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Sister chromatid exchange in selected horse breeds *(Equus caballus)*

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Abstract

In studies of chromosome instability, the sister chromatid exchange (SCE) test is a particularly sensitive cytogenetic assay for detecting DNA damage. SCE tests of chromosome instability were performed in the group of 6 horse breeds (Pure-bred Arabian, Malapolski horse, Polish noble half-bred, Polish cold-blooded, Hucul and Polish Konik). The chromosome preparations were obtained from our *in vitro* culture of peripheral blood lymphocytes stained using the FPG technique. The mean number of SCEs/cell in the analysed population of horses was 5.14±1.44. The mean frequency of SCEs in the 6 analysed horse breeds varied depending on the breed. Statistically significant differences were observed between the horse breeds ($P<0.01$).

No statistically significant differences in the number of SCEs per cell were found between the males and females (5.10±1.34 and 5.20±1.52, respectively). The horses were also assessed for the number of SCEs/cell in relation to the age of the animals. The differences between the age groups were statistically significant ($P<0.01$).

Keywords: sister chromatid exchange (SCE), chromosome, horse

Zusammenfassung

Schwesterchromatidaustausch in ausgewählten Pferderassen *(Equus caballus)*

Bei Untersuchungen der Chromosomeninstabilität ist der Schwesterchromatidaustausch (SCE)-Test ein besonders empfindlicher cytogenetischer Test zum Nachweis von DNA-Schäden. In der Gruppe von 6 Pferderassen (reinrassiger Araber, Malapolski-Pferd, Edles-Polnisches-Halbblut, Polnisches Kaltblut, Hucul und Polnisches Konik) wurden SCE-Tests auf Chromosomeninstabilität durchgeführt. Die Chromosomen-Präparate wurden aus unserer *in vitro* Kultur peripherer Blutlymphozyten erhalten, die unter Verwendung der FPG-Technik gefärbt waren. Die durchschnittliche Anzahl von SCEs/Zelle in der untersuchten Population von Pferden betrug 5,14±1,44. Die durchschnittliche Häufigkeit der SCEs in den 6 untersuchten Pferderassen variierte je nach Rasse. Zwischen den Pferderassen wurden statistisch signifikante Unterschiede ($P>0,01$) festgestellt.

Zwischen den männlichen bzw. weiblichen Tieren wurden keine statistisch signifikanten Unterschiede in der Anzahl der SCEs (5,10±1,34 bzw. 5,20±1,52) festgestellt. Die Pferde wurden auch im Hinblick auf die Anzahl von SCEs/Zelle in Relation zum Alter der Tiere bewertet. Die Unterschiede zwischen den Altersgruppen waren statistisch signifikant ($P<0,01$).

Schlüsselwörter: Schwesterchromatidenaustausch (SCE), Chromosom, Pferd

Introduction

The genome and karyotype of the domestic horse (*Equus caballus*) are not as well described as the genomes of other farm animals. The domestic horse karyotype is characterised by a chromosome diploid number equal to 64. Among the 32 chromosome pairs, 31 are autosomes (13 metacentric and submetacentric pairs and 18 acrocentric pairs). The 32nd pair are sex chromosomes. Chromosome X is one of the largest chromosomes in the karyotype, while chromosome Y is one of the smallest (Richer *et al.* 1990, Bowling *et al.* 1997, Di Meo *et al.* 2009, Wade *et al.* 2009).

The horse is an exceptional animal as compared with other farm animals. Nowadays, it is used more for recreation and sport rather than as a beast of draught. The market value of horses is very high in certain cases and depends on their sports performance and exterior. In comparison with other farm animals, horse breeding is relatively difficult and expensive. This is connected with the comparatively low reproductivity of those animals (Golisch *et al.* 1986, Bugno & Słota 2007). The reasons for failure in reproduction are fertility-reducing chromosome anomalies and genetic disorders that appear during the gametogenesis of one of the parents and affect the development of embryos. The cytogenetic investigations that have been undertaken primarily concerned inadequate number (aneuploidies) or structure (translocations) of chromosomes (Bugno & Słota 2007, Lear & Bailey 2008, Di Meo *et al.* 2009). There are few reports available on chromosome instability in horses (Rubes *et al.* 1992).

The investigation of chromosome instability consists in analysing the metaphases in which various types of chromosome damage are determined. A number of cytogenetic tests are used for this purpose. The basic diagnostic test of DNA damage is the sister chromatid exchange test (SCE). This test makes it possible to assess the genotoxic effect of mutagenic and carcinogenic chemical compounds on chromosome structure. Sister chromatid exchange consists in swapping homologous chromatid segments of the same chromosome. Such exchanges take place during the cell growth cycle. After replication, when the condensed sister chromatids are combined in pairs, the swapping occurs between identical DNA sequences that are close to each other. SCE is the result of semiconservative DNA replication from the damaged matrix and can take place only if the changes in the DNA are not removed before the cell enters the S phase (Wójcik *et al.* 2004, Bayani & Squire 2005). SCE can be recognised as sudden breaks in the continuity of staining patterns of two chromatids of the same chromosome. Sister chromatid exchanges are induced by many factors that disturb DNA structure, metabolism and repair mechanisms. The frequency of SCE increases as a result of DNA damage by factors that inhibit the progression of the replication fork in replicons (Sonoda *et al.* 1999). Faulty systems of DNA damage repair (e.g. homologic recombination) also cause SCEs (Sonoda *et al.* 1999, Wilson & Thompson 2007).

The present research is the first to deal with sister chromatid exchange in horses. The lack of cytogenetic studies on sister chromatid exchange in horses made us decide to perform the analyses presented in this paper.

The purpose of the analyses was to investigate sex-, breed- and age-dependent sister chromatid exchanges in the chromosomes of the following horses: pure-bred Arabians, Malapolski horses, Polish half-bred, Polish cold-blooded, Huculs and Polish Koniks.

Material and methods

The evaluation of chromosome instability using the SCE test was performed for the group of horses representing 6 breeds: pure-bred Arabians, Malapolski horses, Polish half-bred, Polish cold-blooded, Huculs and Polish Koniks that originate from the domestic horse *(Equus caballus)*.

Each pedigree group was represented by 10 animals of both sexes: pure-bred Arabians (9 mares and 1 stallion), Malapolski horses (5 mares and 5 stallions), Polish noble half-bred (6 mares and 4 stallions), Polish cold-blooded (5 mares and 5 stallions), Huculs (5 mares and 5 stallions) and Polish Koniks (7 mares and 3 stallions). The age of the horses ranged from 1 year to 15 years. The horses were divided into two age groups. The 1st group comprised horses of up to 6 years of age, while the 2nd one consisted of horses older than 6 years. For each animal 20 metaphases were analysed. The chromosome preparations were obtained from our *in vitro* culture of peripheral blood lymphocytes. In the 24th hour of culture duration we added 10 µg/ml of BrdU. The differentiation staining of sister chromatids was carried out using the FPG technique described by Kihlman & Kronborg (1975). The staining procedure included the following steps: 1 h of 0.01 % RNase treatment at 37 °C, 1 h of incubation in the solution of 0.5×SSC (0.75M sodium chloride +0.075 sodium citrate; pH=7.0) with the addition of the Hoechst solution (stock solution: 0.5 mg of Hoechst 33 258/1ml of ethanol), 1 h of UV treatment, 24 h of incubation at 4 °C in darkness, half an h of UV treatment, 2 h of incubation at 58˚C, 1 h of Giemsa staining.

The final preparations were subjected to microscope and digital image analysis in order to determine the number and places of sister chromatid exchanges. The results were also statistically analysed using Statistica 9.0 software. For comparing the mean values the Student's t-test (age, sex) and the F test (breed) were used.

Results

The karyotype of the horses was analysed for the exchange of labelled DNA segments between sister chromatids in the chromosomes. Figure 1 depicts the metaphase plates with identified sister chromatid exchanges in the 6 horse breeds.

In the horse population under analysis, the mean number of SCEs/cell in the 1 200 investigated cells and 76 800 chromosomes was 5.14±1.44. A higher mean value was observed for the females 5.20±1.52 than males 5.10±1.34 but the difference was not statistically significant.

The SCE/cell distribution in the 6 horse breeds varied depending on the breed. No significant difference in the mean number of SCEs/cell was found between the Polish Konik and the Arabian horse. The mean values for the other breeds were statistically significant ($P<0.01$). The highest number of SCEs/cell was observed in the Malapolski horses 6.58±0.90, and the lowest in the Polish Koniks 3.63±0.70 (Table 1). The differences in the mean number of SCEs/cell between the particular breeds are depicted in Figure 2.

The horses were also examined for the number of sister chromatid exchanges per cell and chromosome in relation to the age of the animals. A higher mean number of SCEs/cell was observed in the group of horses of more than 6 years of age (5.77±1.35) as compared with the first age group (4.62±1.31). The differences were statistically significant ($P<0.01$) (Table 2).

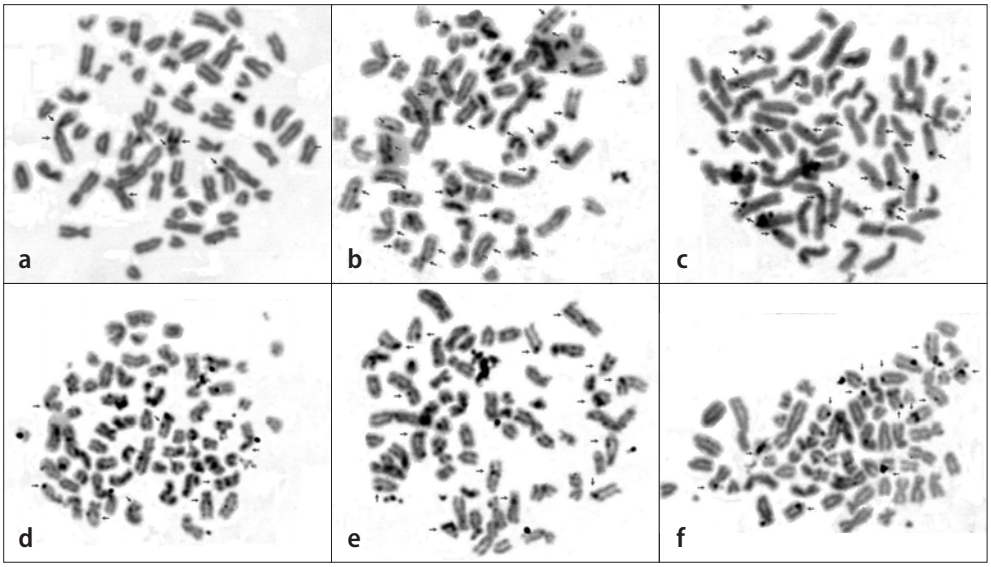

Figure 1
Chromosome metaphase plates for the 6 horse breeds – SCE staining (a - Pure-bred Arabian, b - Malapolski, c - Polish noble half-bred, d - Polish cold-blooded, e - Hucul, f- Polish Konik). SCEs indicated by arrows.

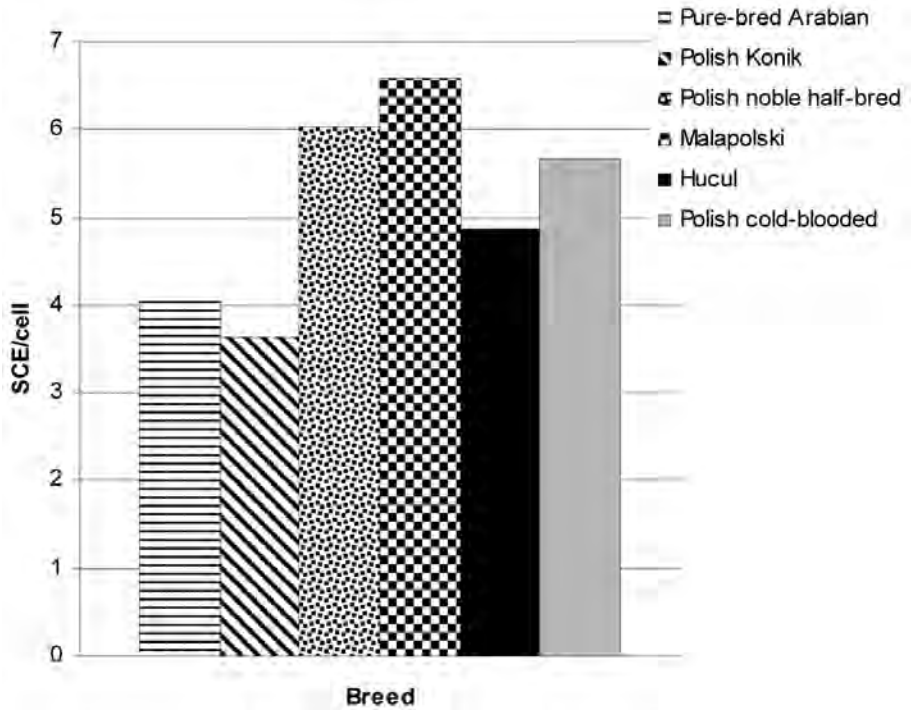

Figure 2
Distribution of SCEs/cell for the analysed horse breeds.

Table 1
SCE distribution in the horse chromosomes, depending on the breed

Breed	Number of SCE cell^{-1} Mean±sd
Pure-bred Arabian	$4.05^{A} \pm 0.54$
Malapolski	$6.58^{C} \pm 0.90$
Polish noble half-bred	$6.04^{BC} \pm 0.85$
Polish cold-blooded	$5.66^{BC} \pm 1.34$
Hucul	$4.87^{AB} \pm 1.41$
Polish Konik	$3.63^{A} \pm 0.70$

^{ABC} the mean values marked with different letters are statistically different

Table 2
SCE distribution in the horse chromosomes depending on the age

Breed	No. of animals	No. of cells	No. of chromosomes	Number of SCE cell^{-1} Mean±sd
Horses of up to 6 years	33	660	42 240	4.62±1.31
Horses of more than 6 years	27	540	34 560	5.77±1.35**

**significant with $P<0.01$

Discussion

The molecular mechanism of SCE is not entirely known and constitutes the subject of much contemporary research. SCE is induced by many factors that disturb DNA structure, metabolism and repair mechanisms. Unfortunately, a strong inducer of SCE is BrdU itself, as it replaces thymidine. The higher the concentration of BrdU, the more difficult it is to determine the frequency of spontaneous SCE. According to Wilson & Thompson (2007), spontaneous SCEs are those that take place with a very low or zero level of BrdU. Leinbenguth & Thiel (1986) considered the concentration of 15-30 µg/ml to be the optimal dose of BrdU, Vijh *et al.* (1992), Di Berardino *et al.* (1995, 1996) and Arias (2000): 5µg/ml, whereas Ciotola *et al.* (2005) and Peretti *et al.* (2006, 2008): 10 µg/ml. Based on the results of the above authors, in experiments we applied the 10 µg/ml dose of BrdU in order to obtain spontaneous SCEs.

An important factor that affects the frequency of SCEs in man is the sex (Margolin & Shelby 1985). Husum *et al.* (1986) found that women had 0.5 SCE per cell more than men. Wulff & Niebuhr (1985) identified a higher number of SCEs in XX cells than in XY cells of human chimeric twins.

As regards farm animals, the following scientists: Di Meo *et al.* (1993) who analysed chromosomes in goats, Iannuzzi *et al.* (1991b) in cattle, Di Meo *et al.* (2000) in sheep, Ciotola *et al.* (2005) in cattle and Ritter & Golisch (1991), Peretti *et al.* (2006) in pigs did not identify any statistically significant differences in the mean values of sister chromatid exchanges in males and females. In experiments we observed differences in the number of SCEs between females than males. However, as in the case of the above scientists, the differences were not statistically significant.

Extensive research showed correlations between the number of SCEs and the breed of the analysed animal species. Catalan *et al.* (1995) for the Fleckvieh and Pirenaica & Iannuzzi

et al. (1991a) for the Podolian, Friesian and Romanga breeds observed varying frequencies of SCE. Also, Ciotola *et al.* (2005) who compared their results with the results of Ianuzzi *et al.* (1991a) and Di Berardino & Shoffner (1979) (acc. to Ciotola *et al.* 2005) found differences of SCE frequency between the Agerolese, Podolian, Romagna and Holstein Friesian cattle breeds. Ritter & Golisch (1991), Peretti *et al.* (2006) also concluded that the breed had a significant influence on the frequency of SCE. Peretti *et al.* (2006) compared the results of their experiments with Rubes (1987) results. The Casertana pig breed analysed by Peretti *et al.* (2006) had higher SCE frequency than the Landrace breed described by Rubes (1987) (acc. to Peretti *et al.* 2006). On the other hand, Di Meo *et al.* (2000), who investigated the Comisana and Laticauda breeds of sheep, did not identify any effect of the breed/race on SCE frequency.

The present research involving the analysis of the frequency of sister chromatid exchanges in the 6 different horse breeds showed that the breed had a significant effect on the SCE number. Only between 2 breeds (Pure-bred Arabian/Polish Konik) no statistically significant differences were found.

A low number of SCEs was observed for the Polish Konik and the Hucul. These breeds are very rare and autochthonous. Their genome is more stable in comparison with other breeds of the same species. A low number of SCEs in comparison with the other horse breeds was also observed in the case of the pure-bred Arabians. The variation in the number of SCEs can be due to the different intensity of selection of those breeds as well as the stud system.

Little information is available on the influence of age on SCE frequency. Peretti *et al.* (2006) concluded that the age had a significant influence on the frequency of SCE. In their experiments on pig groups: below 1 year of age and more than 1 year of age, the authors identified statistically significant differences between the groups. In the older animals the frequency of SCEs was much higher. Such correlation was also observed in humans (Sinha *et al.* 1985, Lazutka *et al.* 1994, Husum *et al.* 1986). Our own studies revealed that the age was a significant factor affecting the number of SCEs. A higher number of sister chromatid exchanges was observed in the group of horses of more than 6 years of age.

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