

CHROMOSOME CHANGES IN CATTLE ON THE FARMS IN SERBIA

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In the paper we presented five-year investigations of numeric and structural changes in cattle karyotype on five farms and two centers for reproduction in Serbia. There were 371 breeding animals (215 male and 156 female), out of which 267 Holstein Friesian breed (193 male and 74 female), 62 Simmental (17 male and 45 female) and 42 Grey Steppe breed (5 male and 37 female). Cultivating of lymphocytes and karyotype analyses, according to the international standards for karyotypization of domestic animals, were applied. The aim of the investigation was to test genetic material on chromosome level of animals introduced into reproduction. The following changes were discovered in the karyotype: 6 animals of Holstein-Friesian breed were with chimeras $2n=60XX/XY$ and there was one Robertson's translocation in Simmental breed. Structural changes as breakage and a ring were discovered in two animals of Holstein-Friesian and Simmental breed raised in the area of bombing in Serbia. The animals of Grey Steppe breed had normal karyotype. There is a total of 9 animals with changes (2.42%), while 362 were with normal karyotype, total 97.57%. According to the results, it may be concluded

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that cytogenetical attestation of the breeding animals will have to be continued for the purpose of protecting the genofond on cattle farms.

Key words: cattle, cytogenetic attestation, himeras, karyotype, translocation

INTRODUCTION

Investigation on numeric and structural changes in karyotype of cattle in former Yugoslavia started by introducing applicative veterinary cytogenetics in cattle breeding in Europe. In the 80's changes in chromosomes of different cattle breeds were discovered, especially among Simmental cattle (SOLDATOVIĆ *et al.*, 1977). In 1995 Scientific Veterinary Institute from Novi Sad established a laboratory for cytogenetic studies of domestic animals with the aim to apply correct selection and make financial contribution to cattle breeding. Despite all the difficult circumstances the laboratory faced, 2200 karyotype analyses on swine, cattle, horses and dogs have been performed in five-years work. The results of the investigations were presented on national and international symposia and were published in national and international journals (KOŠARČIĆ *et al.*, 1996; 1997; 1998; 2000). All the investigations performed so far point that there is a need for introducing program for eradication of animals with chromosome aberrations and creating health stock of cattle breeding in Serbia. In some countries there are legislative regulations for monitoring cytogenetic aberrations of breeding flocks (ERNST and ŽIGAČEV, 1990). Having in mind these facts, some farms in northern parts of Serbia started chromosome investigations on bull mothers and male animals introduced in reproduction. Cooperation between the laboratory for cytogenetics at the Research Institute for Animal Breeding and Nutrition from Hereceghalom, Hungary and the Scientific Veterinary Institute from Novi Sad, Serbia has been established.

The aim of this paper is to present the results of investigating numeric and structural changes on chromosome in cattle karyotype on some farms in Serbia.

MATERIAL AND METHODS

Animals. Karyotype in 371 animals from Serbia (215 male and 156 female) has been investigated over a period of five years. The following breeds were chosen: Holstein Friesian 267 (193 male and 74 female) animals; Simmental 62 (17 male and 45 female) and Grey Steppe 42 (5 male and 37 female).

Chromosome preparation. - For cytogenetic analyses blood was taken from vein jugulars and culture of lymphocyte was cultivated according to the modified method (MOORHEAD *et al.*, 1960). Components for the culture were:

- 200.0 ml TC=199 Medium (Sigma)
- 40.0 ml Fetal Calf Serum (NIVNS, N. S., Yu)
- 1.5 ml PHA - P (Sigma)
- 25 ml Heparin - Na (ICN)
- 25000 i.u. Benzylepenicillin - substrate (ICN)

From this medium we used 3 ml and establish culture from 0.5 ml blood and incubated at 38°C for 72 hours. Cell division was stopped with 4.0 microgram of Colchine (Sigma). The cells were treated with hypotonic solution of 0.56% KCl and fixation was repeated several times with solution of methanol and acetic acid 3:1. Preparation of cells with 60% acetic acid was performed on the following day on cold glass microscope slides. Presence of metaphases and mitotic index was determined by phase contrast microscopy. For the purpose of correct analyses of karyotype we used GTG - band technique according to SEABRIGHT (1971) and 5% staining by Giemza. We analyzed five samples per each animal. An international scheme for karyotypization for domestic animals was used (ISCNDA, 1990). The analyses of 100 metaphases was performed with light microscope at 1000 X magnification. Camera Olympus was used for photos.

RESULTS AND DISCUSSION

The results of chromosome changes in cattle in Serbia in period 1996-2000 are presented in Table 1. Karyotypes were analyzed in 371 animals. They were checked in phases – by contrast and light microscopy of 1855 samples. 100 metaphases were analyzed and the following results were obtained: normal karyotype was present in 362 animals $2n = 60 XY$, $2n = 60XX$ (97.57%), 9 animals (2.42%) had changes in karyotype, type: himerism, translocation 1/29, brake in chromosome and ring. We discovered 6 bulls that had karyotype distinctive for chimeras because cells with $2n = 60XY$ and $2n = 60XX$ were present (Fig. 1).

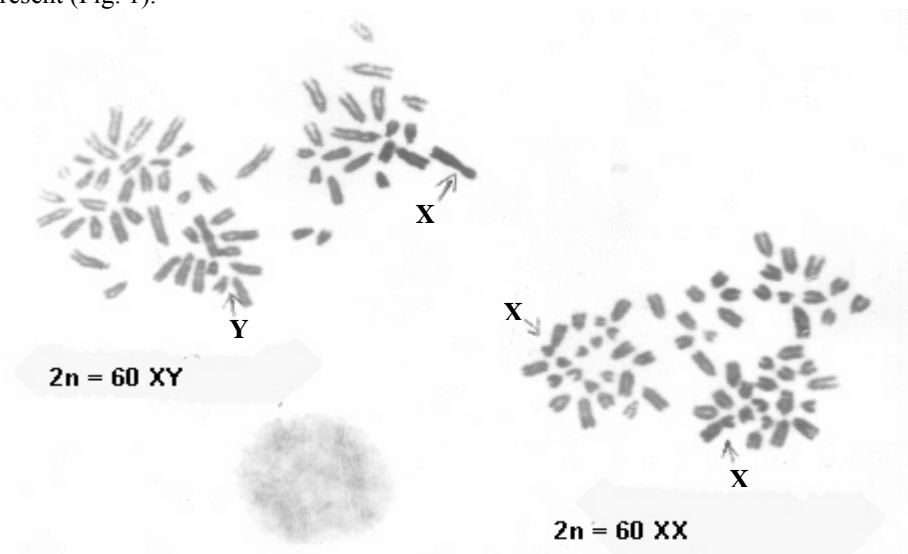


Figure 1. Karyotype of animal No. 1376, Holstein Friesian breed, 1998. PIK BEČEJ, Chimeras $2n = 60XY$, $60XX$

From the evidence we discovered and according to hormonal and cell theory, chimeras are most often found in twins. Among the present races this change was discovered in Holstein Friesian breed where the twins occurs more often than in other breeds. During our work and preparation for introducing into reproduction, these bulls did not satisfy the requirements for reproduction and artificial insemination, and were excluded from the process. The papers on influence of chimeras on quality of sperm were published. Statistically important difference in volume, motility and concentration of spermatozooids between the bulls with normal karyotype $2n=60XY$ and chimera bulls $2n=XX/XY$ were confirmed (REJDUCH *et al.*, 2000).

According to the results (PETUHOV *et al.*, 1985) it is necessary to include chimeras as a criteria for selecting breeding animals.

Some authors think that discovered chimera should be kept in herd and their sexual behavior should be observed, because there is a possibility that these animals give more female offspring (KOVACS, 1978). Because of financial reasons it is necessary to make a program of selection and apply it with the aim to exclude animals that carry chromosome changes and prevent their influence on reproduction and the offspring (HAZAS, 1999).

In Simmental breed we discovered a heterozygote bull with translocation between 1/29 chromosomes (Fig. 2).

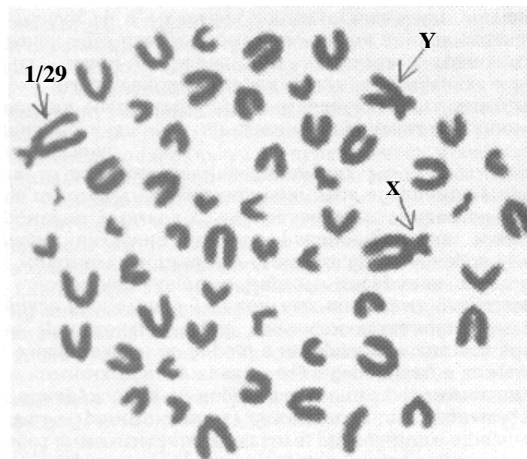


Fig. 2. Translocation 1/29, karyotype of animal No. 3453 Simmental breed, 1999. A.D. Krivaja

This change presents a balanced mutation. The bulls that carry of translocation in gametogenesis, produce balanced and unbalanced spermatozooids, what causes embryo mortality and influences reproduction. GUSTAVSSON and ROCKBORN (1964) discovered it in Europe and since then its frequency, as well as its presence, is followed in the countries with developed cattle breeding. The first translocation in domestic cattle in Serbia was discovered and described by

SOLDATOVIĆ (1971). Research on Robertson's translocation, the obtained results and districts where they may be found were published in a monograph "Robertson's translocation T 1/29 in karyotype of cattle" (VUČINIĆ *et al.*, 1995). Animals with this changes on chromosomes are excluded from reproductive exploitation, what in many countries is regulated by law (ERNST and ŽIGAČEV, 1990).

The results obtained from the animals in the area close to refinery in Novi Sad that was bombed, point out that changes on chromosomes, like break and rings, are the consequence of some genotoxic agents. Structural changes like izochromatide break were discovered on the fifth pair of chromosomes (Fig. 3).

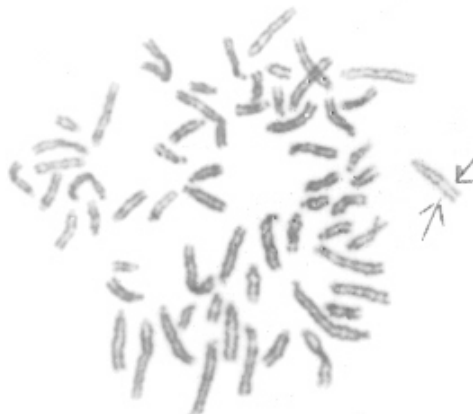


Fig. 3. Karyotype of animal No. 2184, Holstein Friesian breed Izochromatide break on the fifth chromosome

On 100 examined metaphases we discovered 37% with the mentioned change. It was noticed that this structural change occurred *de novo* possibly under the influence of a harmful agent which appeared in the environment due to incomplete burning of oil. Similar results in damages of chromosome structures in cattle were found by LAMBI *et al.* (1999).

Its permanent and hereditary features must be followed on the offspring, which is the aim of the further investigations.

The discovered ring change, which is very common after radiation, that was present in 5% of the examined cells, convinced us that the farms was in radiation zone.

CONCLUSION

From the obtained results we can conclude:

- there is a need for legislative regulations concerning control and selection of all the animals that are introduced in reproduction;
- changes on chromosomes type chimeras were discovered only in Holstein Friesian breed, what can be explained with greater number of twins;

- translocation between the first and twenty-ninth chromosome was discovered only in Simmental breed;
- all the animals Grey Steppe breed had normal karyotype $2n=60XY$, $2n=60XX$;
- animals with structural changes on chromosomes were in area where harmful matters were released in incomplete oil burning, what points to genotoxicity;
- it is necessary to introduce eradication program on chromosome aberration in Serbia and to start a process of introducing law regulations regarding this matter.

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PROMENE U HROMOZOMA GOVEDA NA FARMAMA U SRBIJI

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Izvod

U radu su prezentovana petogodišnja istraživanja numeričkih i strukturnih promena u kariotipu goveda na pet farmi i dva centra za reprodukciju u Srbiji. Analizirana je 371 priplodna životinja i to 215 muških i 156 ženskih grla, HolsteinFriesion rase 267 (193m i 74ž), Simmental 62 (17m i 45ž) i Grey Steppe rase 42 (5m i 37ž). Korišćen je uobičajen metod za uspostavljanje kulture limfocita i analizu kariotipa prema međunarodnim standardima za karotipizaciju domaćih životinja. Cilj ovih istraživanja je provera genetičkog materijala na hromozomskom nivou grla koja se uvode u reprodukciju. Otkrivene su sledeće promene u kariotipu: 6 grla sa himerizmom $2n=60XX/XY$ HolsteinFriesian rase i jedna Robertsonova translokacija u Simmental rasi. Strukturne promene tipa prekida i ringa otkrivene su kod dve životinje HolsteinFriesian i Simmental rase koje su bile u zoni dejstava za vreme bombardovanja SR Jugoslavije. Sva grla Grey Steppe rase imala su normalan kariotip. Ukupno je 9 životinja sa promenama ili 2,49% dok normalan kariotip imaju 362 ili 97,57 %. Prema dobijenim rezultatima možemo zaključiti da je neophodno nastaviti citogenetičku atestaciju priplodnih životinja iz ekonomskih razloga i u cilju zaštite genofonda na farmama goveda.

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