Iranian Journal of Veterinary Research, Shiraz University, Vol. 13, No. 2, Ser. No. 39, 2012

Scientific Report

Sister chromatid exchange analysis in some Holstein bulls

Azimi Dezfouli, S. M.*

Department of FMD, Razi Vaccine and Serum Research Institute, Karaj, Iran

***Correspondence:** S. M. Azimi Dezfouli, Department of FMD, Razi Vaccine and Serum Research Institute, Karaj, Iran. E-mail: m.azimi@rvsri.ir

(Received 8 May 2011; revised version 8 Jan 2012; accepted 29 Jan 2012)

Summary

Chromosomal appearance of 12 Holstein bulls selected for artificial inseminations were examined by sister chromatid exchanges (SCEs). For differential staining of sister chromatids bromodeoxyuridine (BrdU) was inoculated in lymphocyte cultures. The mean, maximum and minimum number of SCE per cell were determined 6.8 ± 1.14, 8.3 ± 1.1 and 5.7 ± 1.5, respectively. SCE frequencies of all animals were in the normal level. Also, evaluation of different breeding condition and age range did not show any significant statistical ($P>0.05$) effect on SCEs rates.

Key words: Sister chromatid exchange, Bull, Bromodeoxyuridine

Introduction

Sister chromatid exchanges (SCEs) involve breakage of both DNA strands, followed by an interchange of whole DNA duplexes and reunion homologues chromosomes. Evaluation of SCEs is a valuable test for analysing the effects of different agents on DNA (Sivikova *et al*., 1999). Most of the SCE studies have been carried out in humans and rodents. However, these studies should be considered as an important genetical evaluating test for farm animals, especially ruminants which make up the main part of human food (Iannuzzi *et al*., 1991; Catalan *et al*., 1995).

Sister chromatid exchange assay is a short-term test and requires some means of differentially labeling sister chromatids, which can be achieved by incorporation of BrdU into DNA for two cell cycles. Newly synthesized strand will be stained pale and differentiated from a conserved one. By this way, any reciprocal exchanges between two sister chromatids can be distinguished easily.

Materials and Methods

A group of 12 clinically healthy Frisian bulls which were candidates for artificial insemination services were selected. The animals were reared in three different provinces including Tehran, Isfahan, Golestan (Table 1).

Lymphocyte culture was carried out as previously described (Di Berardino and Shoffner, 1979). For differential staining of sister chromatids, 24 h before harvesting 10 µg/ml halogenated analogue of thymidine (BrDU) was inoculated to cultures. The mitotic spreads were stained with Hoechst 33285 (0.5 µg/ml) for 15 min. Then the mounted slides were exposed to UV for 24 h. The chromosomal complements were stained by 3% Giemsa solution for 30 min. In order to determine SCEs number, 30 intact metaphase complements were examined by Zeiss Photomicroscope and photographs with low sensitivity (ASO 100). Statistical analysis of SCEs variation was done by SPSS package (version 14) using ANOVA method. Rearing animals in different provinces was considered as three

groups and SCE rates were evaluated between groups and within groups.

Table 1: Number of examined bulls and SCE rate per lymphocyte in each province

Group	Province	SCE/cell (Mean)	Age (Month)
1	Tehran	8.3 ± 1.1	18
		8.2 ± 1.08	19
		5.7 ± 1.61	21
		5.9 ± 0.94	25
		5.7 ± 1.85	29
2	Isfahan	8.2 ± 1.59	22
		6.2 ± 2.01	24
		6.1 ± 1.51	27
3	Golestan	7.9 ± 1.03	19
		5.7 ± 1.91	20
		8.1 ± 0.87	25
		5.7 ± 1.5	28

Results

Chromosomal evaluation of selected animals by SCE staining revealed normal karyotype (2n = 60, XY) without any chromosomal gaps, breaks and fragments.

The SCE number in each animal was reported in Table 1. The mean value (±SD) per chromosomal complement was calculated 6.8 ± 1.14; and the means of SCEs in selected bulls from Tehran, Isfahan, and Golestan were determined 6.76 ± 1.22, 6.83 ± 0.97 and 6.85 ± 1.15, respectively. Also, maximum and minimum SCE were found to be 8.3 ± 1.1 and 5.7 ± 1.5 in Tehran and Golestan provinces, respectively.

Statistical analysis did not show any significant variation among the bovines within and between different provinces ($P>0.05$).

Discussion

Previous studies showed that the average of SCEs in normal cattle treated by a permissible concentration of BrdU (10 µg/ml) is 5 up to 14 exchanges per cell (Pathak *et al*., 1977; Popescu, 1978). Significant deviations in the frequency of SCE may indicate a pathological condition and damage to the genome (Nicolae *et al*., 2009).

Di Berardino and Shoffner (1979), and Iannuzzi *et al*. (1991) determined a mean value of 5.4 ± 2.1 and 7.1 ± 3.3 SCE/cell in the American and Italian Friesian breed, respectively. In the present study, a safe dose of Brdu (10 µg/ml) was used and the SCE rate was found to be 6.8 ± 1.14, near the level of previous studies. The SCEs number of the bulls in each province based on age as a variable did not show any significant fluctuation. Sampling of the young bull and low differences of age may have caused this result. Catalan *et al*. (1995) confirmed that age less than 3 years did not have a noticeable effect on SCE in cattle.

In another study, the number of SCE in cows from the industrial region did not significantly vary from the agricultural region in spite of the persistence of different breeding conditions (Parada and Jaszczak, 1993). Our findings, in agreement with this study, did not show any significant relation between SCEs frequencies and rearing condition. That means these animals did not have contact with harmful genotoxic materials which can cause DNA fragility, instability and SCE elevation.

References

Catalan, J; Moreno, C and Arruga, M (1995). Sister-chromatid exchanges in cattle: breed, sex and BrdU dose effects. Mutat. Res., 331: 205-211.

Di Berardino, D and Shoffner, R (1979). Sister chromatid exchange in chromosomes of cattle (*Bos taurus*). J. Dairy Sci., 62: 627-632.

Iannuzzi, L; Di Meo, GP; Perucatti, A; Ferrara, L and Gustavsson, I (1991). Sister chromatid exchange in chromosomes of cattle from three different breeds reared under similar conditions. Hereditas. 114: 201-205.

Nicolae, I; Enculescu, M; Vidmichi, D; Paraschivescu, M; Bota, A and Hârceaga, L (2009). Sister chromatid exchenges in river buffalo female with chromosomal fragility. Luc. Şti. Zoot. şi Biot., 42: 64-69.

Parada, R and Jaszczak, K (1993). A cytogenetic study of cows from a highly industrial or an agricultural region. Mut. Res./Gen. Tox., 300: 259-263.

Pathak, S; Ward, OG and Hsu, T (1977). Rate of sister chromatid exchanges in mammalian cells differing in diploid numbers. Cell. Mol. Life Sci., 33: 875-876.

Popescu, CP (1978). Sister chromatid exchanges (S.C.E) in normal and abnormal bovine (*Bos taurus* L.) karyotypes. Cytologia. 43: 533-540.

Sivikova, K; Dianovsky, J and Piesova,·E (1999). Chromosome damage in culture bovine peripheral lymphocytes induced by herbicide chloridazxon. Acta Vet. Brno., 68: 105-110.