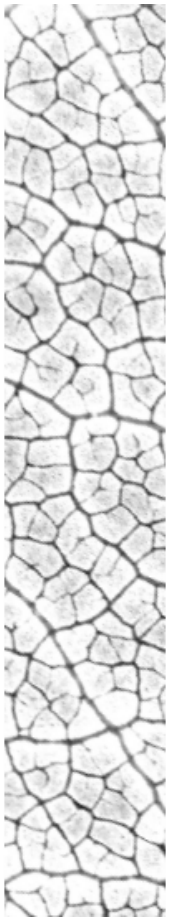

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Screening of the allele pool of the pig populations of various breeds in the Belgorod and Voronezh regions of Russia by the gene of the estrogen receptor gene *ESR1*

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Abstract
We studied populations of boars of two pig breeds (Large White and Landrace) in the Belgorod and Voronezh regions of Russia to identify mutations in the gene of the estrogen receptor *ESR1* (*ESR/PvuII*). The results showed that the frequency of the positive *B* allele was 0.279 in the Landrace and 0.824 in the Large White. An assumption is made that the breeding farms of the indicated region carry out the insufficient selection in Landrace pig populations in relation to maintaining high rates of multiple fertility.

Keywords: *ESR1*, *ESR/PvuII* mutations, pigs

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INTRODUCTION

When creating meat genotypes of pigs, it is important to maintain a high level of reproductive activity. One of the first *DNA* markers to prove the association with pig prolificacy was the estrogen receptor gene. The product of this gene (receptor) implements the action of sex hormones - estrogens. Three types of *ESR* gene polymorphism (*ESR/PvuII*, *ESR/AvaI*, and *ESR/MspII*) were identified; all of them are associated with the reproductive traits of pigs (Rothschild et al. 1996). Of greatest interest is the *ESR/PvuII* polymorphic system located in the first intron of the gene, for which two codominant alleles - *A* and *B*, have been identified. The *B* allele is desirable.

Mutation of this gene leads to a change in the protein estrogen receptor, which is the cause of spontaneous abortion. Studies have shown that the *ESR* gene is reliably associated with nest size in pigs and the effect of the desired alleles varies from 1.15 pigs per nest in the Meishan to 0.42 pigs per nest in the Large White (Rothschild et al. 1996, Short et al. 1997). It is known that homozygous pigs for the desired *B* allele of the *ESR* gene have 0.8-0.9 more piglets than pigs with a different genotype. According to other data, the differences between the *AA* and *BB* genotypes can vary from 0.6 to 3.58 piglets per nest (Chen et al. 2000, Horogh et al. 2005, Kolosov et al. 2016, Isler et al. 2002, Van Rens et al. 2002). However, not all experiments produce an unambiguous result. For example, in the Leningrad region (Russia), the Yorkshire × Landrace crossbreeding group in the group with high prolificacy (16 piglets) had the allele frequencies *A*=0.442 *B*=0.558, with the genotype frequency *AA*=0.218, *AB*=0.447, *BB*=0.335. The group with low prolificacy (7 piglets) had *A*=0.513, *B*=0.487, and genotype frequencies *AA*=0.262, *AB*=0.501, *BB*=0.237 (Serdjuk et al. 2015). Such small differences probably indicate that other genetic factors, as well as breed affiliation, influence fertility.

Objective. To study the frequency distribution structure of alleles of the estrogen receptor gene (*ESR1*) in pig populations of the Belgorod and Voronezh regions of the Russian Federation.

MATERIALS AND METHODS

PCR-RFLP (Polymerase chain reaction-restriction fragment length polymorphism) method was used for analysis. *DNA* was isolated from earmarks fixed in 70% ethanol. For this, a *DNA*-extran 2 Proteinase K kit (Synthol, Russia) was used. The isolation procedure was carried out according to the manufacturer's protocol. For amplification, primers were used synthesized by the same company (Short et al. 1997):

F: 5'-CCTGTTTTTACAGTGACTTTTACAGAG-3'
R: 5'-CACTTCGAGGGTCAGTCCAATTAG-3'

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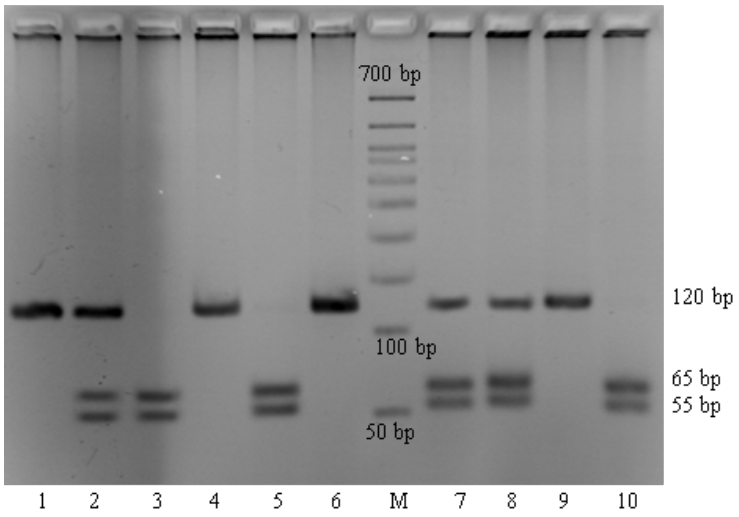

Fig. 1. Determination of *ESR1/PvuII* polymorphism by *PCR–RFLP*: M - *DNA* marker; lanes 1, 4, 6, 9 – homozygous genotype *AA* (120 bp); lanes 2, 7, 8 – heterozygous *AB* genotype (120 bp, 65 bp, 55 bp); lanes 3, 5, 10 – homozygous genotype *BB* (65 bp, 55 bp). The preferred genotype is *BB*

Table 1. Test results of a pig population to identify mutations in the estrogen receptor gene *ESR1*

Breed	N	Genotype frequency			Allele frequency	
		AA	*AB*	*BB*	*A*	*B*
Landrace	48	0.625	0.312	0.062	0.721	0.279
Large White	68	0.147	0.059	0.794	0.176	0.824

For *PCR*, the reaction mixture was used with a final volume of 20-25 µl, including 50 to 100 ng *DNA*, primers in an amount of 10 to 25 pM, 200 µM each of *dNTP*, 1x buffer (10 mM Tris pH 8.6, 50 mM KCl, 0.1% tween-20), 1.5 mM $MgCl_2$ and 1.3-2.5 un. act. *Taq* polymerase.

Amplification conditions: 94ºC - 5 min.; 35 cycles (94ºC - 30 sec., annealing - 58ºC - 40 sec., elongation - 72ºC - 40 sec.) 72°C - 5 min.

The restriction of amplification products lasts for 12 hours at 37°C using the restriction enzyme *PvuII* (SibEnzyme, Russia). Fragmentation after restriction analysis was performed on a 4% agarose gel. An electrophoregram with identified *DNA* fragments is shown in **Fig. 1**.

A total of 116 boars of two breeds were studied - Large White and Landrace.

RESULTS

The results of the analysis of the distribution of genotypes and alleles of *ESR/PvuII* are presented in **Table 1**.

According to the data obtained, the *A* allele dominates in the Landrace populations, and the desired *B* allele dominates in the Large White population. We think that systematic breeding work is being carried out in the breeding farms of the studied regions for the Large White to reproduce the pigs with the *B* allele genotype. With regard to the Landrace population, such work is probably not enough.

For comparison, below are the frequency distribution of genotypes and alleles of *ESR/PvuII* in populations of different breeds of different regions.

Table 2. Frequency distribution of *ESR/PvuII* genotypes and alleles in pig populations of different regions

Breed	Genotype frequency			Allele frequency		Region, link to the author
	AA	*AB*	*BB*	*A*	*B*	
Large White	0,250	0.620	0.130	0.560	0.440	Ukraine [9]
Large black	0.840	0.140	0.020	0.780	0.220	Ukraine [9]
Maishan	0.200	0.800	0.00	0.600	0.400	Ukraine [9]
Pietren	0.800	0.100	0.100	0.830	0.170	Ukraine [9]
Poltava meat	0.890	0.070	0.040	0.930	0.070	Ukraine [9]
Landrace	0.612	0.376	0.012	0.800	0.200	Taiwan [10]
Yorkshire	0.149	0.529	0.322	0.414	0.586	Taiwan [10]
Duroc	0.774	0.226	0.000	0.887	0.113	Taiwan [10]
Landrace, Yorkshire	0.720	0.022	0.060	0.834	0.166	Thailand [11]
Landrace × Large White	0.327	0.584	0.089	0.619	0.381	Croatia [12]
Polish Large White	0.384	0.485	0.131	0.626	0.374	Poland [13]
Polish Landrace	0.848	0.152	0.0	0.924	0.076	Poland [13]
Landrace	0.495	0.429	0.762	0.710	0.290	Belarus, [14]
Yorkshire	0.296	0.580	0.123	0.590	0.410	Belarus, [14]
Duroc	1.0	-	-	1.0	-	Belarus, [14]
Belarusian black pied	0.580	0.360	0.060	0.760	0.240	Belarus, [14]
Large White × Landrace	0.820	0.140	0.040	0.890	0.110	Ukraine, [15]
Large White	0.075	0.612	0.313	0.383	0.617	Russia, Rostov region [7]

Subject to the presented results, the *A* allele dominates in most populations. The exception was only the Yorkshire population from Taiwan (Dong et al. 2015, Ismail 2014, Lugovoi et al. 2017) and the Large White population from the Rostov region (Russia) (Kolosov et al. 2016).

CONCLUSION

Thus, the data obtained demonstrate an emerging trend in breeding aimed at increasing the frequency of the *B* allele of the estrogen receptor gene (*ESR/PvuII*), especially in the Large White populations.

SUMMARY

The screening of the estrogen receptor gene allele pool in 116 boars of the Belgorod and Voronezh regions (Russia) showed the predominance of the *A* allele in the landrace population and the predominance of the *B* allele in the Large White breed. We believe that the result demonstrates insufficient attention of breeders of the studied regions in relation to the increase in the reproductive activity of the Landrace breed. The Large White population demonstrates a clear selection of individuals towards the preservation of genotypes associated with prolificacy.

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