COMPREHENSIVE APPROACH TO DEFINITION OF GENETIC AND REPRODUCTIVE CAPACITY OF BOARS USING CYTOGENETIC AND BIOTECHNOLOGICAL METHODS

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The article presents data on research fertilizing ability of boar spermatozoa of different breeds based on modeling the process of gametes interaction in terms in vitro and cytogenetic evaluation of chromosomal polymorphism of somatic cell producers.

Oocytes, sperm, in vitro, fertilization, embryo, karyotype, chromosome aberrations, pigs.

The intensification of animal production significantly depends on the correct system forming herds, usage and livestock breeding sires. Monitoring and evaluation of reproductive traits of boars with conventional methods in quality offspring gives complete data on the morphofunctional condition of the body, the impact of the karyotype instability in reproductive ability bulls, forming multiple pregnancy values in paired sows and their piglets exit. Common evaluation methods are complicated by the fact that a number of inherited phenotypic abnormalities occur only in adult animals obtained from parents-carriers with hidden genetic defects [3]. Therefore, for a complete description of genetic and reproductive potential of sires it’s appropriate to conduct comprehensive monitoring, part of which is to analyze the karyotype test animals and fertilizing ability of sperm by the method of fertilization in vitro.

The preventive diagnosis of genetic potential of boars in commercial breeds using cytogenetic methods provides an estimate mutability of environmental factors, to determine the degree of influence of chromosomal abnormalities in productive and reproductive characteristics of animals, their viability, the probability of genetic risk on account of the frequency and spectrum of chromosomal mutations in somatic cells [8]. According to the "Instructions for

artificial insemination of pigs" ability fertilizing boar semen should be checked at least five ejaculates and twenty inseminations of sows [9], due to the high costs and time. Modeling of the interaction of female and male gametes in in vitro conditions allows quickly, objectively, with less ability to assess fertilizing sperm sires compared with the use of artificial insemination [1, 10]. Thus, in one experiment 100 oocytes matured in vitro can be inseminated with sperm from one boar and in 6 days we can have results of fertilizing ability of sperm.

Thus, implementation of livestock integrated approach to identifying and predicting genetic and reproductive potential sires based on conducting sustained cytogenetic screening of productive commercial animal breeds with hidden unstable karyotype, mutational variability which will manifest itself in the offspring and future generations while animals in with elevated levels of genotoxic factors and testing fertilizing ability of sperm by the method of fertilization in vitro, will enable in short conducting an objective assessment of the breeding value of animals.

The aim was to evaluate the reproductive traits of boars of different breeds using cytogenetic and biotechnological methods.

Materials and methods of the research. The objects of the research were three boars aged 2 years breed of large white, Petren and PIC−337 beef synthetic lines L−65 that are typically kept under Ltd. «Pryluky pedigree stock-breeding».

Methods of obtaining cytogenetic preparations of peripheral blood lymphocytes included the following sequence of steps: blood collection, transportation, preparation of sterile nutrient bottles, cooking preparations, color, analysis of metaphase plates, photographs [11]. During the research determined the percentage of metaphase plates with quantitative abnormalities (aneuploidy), cells with asynchronous disjunction of centromere areas of chromosomes and structural disorders − breaks chromosomes. 100 metaphase plates were analyzed in each animal.

Micronucleus test was performed on the same preparations, counting dual lymphocytes (AH), mononuclear cells with micronuclei (MN), mitotic index (MI).

Oocyte-cumulus complexes were obtained from slaughtered ovaries of pigs of large white breed. Oocytes matured in vitro in medium TC 199 with the addition of 20% estrusal serum of cows and 3−5x10^6 granulosa cells/ml (granulosa cells obtained from follicles with a diameter of 3−4 mm without atretic changes and morphologically normal oocytes). Oocyte-cumulus complexes of pigs were cultivated for 45 hours at + 38,8° C and 4% CO_2 in air. For in vitro fertilization, ejaculated sperm of boars was used. Motile sperm was selected by the ascent (swim-up) in TALP medium without calcium ions [12]. Joint incubation of in vitro matured oocytes and selected by swim-up sperm was performed for 20 hours in a modified environment Tirode (TALP) supplemented with 10 μg/ml heparin, 20 μM penicillamine, 10 μM hypotaurine and 1 μM epinephrine. The washed sperm from the expected zygotes were cultivated in vitro in NCSU-23 medium. Cytogenetic preparations of oocytes and embryos nuclei were prepared by a modified method of A.K. Tarkowski [13]. The preparations were stained with 2% solution of the dye Giemsa and analyzed them under a light microscope with an increase oc.10 ×ob.100.

Morphological analysis of sperm in the presence of abnormal forms was carried out under a microscope by the conventional method.

Statistical analysis of the data was performed by standard methods [5] using the computer program «Microsoft Excel».

Results of the research. In order to develop a method of comprehensive evaluation of sires using biotechnology and cytogenetic techniques in biotechnology reproductive system of farm animals it was performed an evaluation of sperm fertilizing ability of three breeds of boar large white, Petren and PIC−337 beef synthetic lines L−65 on the basis of fertilizing method of matured in vitro oocytes and cytogenetic evaluation of chromosomal polymorphism in somatic cell of producers. The audit of fertilizing ability of boar spermatozoa of breed large white (№77376), Petren (№70858), PIC−337 hybrid in terms of in vitro it was revealed that the level of cleavage of pig embryos for insemination using in vitro matured oocytes of ejaculated boar sperm PIC-337 hybrid was the highest (62,0%)

and exceeded by 6,0% and 1,2% of the corresponding rates sires breeds of large white and Petren but significant difference between the fertilizing ability of boar gametes after insemination of in vitro matured oocytes were not found (Table 1, Fig. 1).

1. Quantitative indexes of reproductive ability of spermatozoa of boar

Species, individual number of boar	Ejaculate volume, ml	Sperm concentration billion/ml	Total of pathological form, %	Total of insemination eggs, n (%)	Level of cleavage embryos in vitro, n (%)
Petren (77376)	273	1,49	2,2	51	31[a](60,8±6,84)
Large white (70858)	215	1,12	2,4	50	28[a](56,0±7,02)
PIC 337 beef synthetic lines L-65	168	1,09	1,4	50	31[a](62,0±6,86)

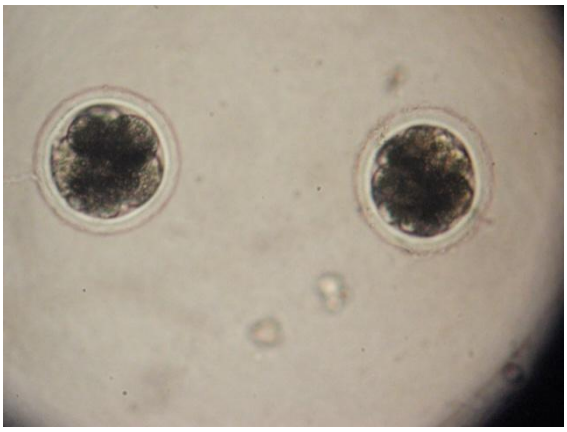

Figure 1 Pig embryos formed after fertilization with ejaculated sperm of matured oocytes in vitro, (magnification, 400x)

The impact of artificial insemination of females depends on the quality of sperm, which was obtained from a nursery. Assessment of sperm enables first determine its biological usefulness, that ensure fertilization and obtain healthy offspring. However, a common characteristic of semen (ejaculate volume, sperm motility, sperm concentration) can not always explain the reason for its low fertilization ability. A morphological analysis of ejaculate sires was investigated for the presence of abnormal sperm forms and it was found presence of sperm

morphological abnormalities as abnormal acrosome in different ratios, with distal cytoplasmic droplet, free head and twisted flagellum (Fig. 2) [4]. Yes, sire breed Peitrain, great white and PIC−337 hybrid abnormal sperm were 2,2%, 2,4% and 1.4%, respectively, does not exceed the allowable [2] (Table 2).

It is known that in animals ejaculate sperm immature germ cells can be found. It was identified that in individuals with impaired spermatogenesis the number of immature germ cells dramatically increases and reduces the fertilizing ability of sires [7]. Analysis of the results of the studies showed the absence of immature germ cells in the ejaculate of sires which were studied.

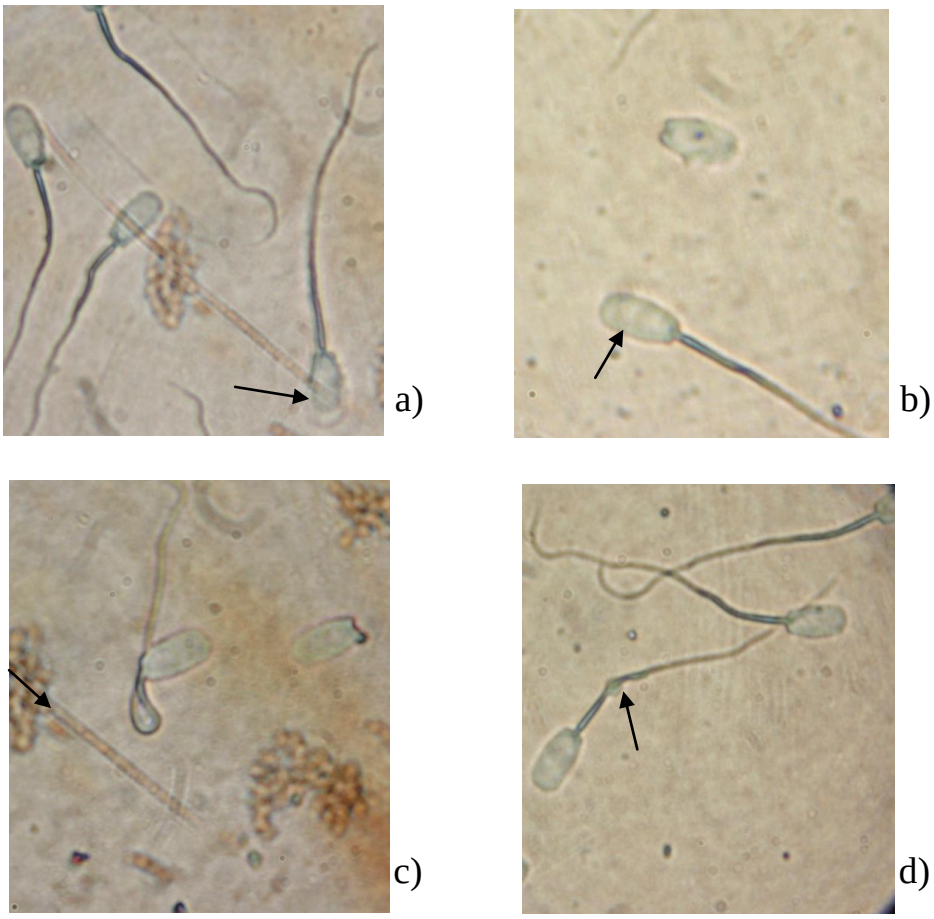

Figure 2. Abnormal forms of sperm in the ejaculate sires: a) bulging acrosome; b) free head; c) twisted flagellum; d) distal cytoplasmic droplet (magnification, 1000x)

2. Distribution of abnormal sperm forms of boars

Species	Total sperm analysis, n	Distal cytoplas-matic drop, n	Patholo-gacal acrosome, n	Free head, n	Spin flagellum, n	Total pathological forms, %
Petren	500	2	1	4	4	2,2
Large white	500	3	-	3	6	2,4
PIC 337 beef synthetic lines L-65	500	1	1	-	5	1,4

For a more complete description of the reproductive properties of sires it is appropriate to conduct cytogenetic monitoring of animal karyotype (Fig. 3). The results of cytogenetic analysis of peripheral blood lymphocytes in studied animals showed the presence of genomic and structural chromosomal variability (Table 3). Quantitative violation of chromosomes manifested as aneuploidy. The lowest index of variability was typical for boar breeding PIC−337 hybrid and was 2,3%, and the highest − in large white boar breed that met the 4,0% rate.

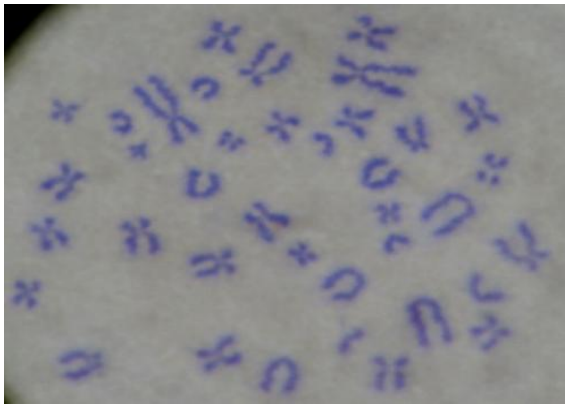

Figure 3. Karyotype of boar breed of white, (2n = 38), (magnification, 1000x)

3. Evaluation of chromosomal polymorphism of somatic cells of boars

Breed	Age, years	Aneuploidy, %	Asynchronous disjunction of centromere areas of chromosomes, %	Chromosomal breaks, %	Chromatid breaks, %	MN, ‰	MI, ‰
Petren	1,5	3,3	2,7	2,05	0,9	3	6.3
Large white	1,5	4,0	2,3	1,6	1,0	2	1,9

PIC 337 beef synthetic lines L-	1,5	2,31	2,05	1,3	0,63	-	4,4

The frequency of aneuploidy was 2,3 – 4,0% and corresponded to the spontaneous level, which is typical for the species Sus scrofa [6]. Background of numerical chromosome disorders is asynchronous cleavage of their centromeric areas. According to the results it was found that the frequency of asynchronous cleavage of centromeric regions of chromosomes in studied male pigs was 2,05 – 2,7%. Structural violations of chromosomes were represented by chromosomal and chromatid breaks and fluctuated within 1,3 – 2,05% and 0,63 – 1,0%, respectively. The difference of averages for quantitative disorders of chromosomes (aneuploidy), asynchronous splitting centromeric areas and structural chromosome abnormalities (chromosomal and chromatid breaks) between species was unreliable and did not exceed spontaneous level characterized for hogs of these species [11].

For more complete assessment of somatic mutagenesis in studied boars a micronucleus test was used. In sires the frequency of lymphocytes with micronuclei (MN) was 2 − 3 ‰, which did not exceed the parameters of cytogenetic indicators of domestic pigs for spontaneous mutagenesis. Dual-lymphocytes in the blood of studied boars were absent. The obtained results confirm the expression of low levels of spontaneous mutations and found no differences interbreed.

To establish an associated connection between instability of karyotypes and reproductive functions of male pigs a correlation analysis was done. The results showed negative correlation among aneuploidy, asynchronous difference in centromeric areas of chromosome, chromatid breaks and feritilizing ability (Table 4).

It was discovered likely negative correlation between aneuploidy and fertilizing ability of sperm sires (P> 0,95). It was also found a negative correlation link between fertilizing ability of sperm and asynchronous disjunction of

centromere areas of chromosomes and chromatid breaks of chromosomes. This means that with an increase in the frequency of cells with chromatid breaks and asynchronous disjunction of centromere areas of chromosomes tends to decrease fertilizing ability in animals. Chromosomal breaks negatively correlated with ejaculate volume, sperm concentration and percentage of probable reliability of pathological forms (P> 0,999).

4. Correlation between karyotype instability and reproductive functions of boar

Correlating signs	Chromosomal abnormalities			
	Aneuploidy, %	Asynchronous disjunction of centromere areas of chromosomes, %	Chromosome breaks, %	Chromatid breaks, %
Volume ejaculate	0,5328	0,9974	-0,6657	0,6613
Concentration sperm	0,1654	0,9480	-0,3266	-0,1822
Percentage of pathological forms	0,9726	0,6628	-0,9977***	0,9973
Fertilizing ability	-0,9080*	-0,0576	0,8260	-0,8294

*-P>0,95;***-P>0,99

Conclusions and recommendations for further research. Thus, analyzing the obtained results it was revealed the unreliability of the difference of averages between quantitative and structural chromosomal disorders in boars of different breeds. It was found an associative link between karyotype instability of sires and the percentage of abnormal sperm forms and fertilizing ability of boars.

Further research will be aimed at determining the effect of chromosomal polymorphism of germ cells to fertilizing ability of sperm sires in vitro.

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