

REVIEW OF INVESTIGATIONS ON INFLUENCE OF ENVIRONMENTAL CHEMICAL CONTAMINANTS ON HEREDITARY BASE AND REPRODUCTIVE CAPACITIES OF LANDRACE BREED BOARS FROM PIG FARM, CENTER FOR REPRODUCTION AND ARTIFICIAL INSEMINATION AND BIOSECURITY MEASURES IN SERBIA

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Summary

In this article we described two experiments. Our investigations were aimed at following: establishing possible onset of hereditary base changes in the boars originating from the farms located in the vicinity of the industrial zone as well as at identification of possible presence of heavy metals in the reproduction organs of the boars.

First experiment. Thus, our cytogenetic studies included boars (n=40). The boars were selected based on the history data. The former was followed by analysis of feed samples consumed by the boars and boar semen analysis. The analyzed samples of boar feed and semen (n=5) were analyzed in 100A digester made by »Tekator« using concentrated nitric acid and hydrogen peroxide. Heavy metal content was determined using absorption spectrometry. In six boars originating from the farm »A« aneuploid cell content ranging from 1.8% to 20.0% was detected. Polyploid cells were found only in 2 boars, ranging between 1.0 % and 1.1%. The cells with structural chromosomal aberrations were evidenced only in two boars, with their percentages being 1.0% and 8.9%, respectively. The identified cells with structural chromosomal aberrations were of the monochromatid break type. We have also analyzed karyotype in 34 boars from the farm »B«. The aneuploid cells ranging between 2.0% and 38.0% were evidenced. In 29 boars, the aneuploid cell count was not higher than 10.0%. Polyploid cells were evidenced in 16 boars in the percentages ranging between 1.0% and 6.0%. The cells with structural chromosomal aberrations were evidenced in 10 boars, with their percentage ranging between 1.0% and 7.0%. Transformed karyotype leads to reduction of fertility, which was confirmed by our findings.

Second experiment. Five boars used for reproduction that consumed the analyzed feed underwent semen analysis in order to determine presence of heavy metals using the method of absorption spectrometry. The obtained results indicated that the metals were transported into the reproductive organs, with the highest level of lead 0.36mg/kg, cadmium 0.0013 mg/kg and mercury 0.0021 mg/kg. None of the above-mentioned boars underwent cytogenetic analysis.

Key words: boars, cytogenetic analysis, environmental pollution

The paper presents a review of two experiments. The first experiment describes effects of environmental contaminants on boar karyotype alterations. The second experiment describes presence of heavy metals in boar semen.

The most common karyotype alterations in domestic pig are reciprocal translocations and Robertsonian fusions of the acrocentric autosomes. (1, 8, 9, 10, 12, 13, 15, 18, 20). All the above listed authors report untreatable reproductive disorders as major consequences of these karyotype alterations, which necessitate mandatory exclusion of the carriers of the anomaly from reproduction in order to prevent their subsequent emerging in population. The same authors stressed that karyotype alterations may develop under the influence of chemicals that may be found in feed, water or generally in the living environment of the studied animals.

Our study was aimed at determining possible onset of hereditary base alterations in boars living in chemically contaminated environment as well as at establishing possible presence of heavy metals in boar semen. Namely, it has been notorious that heavy metals represent severe risk for living systems since they react with organic molecules thus changing their structure and function. Heavy metals penetrate into the organism via the airways, gastrointestinal tract or through the skin.

Materials and methods

Karyotypes were analyzed in the total of 40 breeding boars. Analysis of karyotypes obtained from peripheral blood lymphocytes was performed using the method according to Moorhead et al. (1960) as well as the method subsequently modified by Evans and I Riordanova. Lymphocyte cultures were prepared with Parker 199 and nutritive medium with addition of sterile inactive calf serum. The cultures were grown in penicillin vials at 37°C. The quantity of 3.5 ml of Parker 199 medium, 1.5 ml of sterile inactivated calf serum, 0.5 ml of bacto-phyto hemagglutinin and 8 drops of whole venous blood were poured into the vials. Bacto-phyto hemagglutinin was used for transformation of lymphocytes to the blastoid cells in which potent mitotic activity starts. The cultures were kept 72 hours at 37°C. Two hours before processing of the lymphocyte culture cytostatic choline was added for halting of cell division in metaphase. After elapse of this period of time, vial content was transferred into graduated tubes and centrifuged, to be decanted and resuspended thereafter. The cells were kept in the hypotonic solution for 40 minutes at room temperature and the solution was centrifuged after that. After centrifuging, supernatant is decanted and freshly prepared fixing agent *methylicus-acidum : acetatum glaciale* in 3: 1 ratio was added to the precipitate. The preparations were Giemsa-stained.

In the second experiment, boar semen was analyzed when semen specimens were digested with concentrated nitric acid and hydrogen peroxide using digester 100A manufactured by "Tekator". Heavy metal content was determined using atomic absorption spectrometry.

Results and discussions

Karyotype analysis was performed in 24 boars from the pig farm A. Percentage of evidenced aneuploid cells ranged between 2.0% and 38%. In 29 boars, percentage of aneuploid cells did not exceed 10%. Percentage of polyploid cells evidenced in 16 boars ranged between 1.0% and 6.0%. Percentage of cells with structural chromosomal aberrations evidenced only in 10 boars ranged between 1.0% and 7.0% (Table 1, fig.1)

Table 1

Cytogenetic analyses breeding boars from pig farm "A"

Number of boars	Total number of cells	Number(%) of diploid cells	Number(%) of aneuploid cells	Number(%) of poliploid cells	Number(%) of cells with structural chromosome aberrations
1	100	87(87.0)	12(12.0)	1(1.0)	1(1.0)
2	100	92(92.0)	2(2.0)	6(6.0)	-----
3	100	81(81.0)	16(16.0)	3(3.0)	7(7.0)
4	100	90(90.0)	9(9.0)	1(1.0)	-----
5	100	91.(91.0)	9(9.0)	-----	-----
6	100	93(93.0)	7(7.0)	-----	-----
7	100	89(89.0)	10(10.0)	1(1.0)	-----
8	100	94(94.0)	6(6.0)	-----	-----
9	100	96(96.0)	4(4.0)	-----	-----
10	100	91(91.0)	9(9.0)	-----	-----
11	100	97(97.0)	3(3.0)	-----	-----
12	100	59(59.0)	38(38.0)	3(3.0)	6(6.0)
13	100	94((94.0)	6(6.0)	-----	-----
14	100	95(95.0)	4(4.0)	1(1.0)	-----
15	100	89(89.0)	11(11.0)	-----	-----
16	100	89(89.0)	10(10.0)	1(1.0)	2(2.0)
17	100	92(92.0)	8(8.0)	-----	2(2.0)
18	100	96(96.0)	4(4.0)	-----	-----
19	100	90(90.0)	9(9.0)	1(1.0)	1(1.0)
20	100	92(92.0)	7.(7.0)	1(1.0)	-----
21	100	96(96.0)	4(4.0)	-----	-----
22	100	95(95.0)	5(5.0)	-----	1(1.0)
23	100	89(89.0)	10(10.0)	1(1.0)	1(1.0)
24	100	89(89.0)	10(10.0)	1(1.0)	2(2.0)
25	100	78(78.0)	18(18.0)	4(4.0)	-----
26	100	92(92.0)	8(8.0)	-----	-----
27	100	93(93.0)	7(7.0)	-----	1(1.0)
28	100	96(96.0)	4(4.0)	-----	-----

29	100	95(95.0)	5(5.0)	-----	-----
30	100	98(98.0)	2(2.0)	-----	-----
31	100	94(94.0)	6(6.0)	-----	-----
32	100	92(92.0)	6(6.0)	2(2.0)	-----
33	100	93(93.0)	6(6.0)	1(1.0)	-----
34	100	94(94.0)	5(5.0)	1(1.0)	-----

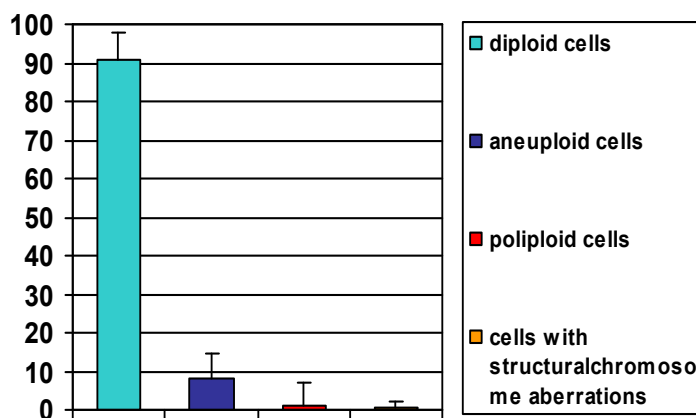


Fig. 1. Procent values: diploid, aneuploid, poliploid cells, and cells with structural chromosome aberrations

Karyotype analysis was performed in 6 boars from the Center for Reproduction and Artificial Insemination. Percentage of evidenced aneuploid cells ranged between 1.8% and 20.0%. Aneuploid cells were not evidenced in one of the boars. Percentage of polyploid cells evidenced in two boars ranged between 1.0% and 1.1%. Percentage of cells with structural chromosomal aberrations evidenced only in two boars ranged between 1.0% and 8.9% (Table 2, fig. 2)

Table 2

Cytogenetic analyses breeding boars from Center for reproduction and artificial insemination

Number of boars	Total number of cells	Number(%) of diploid cells	Number(%) of aneuploid cells	Number(%) of poliploid cells	Number (%) of cells structural chromosome aberrations
1	80	78(97.5)	2(2.5)	-----	-----
2	76	76(100.0)	-----	-----	-----
3	56	55(98.2)	1(1.8)	-----	-----

4	90	71(78.9)	18(20.0)	1(1.1)	8(8.9)•
5	100	89(89.0)	10(10.0)	1(1.0)	1(1.0)••
6	66	63(95.4)	3(4.6)	-----	-----

Chromosome tranlocations•
Chromosome phragments••

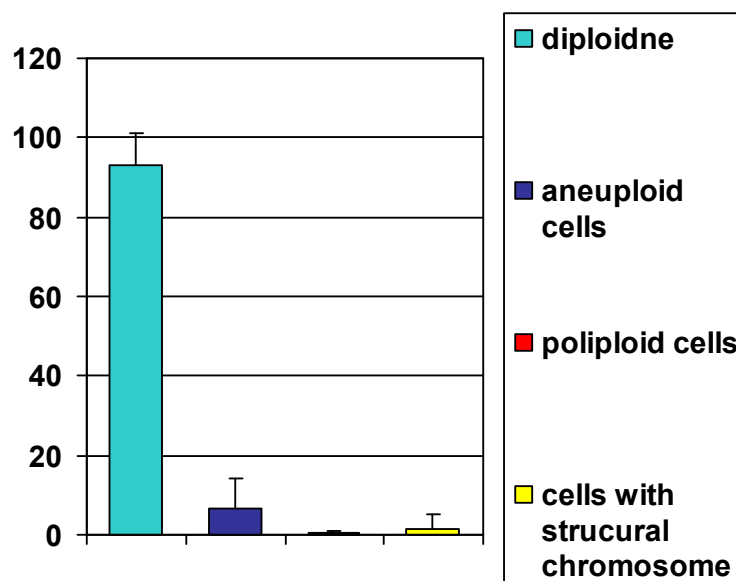


Fig. 2. Percentage values diploid, aneuploid, poliploid cells and cells with structural chromosome aberrations

The incidence of cells with structural chromosomal aberrations, mostly of the monochromatid breaks type as well as balance translocations was somewhat higher. Chromatid breaks detected in 10 boars, tended to emerge as the result of the effects of the chemicals found in the living environment. The incidence is considered to be rather high and thus it may result in reproduction disorders.

We believe that detection of carriers of the structural balance karyotype alterations is important in breeding heads. It is particularly important for artificial insemination programs, since the alterations may spread through the population and eventually result in reproduction disorders in the following generations. Therefore, we suggest timely elimination of heads with balance karyotype alterations from reproduction.

The obtained results suggested that heavy metals were transported from the circulation into the reproductive organs of boars, with lead quantity being the highest with 0.36 mg/kg, followed by cadmium with 0.0013 mg/kg and mercury with 0.0021 mg/kg (fig. 3)

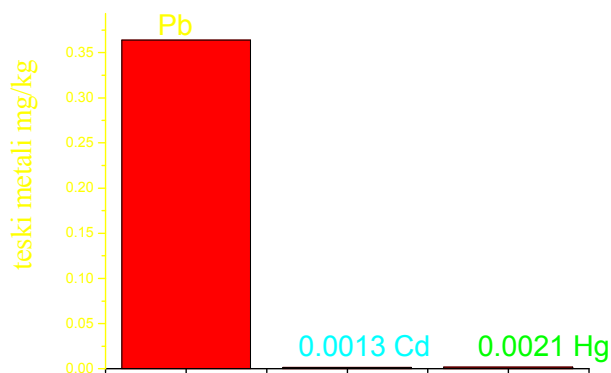


Fig. 3. Heavy metals in semen boars

Our published studies also addressed application of biosafety measures on the pig farms (2, 5, 6). Planned application of biosafety measures on the pig farms should be the result of logical reasoning and timely applied measures in the specific environment as well as understanding of epidemiological situation with recognition of the concrete environmental threats and weaknesses of the manufacturing technology. Introduction of Hazard Analysis and Critical Control Point (HACCP) and sanitation protocol has led to logical resolution for prevention of introduction and spreading of the infectious diseases on the breeding farm (17). Namely, it has been well known now that biosafety, well-being, good manufacturing practice and risk analysis at the critical control points are highly important elements in intensive pig production, which includes high concentration of animals in relatively small spaces. Implementation of biosafety measures is crucial for protection of pig health and success of the production itself. Biosafety plans are essential for prevention of the diseases and unwanted situations as well as for improvement of business. (19). Emergency plans are also being prepared for more rapid responding to unwanted events aimed at timely continuation of production. Assessment of biosafety based on indicators (isolation as a measure of farm biosafety, quarantine, assessment of health status of the breeding herd, attitude of staff toward equipment, control of movement and turnover, regime of visits, control of feed and feeding equipment, cleaning, removal of carcasses, attitude toward other farm animals, bird and rodent control), should be a routine mechanism for assessment of biosafety on a farm that may point out to direction of further measures and their possible improvement (11, 16).

Conclusions

Identification of chromosomal aberration carriers will assure their elimination from the reproduction program. Carriers of the hereditary anomalies most commonly have balanced alterations of the karyotype, they are heterozygotic and thus the changes are not observed on the phenotype and the consequences are becoming manifest in the offspring in the form of different disorders, both physiological and production characteristics-related. Identification of the carriers is particularly important in artificial insemination programs. In the era of wide application of artificial insemination when semen obtained from one breeding boar may be used for fertilization of a large number of sows, identification of carriers of the hidden anomalies represents important task during selection of breeding units.

Heavy metals represent a particular threat for living organisms since they react with organic molecules thus changing their structure and function. Heavy metals penetrate into the organism via the airways, gastrointestinal tract or through the skin. The results of our years-long studies indicate possibility of contamination of feed with heavy metals and their depositing into the animal organism as well as their negative effects on the reproductive capacity of the domestic animals. Toxicity of heavy metals generally leads to forming of free radicals and inhibition of antioxidative defense enzyme activity as well as glutathione oxidation and production of malondialdehyde (MDA) as a marker of oxidative stress. The toxicity is the result of their capacity to form covalent bonds with sulfhydryl groups of biomacromolecules or to displace certain cofactors, thus inhibiting activity of certain enzymes (3).

Veterinary medicine has a highly important role in animal husbandry, protection of animal and human health and environmental protection. Each day we are faced with the question how to produce as much as possible food that will indeed be safe and suitable for human consumption. For this reason, health protection of farm animals more based on preventive than on therapeutic measures with veterinary medicine measures as its integral part, may assure a concept that will provide at the same time lucrative production and multi-level control of toxic agents.

Our recommendation for the industrial-scale farms is application of measures aimed at reduction of risks associated with heavy metal effects for the purpose of introduction of multi-stage quality monitoring of raw materials and finished products as well as application of appropriate protectors against toxic effects of these agents (4).

Acknowledgments

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References

1. **Bojkovski, J.**, Efekti faktora sredine na kariotipske varijacije sisara. Doktorska disertacija. Univerzitet u Beogradu, Fakultet veterinarske medicine 1994.
2. **Bojkovski, J., Radojčić, Biljana, Petrujkić, B.**, Savremeni aspekti u dijagnostici i terapiji uzgojnih bolesti svinja. "Proceedings work shop;" Clinica Veterinaria" Ohrid, 2005, 251-257.
3. **Bojkovski, J., Borozan, Sunčica, Hristov, S., Stanković, B., Jaksimović-Todorović, Mirjana, Davidović, Vesna**, Uticaj kontaminenata na zdravstveno stanje farmskih životinja. Dobrobit životinja i biosigurnost na farmama str. 251-258. Međunarodna konferencija o dobrobiti i biosigurnosti na farmama u Srbiji, Zemun 14. i 15. novembar. 2007 Monografija Univerzitet u Beogradu, Poljoprivredni fakultet
4. **Bojkovski, J., Radojčić, B., Petrujkić, T., Borozan, S.**, A contribution to insight of the most important etiological factors with influence of farm animal health in Serbia Proceedings of the International Symposium on new research in biotechnology, Bucharest, Romania, Biotechnology, seria F, Special volume, 2008, 101-114.
5. **Bojkovski, J., Stanković, B., Petrukić, T., Radojčić, B.**, Uzgojne bolesti, telesna kondicija i biosigurnosne mere na farmi svinja industrijskog tipa Veterinarski žurnal Republike Srpske, 2009, IX, 1. 43-52.
6. **Bojkovski, J.**, Prilog poznavanju uzgojne patologije svinja sa osvrtom na biosigurnosne mere. Zbornik radova VII Simpozijuma Zdravstvena zaštita, selekcija i reprodukcija svinja, Srebno jezero, 2009, 57-64.
7. **Ducos Berland, H.H., Bonet, A., Calgaro, S., Billoux, S., Mary, N., Garnier-Bonnet, A., Darre, R., Pinton, A.**, Chromosomal control of pig populations in France. 2002-2006 survey, Genet Sel Evol, 2007, 39, 583-597.
8. **Gustavson, I.**, Chromosomes of the pig, Adv. Vet. Sci. Comp. Med, 1990, 34, 73-107.
9. **Gustavson, I., Jönsson, L.**, Stillborns, partially monosomic and partially trisomic in the offspring of a boar carrying a translocation: rcp(14;15)(q29;q24), Hereditas, 1992, 171, 31-37.
10. **Kuokkanen, M.T. Mäkinen, A.**, Reciprocal chromosome translocations (1p-11q+) and (1p+15q-) in domestic pigs with reduced litter size, Hereditas 1988, 109, 69-73.
11. **Lončarević, A., Maričić, Z., Tosevski, J., Pavlović, I.**, Osnove sistematskog zdravstvenog nadzora i programiranje zdravstvene zaštite svinja u intenzivnom odgoju U: A. Lončarević: Zdravstvena zaštita svinja u intenzivnom odgoju, Izd.: Naučni institut za veterinarstvo Srbije, Beograd, 1997, 517-523.
12. **Madan, K., Ford, D., Polge, C.**, A reciprocal translocation, t(6p+;14q-) in the pig, J. Reprod. Fert., 1978, 53, 395-398.

13. **Parkanyi, V., Fulop, L., Babusik, P., Kuliskova, L.**, Reciprocal chromosome translocation rcp(5q+;15q-) in sterile boar with andrologic finding. Proc 10th Int. Colloq. Cytogenet. Dom. Anim. Utrecht, The Netherlands, 1992, 165.
14. **Petrujkić, T., Bojkovski, J., Radojičić, Biljana, Petrujkić, B.**, Prilog poznavanju reproduktivnog i zdravstvenog statusa svinja intenzivnog uzgoja Veterinarski žurnal Republike Srpske, 2007, VII, 1, 20-29.
15. **Popescu, C.P. Bonneau, M., Tixier, M., Bahri, I., Boscher, J.**, Reciprocal translocations in pigs. Their detection and consequences on animal performances and economic losses, J. Hered, 1984, 448-452.
16. **Stanković, B., Hristov, S., Valčić, M., Petrujkić, T., Bojkovski, J.**, Standardi doborbiti biosigurnosti na farmama goveda i svinja 8 Kongres Veterinara Srbije sa međunarodnim učešćem zbornik referata, Beograd, 2009, 67.
17. **Stanković, B., Hristov, S., Petrujkić, T., Todorović Jaksimović, M., Davidović, V., Bojkovski, J.**, Biosigurnost na farmama svinja u svakodnevnoj praksi, Biotechnology in animal husbandry, 2008, 24, 601-608.
18. **Tzocheva, K.**, A new reciprocal translocation rcp(1p-;11p+) in the karyotype of the boar, Hereditas, 1994, 120, 187-189.
19. **Uhlepnhoop, E.**, Biscurity planning for livestock farms. Doborbit životinja i biosigurnost na farmama, monografija Poljoprivredni fakultet, Zenmun, 2007, 227-237.
20. **Vilagómez, D., Gustavson, A.F., Jönsson, I., Plöen, L.**, Reciprocal chromosome translocation, rcp(7,17)(q26,q11), in a boar giving reduced litter size and increased rate of piglets dying in the early life, Hereditas, 1995, 257-267.