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CYTOGENETICAL AND BIOTECHNOLOGICAL METHODS FOR ASSESSING BOAR REPRODUCTIVE POTENTIAL

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Abstract

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

Key words: oocyte, sperm, in vitro, fertilization, embryo, karyotype, chromosome aberration, pig

Abbreviations: ACCRC – asynchronous cleavage of centromeric regions of chromosomes, DL – dual lymphocytes, LM – lymphocytes with micronuclei, MI – mitotic index

Introduction

The intensification of animal production significantly depends on the correct system of forming herds, selection and livestock breeding sites. Monitoring and evaluation of the 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 the reproductive ability, multiple pregnancy values in paired sows and their piglets. The 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 (Gusev, 2009). 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 of test animals and the fertilizing ability of sperm by the method of *in vitro* insemination.

The preventive diagnosis of the genetic potential of boars in commercial breeds using cytogenetic methods provides an estimate of the 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 (Dzitshyuk, 2009). According to the "Instructions for artificial insemination of pigs" the fertilizing ability of boar semen should be checked at least at five ejaculates and twenty inseminations of sows (Melnik, 2003), due to the high costs and time needed. Modeling of the interaction of female and male gametes in *in vitro* conditions allows quickly and objectively to assess sires fertilizing sperm compared with the use of artificial insemination (Burkat et al, 1992). Thus, in one experiment 100 oocytes matured *in vitro* can be inseminated with sperm from one boar and in 7 days we can have results for the fertilizing ability of sperm.

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

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

Materials and Methods

The objects of the research were three boars aged 2, of Large White, Peitrain and PIC–337 beef synthetic lines L–65 Large White breeds that are typically kept under "Pryluky pedigree stock-breeding" Ltd.

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 (Kopylov et al, 2011). During the research we determined the percentage of metaphase plates with quantitative abnormalities (aneuploidy), cells with asynchronous cleavage of centromeric regions of chromosomes (ACCRC) and structural disorders - breaks chromosomes. 100 metaphase plates were analyzed in each animal. Micronucleus test was performed on the same preparations, counting dual lymphocytes (DL), lymphocytes with micronuclei (LM), mitotic index (MI).

Prepubertal porcine ovaries were collected from slaughterhouses and transported to the laboratory at 20°C. Oocyte-cumulus complexes were obtained from ovaries of slaughtered pigs. Oocytes matured *in vitro* in medium TC 199 with the addition of 20% estrusal serum of cows and $3–5\times10^6$ 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 incubated for 45 h at 38.8°C in an atmosphere of 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 (Katska, 1993). 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 hipotauryn 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. Tarkovsky (Tarkowski, 1966). The preparations were stained with 2% solution of the Gimza and examined under the light microscope at 400× and 1000× magnification. Morphological analysis of sperm in the presence of abnormal forms and examined under the phase-contrast microscope at 400× and 1000× magnification by the conventional method (GOST P 54638-2011, 2013; Milavanov, 1962). Statistical analysis of the data was performed by standard methods using the computer program «Microsoft Excel».

Results and Discussions

In order to develop a method of comprehensive evaluation of sires, biotechnological and cytogenetic techniques in biotechnology reproductive system of farm animals were performed with an evaluation of sperm fertilizing ability of three breeds Large White, Peitrain and PIC–337 beef synthetic lines L–65 Large White breed, 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 Large White, Peitrain, PIC–337 hybrid, in terms of *in vitro* 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% that of the corresponding rates for sires of Large White and Peitrain breeds but significant difference between the fertilizing ability of boar gametes after insemination of in vitro matured oocytes were not found (Table 1).

The impact of artificial insemination of females depends on the quality of sperm, which was obtained from a nursery. Assessment of sperm enables first to determine its biological usefulness that ensure fertilization and obtainment of healthy offspring. However, a common characteristic of semen (ejaculate volume, sperm motility, sperm concentration) cannot

Table 1
Quantitave index of fertile ability of boars' sperm

Breed	Ejaculate volume, ml	Sperm concentration ml billion / ml	Total of pathological form, %	Total of insemination oocytes, n	Level of embryos cleavage in vitro, n %
Peitrain	273	1.49	2.2	51	31a (60.8)
Large White	215	1.12	2.4	50	28a (56.0)
PIC–337	168	1.09	1.4	50	31a (62.0)

always explain the reason for its low fertilization ability. A morphological analysis of sires' ejaculate was investigated for the presence of abnormal sperm forms and we found presence of sperm morphological abnormalities as abnormal acrosome in different ratios, with distal cytoplasmic droplet, free head and twisted flagellum. Sire breed Peitrain, Large White and PIC–337 hybrid had abnormal sperm of 2.2%, 2.4% and 1.4%, respectively which does not exceed the permissible (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 (Dzitshyuk, 2009). Analysis of the results of the studies showed the absence of immature germ cells in the ejaculate of the sires which were studied.

For a more complete description of the reproductive properties of sires it is appropriate to conduct cytogenetic monitoring of animal karyotype. The results of cytogenetic analysis of peripheral blood lymphocytes in studied animals showed the presence of genomic and structural chromosome 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 large white boar breed that met the 4.0% rate.

The frequency of aneuploidy was 2.3–4.0% and corresponded to the spontaneous level, which is typical for the species Sus scrofa (Dzhus, 2012). 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 typical for hogs of these species.

For more complete assessment of somatic mutagenesis in studied boars a micronucleus test was used. In sires the frequency of lymphocytes with micronuclei (LM) 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 fertilizing ability (Table 4). Likely negative correlation between aneuploidy and fertilizing ability of sperm sires was discovered (P>0.95). Also we found a negative correlation between fertilizing ability of sperm and ACCRC and chromatid

Table 2
Distribution of abnormal sperm forms

Breed	Total sperm analysis, n	With bistal cytoplasmatic drop, n	Wist pathologacal acrosome, n	Free head, n	With spin flagellum, n	Total pathological forms, %
Peitrain	500	2	1	4	4	2.2
Large White	500	3	0	3	6	2.4
PIC–337	500	1	1	0	5	1.4

Table 3
Evaluation of chromosomal polymorphism of somatic cells

Breed	Age, years	Aneubloidy, %	ACCRC	Chromosomal breaks, %	Chromatid breaks, %	Lymphocytes with micronuclei, ‰	Mitotic index, ‰
Peitrain	1.5	3.3	2.7	2.05	0.9	3	6.3
Large White	1.5	4	2.3	1.6	1	2	1.9
PIC–337	1.5	2.31	2.05	1.3	0.63	0	4.4

Table 4
Correlation between karyotype instability and reproductive functions of male pigs

Correlating signs	Chromosomal abnormalities			
	Aneuploidy,%	ACCRC,%	Chromosome breaks,%	Chromatid breaks,%
The volume of ejaculate	0.5328	0.9974	-0.6657	0.6613
The concentration of sperm	0.1654	0.9480	-0.3266	-0.1822
The 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

breaks of chromosomes. This means that with an increase in the frequency of cells with chromatid breaks and ACCRC fertilizing ability in animals tends to decrease. Chromosomal breaks are also negatively correlated with ejaculate volume, sperm concentration and percentage of probable reliability of pathological forms (P>0.99).

Conclusions

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. An associative correlation between karyotype instability of sires and the percentage of abnormal sperm forms and fertilizing ability of boars was found.

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