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CHANGES IN KARYOTYPE IN DOMESTIC ANIMALS DISCOVERED ON THE FARMS IN VOJVODINA AND THEIR INFLUENCE ON REPRODUCTION

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New directions in animal husbandry demand raising of animal kinds that are adjusted to intensive way of breeding. In order to accomplish these demands, beside known methods in selection, Cytogenetic control of existing genotypes is needed that has been carried through ten year examination on pig, cattle and stud farms in Vojvodina. Chromosome aberration of numeric polyploidy and aneuploidy but also structural translocation, deletion, duplication, inversion, ring, break and other segregations were discovered. Numeric and structural changes on animal karyotype influenced on reproduction disturbance, phenotype expression, as well as selection program and stability of genofond. Different aspects of reproductive disturbance were noted like for example: small litter, embryo mortality, frequent repeated breeding, abortion, stillbirth and mummified embryo, offspring with anomalities, different kinds of sterility, Analyses of the results obtained from monitoring the herd book and making genealogy show on existence of chromosomepathy

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on our farms. The aim of this work is to inform scientists and experts with the fact that these changes are spreading, especially through among the breeding animals. Therefore genetic control and timely exclusion of chromosome aberration is necessary.

Key words: pig, cattle, cytogenetic, attestation, polyploidy, translocation

INTRODUCTION

Importance and application of genetics in cattle breeding has its roots in the first part of the 20th century, but introduction of cytogenetics as a genetic discipline started with two scientific and expert symposium (1970, 1975) held in Giessen, Germany, and than in France (1975), and three years after in Uppsala, Sweden. Since that time for European cytogenetics biennale has been established and the results are presented every two years. Presentation of the achieved results and determining the international standards for karyotypization of domestic animals occurred in the following sequence: 1980 (Milan, Italy); 1984 (Zurich, Switzerland); 1986 (Warsaw, Poland); 1988 (Bristol, England); 1990 (Tuluz, France); 1992 (Utrecht, Holland); 1994 (Copenhagen, Denmark); 1996 (Saragossa, Spain); 1998 (Budapest, Hungary); 2000 (Brno, Czech Republic); 2002 (Sorrento, Italy); 2004 (Paris, France); 2006 (Lisbon, Portugal). Due to great interest in cytogenetic research in animal genetics in Europe, in our country the first results were published in 1997 (SOLDATOVIĆ B. *et al.*). Contribution to genetic discipline in development of cattle breeding has been given through ten year research at the Scientific Veterinary Institute in Novi Sad, where exists a laboratory for this field. Cytogenetic attestation of breeding animals has been carried out on pig, cattle and horse farms. Genetic material has been controlled on chromosome level and animals with genome changes were excluded from reproduction. The results or these research have been presented on national and international symposium and published in journals (KOŠARČIĆ S. *et al.*, 1997; 1998; 2000).

It is important to stress that cytogenetic control is an important selective measure for accurate choice of genetically health breeding material. Having in mind status and chronology of European cytogenetics, as well as its selective program and control in reproduction, the majority of countries introduced cytogenetic attestation as legislative obligatory (HAZAS G., 1999).

Having this all in mind, the aim of this paper is to inform scientific population about detected chromosome changes in genome of breeding animals and their influence on reproduction on farms in Vojvodina.

MATERIAL AND METHODS

During twenty years of research 1500 of animals were observed on 8 pig farms, 5 cattle farms and 2 stud farms. This made a total of 5% of livestock from

chosen farms in Southern Bačka and Srem district in Vojvodina. Out of this, 535 animals were with the following reproductive disorders: frequent abortion, frequent repeated breading, still birth, mummified fetus, small litters, avital offspring, bad sperm quality, reduced libido, necrospermia, offspring with anomalities, sterility, intersexuality, freemartin, cryptorchidism and neoplasia. Cytogenetic analyses of biological material was carried on 1500 samples on numeric and structural changes in genome and mitotic index. In this work periphery lymphocyte blood and bone marrow cells from the offspring with anomalities were used as a material for chromosome preparation. We used sterile method and by adding anti-coagulase we established cell culture using known and modified methods (MOORHEAD *et at.*, 1960); (BOYUM, 1968); (HARTEY and HORNE, 1985).

Components for the cell culture medium were:

- 4 ml of medium, Ham F 12, TC 199, or RPMI 1640
- 1 ml Fetal Calf Serum (FCS-NIVNS. NS., YU) or Sigma
- 10 mg/ml PHA - P, mitogen, Sigma
- 25000 I.U. Benzylepenicillin- substrate ICN

In this medium 0.5 ml heparized blood was cultivated on temperature 37°C for 72 hours. Cell division was stopped by mitostatics Colhicine (Sigma) 0.5 mg/1ml. After two hours the cells were treated with 0.56% KCL (hypo tonic) at temperature 37°C and fixative 1:3 acetic acid-methanol on room temperature for several times. Preparation was made with 60% acetic acid on the following day (personal experience, better diffusion of chromosomes). Mitotic index was determined by phase-contrast microscope. For better analyses of chromosome we used GTG-bend technique according to SEABRIGHT (1971) and staining by Giemza 5%. Chromosome analyses on numeric and structural changes was done according to Internationals standards for kariotypisation of domestic animals (ISCNDA, 1990). For every animal 5 preparations were made. Degree of changes was observed on 100 metaphases under light microscope, 1000X. Microphotographs were made with Olympus camera.

RESULTS AND DISCUSSION

After performed analyses, out of 535 animals with the symptoms of reproductive disorders, we discovered 48 with numeric and structural changes in karyotype. Weak mitotic activity (mitotic index) was detected in 150 animals. Most of the changes were discovered on pig farms (35 animals) and cattle farms (13 animals), while on horse farms there were other changes in karyotype and reproductive disorders.

From numeric changes we found polipoidy type 4n = 76XY, XY, XY.XY and 5n=95XY (Figure 1.) and aneuploidy type 2n < 38XY, XX and 2n > 60XX (Figure 2.).

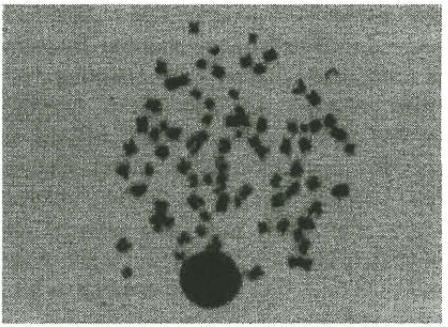

Fig. 1. - Poliploidy 4n = 76XY, karyotype pig

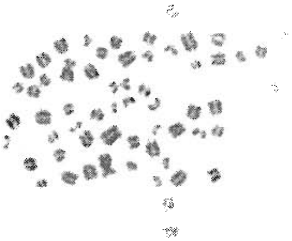

Fig. 2. - Aneuploidy 2n > 60XX, karyotype cattle

This is not a change of constitutional karyotype, because not all the cells were included (only 3-9%), but it was determined that the animals with detected polyploidy (9%) often had abortion. According to Russian cytogenetics (A.B. Bakaj, 1985) this numeric change indicate inclination towards irregular mitosis and meiosis in germinative tissue and such animals are excluded from reproduction. Heteroploidy influences on early embryonal mortality, because unbalanced zygote cannot continue regular division, and this results in abortion (GUSTAVSSON I., 1980). Control of numeric chromosome aberration in cattle production has practical importance, because it is important to know time of estrus, to prevent sperm aging in reproductive tract, since it was detected that this phenomenon influences cases of triploid or haploid-diploid chimeras (MARTIN and CHAVER, 1972), which was confirmed through our research. Aneuploid organisms with constitutional change on karyotype, where some chromosomes are in surplus or in deficit, and genetic balance is disturbed, carry serious phenotype, even teratogenic disorders and end up lethally (Figure 3.).

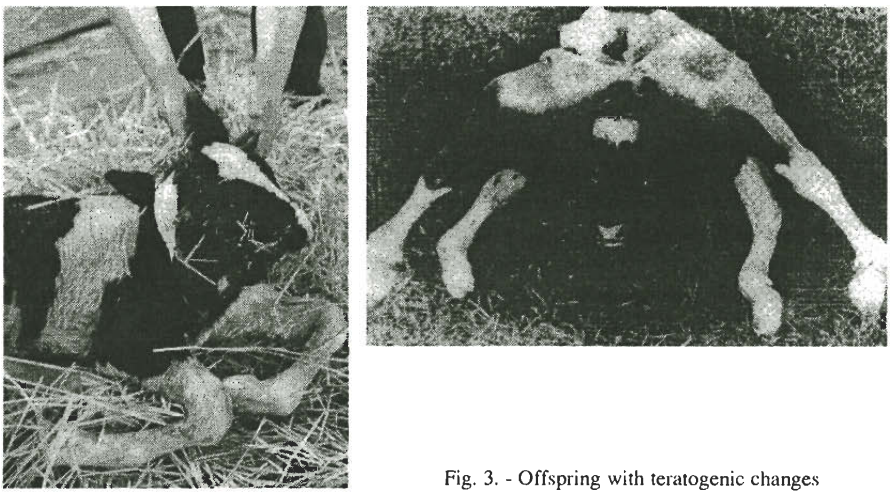

Fig. 3. - Offspring with teratogenic changes

However, aneuploidy where there are more sex chromosome of XXY-trisomy, do not show visible phenotype expression and can be detected with cytogenetic attestation when introduced in reproduction and subjected to additional gonad analyses (MOLTENI L. *et al.*, 1999). Beside above mentioned numeric changes, the following structural changes were discovered in genome: deletion, inversion, break, duplication, reciprocal and Robertsonžs translocation, fragile places, deviations from morphology in certain phases of mitosis and constrains of chromatides. Out of 100 examined metaphases chromosome aberration was ranging form 2% to constitutional karyotype. The most important reproductive disorder was discovered in reciprocal translocation. Translocation between one of homologous chromosome of the first pair on q-arm in region 2 was detected where part of q-arm chromosome from the fifth pair was translocated. This was type 1q+; 15 q- (Figure 4).

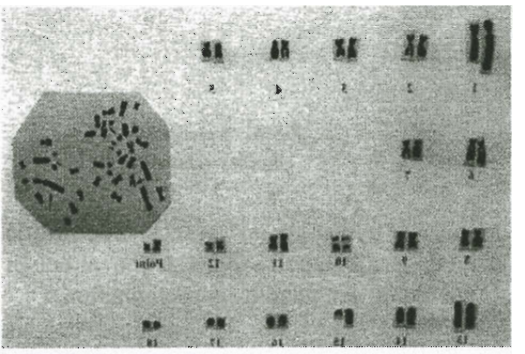

Fig. 4. - Reciprocal translocation 1q+; 15 q- karyogram and karyotype pig

In herd book there was the evidence that this animal had a stillborn offspring. This kind of karyotype change is considered by the European cytogenetics as the most responsible factor in reproductive disturbance, especially for phenomenon of small litters (KONFORTOVA G. D., *et al.*). During the research we discovered Robertson's translocation between 16th and 18th chromosome (Figure 5).

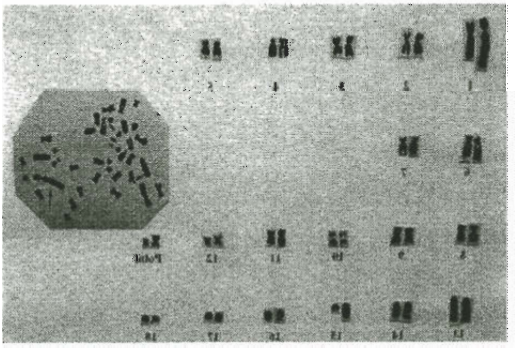

Fig. 5. - Robertson's translocation of 16/18 chromosoms boar

This type of translocation reduces fertility up to 36-50%, because in spermiogenesis arise balanced and unbalanced gametes. It is interesting to stress

that we discovered 5 breeding boars with fusion between the same acrocentric chromosome 16 and 18. Some authors (ASTOCHOVA N. M. *et al.*, 1991) consider this the cause of reduced fertility in male animals. Discovered structural changes of deletion break and deviation from chromosome morphology occasionally influenced on reproductive disorders. We discovered thermal chromatide deletion on q-arm of the 13th chromosome in an animal that used to give birth to mummified offspring (Figure 6.).

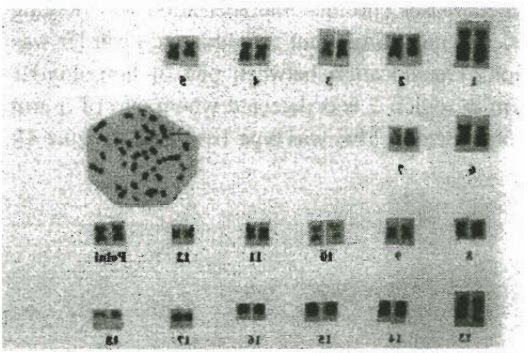

Fig. 6. - Deletion on 13th chromosomeq-arm gilt

There was a considerable number of hymerism and mosaicism on cattle farms. There are different opinions about their influence on reproduction, although our research points that these animals should be excluded from reproduction.

CONCLUSION

- we performed cytogenetic attestation on 5% of pig, cattle and horse farms,
- reproductive disorders were registered,
- discovered numeric and structural changes in kariotype resulted in reproductive disorders,
- on state level it is necessary to introduce selection for genome control of breeding animals.

REFERENCES

ASTACHOVA N. A, VYSOTSKA L. V, and GRAPHODATSKY A. S. (1991): Detailed analysis of a new translation in pig. Genetics - Selection - Evolution 23: Suplement 1, 65-69

BAKAY A. B. (1986): Kariotipi~eskie isledovaniæ selýskohazæystvenih `ivotnih Gosagropom - Moskva SSSR

BOYUM A. (1968): Separation of leukocytes from blood and bone morrow. Scand. J. Clin. Lab. Invest. 21,(Suppl. 97) 31

DI BERARADINO D. H, H. HAYES, R. FRIES and S. LONG (1990): ISCNDA-International System for Cytogenetic Nomenclature of Domestic Animals, Cytogenetic Cell. Genet. 53, 65-79.

GUSTAVSSON I. (1980): Chromosome aberation and thier influenca on the reproductive performance of domestic animals - a review. Z. Tierzüchtg. Züchtgsbiol. 97, 176-267

HAZAS G, A. KOVACS and P. KARAKAS (1990): Decrease of percentage of carriers of bovine hereditary chromosome abnormalites, Hungarian journal of animal production, 48 (1), 115- 116

HARTLEY S. E. and HORNE M. T. (1985): Cytogenetic techniques in fish genetics. J. Fish. Biol. 26, 575-582

KONFORTOVA G.D. (1995): A new reciprocal translation (7g^{+} : 15g^{-})in the domestic pig. Cytogenet. Cell. Genet.71, 285-288

KOŠARČIĆ S, M. GAGRČIN, R. KLJAJIĆ and S. BOBOŠ (1996): Značaj uvođenja citogenetičke atestacije priplodnih grla, Veterinarski glasnik, 50, (7-8), 473-480

KOŠARČIĆ S, M. GAGRČIN, D. KOŠARČIĆ, S. LAZIĆ and M. SARAČ (1997): Uloga i značaj citogenetskih istraživanja (određvanje kariotipa) kod svinja, Biotehnologija u stočarstvu, 13, (1-2), 19-24

KOŠARČIĆ S, D. KOŠARČIĆ and M. KOVAČEVIĆ (2000): The possible assotiation of structural chromosome aberrations with reproductive disturbances in the breeding pig. Acta veterinaria, 50, (2-3), 155-162

MARTIN P. A and SHAVER E. L. (1972): Sperm aging in utero and chromosomal anomalies in rabbit blastocites. Develop. Biol, 28, 480- 486

MOORHEAD P. S, P. C. NOWEL, W. J. MELLMAN, D. M. BATTIPS and D. A. HUNGERFORD (1960): Chromosome preparations from leukocytes cultures from peripheral blood. Exp. Cell. Res, 20, 613

MOETENI L, A. DE GIOVANINI MACCHI, D. MEGGIOLARO, G. SIRONI, F. ENICE and P. POPESCU (1999): New cases of XXY constitution in catle, Animal rep. Sci, 55, 107-113

SEABRIGHT M. (1971): A rapid banding technique for human chromosomes, Lancet , 2, 971- 972

SOLDATOVIĆ B, Z. PROLIĆ and M. CVETKOVIĆ (1977): Značaj citogenetike u dijagnozi kongenitalnih anomalija goveda. Veterinarski glasnik, 1, 33-39

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PROMENE U KARIOTIPU DOMAĆIH ŽIVOTINJA OTKRIVENE NA FARMAMA U VOJVODINI I NJIHOV UTICAJ NA REPRODUKCIJU

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Izvod

Novi pravci u stočarskoj proizvodnji zahtevaju uzgoj životinjskih vrsta sa visokim stepenom prilagođenosti na intenzivan način gajenja. Za ostvarenje navedenih zahteva pored poznatih metoda u selekciji neophodna je citogenetička kontrola postojećih genotipova, koja je sprovedena kroz desetogodišnja ispitivanja na farmama svinja, goveda i ergelama konja u Vojvodini. Otkrivene su hromozomske aberacije od numeričkih tipa poliploidije i aneuploidije do strukturnih tipa translokacija, delecija, duplikacija, inverzija, ringa, prekida i raznih oblika segregacije. Numeričke i strukturne promene u kariotipu životinja uticale su na poremećaj reprodukcije, fenotipsku ekspresiju, kao i na selekcijske programe i stabilnost genofonda. Registrovani su razni vidovi poremećaja u reprodukciji kao što su: mala legla, embrionalni mortalitet, česta povađanja, spontani pobačaji, mrtvorođeni i mumificirani plodovi, potomci sa anomalijama, razni oblici steriliteta. Analiza rezultata praćenje matične evidencije i sačinjavanje rodoslova ukazuju na postojanje hromozomopatija na našim farmama. Cilj ovog rada je upoznavanje naučne i stručne javnosti sa činjenicom da se otkrivene promene šire, posebno preko priplodnih grla i zato je potrebna genetska kontrola i blagovremeno isključenje hromozomskih aberacija.

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