89.

WÓJCIK E., SMALEC E. 2013. The effect of environmental factors on sister chromatid exchange incidence in domestic horse chromosomes (*Equus caballus*). Folia Biol. (Kraków) **61**: 199-204.

WÓJCIK E., ANDRASZEK K., SMALEC E., KNAGA S., WITKOWSKI A. 2014. Identification of chromosome instability in Japanese Quail (*Coturnix japonica*). Br. Poult. Sci. **55**: 435-441.

YERLE M., ECHARD G., GILLOIS M. 1987. The high-resolution GTG-banding pattern of rabbit chromosomes. Cytogenet. Cell Genet. **45**: 5-9.