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Association of polymorphisms in estrogen and prolactin receptor genes with reproductive traits in sows of rare breeds

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Reproductive performance is a key factor influencing the sustainability and economic viability of pig breeding, particularly in rare and endangered breeds. This study investigates the associations between polymorphisms in the estrogen receptor 1 (ESR1) and prolactin receptor (PRLR) genes and reproductive traits in three rare pig breeds: Myrhorod, Poltava Meat, and Welsh. A total of 61 sows were examined, including 20 Myrhorod, 20 Poltava Meat, and 21 Welsh pigs. DNA was extracted from bristle samples, and genotyping for PvuII (ESR1) and AluI (PRLR) polymorphisms was conducted using PCR-RFLP analysis. The study assessed key reproductive indicators such as litter size, number of piglets born alive, piglet survival rate at weaning, weaning litter weight, and selection index of reproductive qualities of sows (SIRQS). Statistical analysis was performed to evaluate breed-specific differences and genotype-trait associations. Results indicate that the Myrhorod and Poltava Meat breeds exhibit lower reproductive performance compared to Welsh pigs, with significantly fewer piglets born alive and at weaning. Notably, the Myrhorod breed displayed a higher individual piglet weight at weaning, suggesting a trade-off between litter size and piglet growth rate. A significant negative correlation was observed between the number of piglets per litter and individual piglet weight, which became more pronounced at weaning. Genetic analysis revealed strong associations between ESR1 and PRLR polymorphisms and reproductive traits. The ESR1 BB genotype was linked to superior reproductive performance across all breeds, with the highest SIRQS values and larger litter sizes, while sows with the AA genotype exhibited significantly smaller litters. In contrast, the PRLR AA genotype was associated with increased piglet birth weight, confirming its role in early developmental traits. The findings underscore the importance of molecular genetics in improving reproductive efficiency, particularly in endangered breeds like Myrhorod pigs, where maintaining genetic diversity while enhancing productivity is a key challenge. The study highlights the necessity of integrating marker-assisted selection and genomic selection strategies to optimize fertility traits and ensure the long-term sustainability of small-population breeds. Further research is needed to explore additional genetic markers influencing reproductive traits and to refine breeding programs that balance genetic conservation and economic viability in rare pig breeds.

Keywords: pig breeding; genetic marker; ESR1; PRLR; litter size; fertility; piglets.

Introduction

Increasing the productivity of farm animals is a problem of global importance, since, together with increasing production efficiency, it contributes to the conservation of resources and minimizing the negative impact of livestock production on the environment (Kyryliuk et al., 2021; Zhyvko et al., 2022; Hnatenko et al., 2024). An essential stage in pig breeding is the evaluation of animals, which enables the determination of their productive potential. Traditional zootechnical methods for assessing sow productivity often lack sufficient accuracy and completeness. Moreover, assessments based solely on phenotypic evaluation may be influenced by paratypic factors, complicating the objective determination of an animal's breeding value (Voitenko et al., 2019; Davoudi et al., 2022). The development of index selection approaches and the Best Linear Unbiased Prediction (BLUP) model has led to improved breeding outcomes, particularly for traits with high heritability. However, to gain a deeper understanding of the relationship between an animal's genotype and phenotype, as well as to enhance breeding for traits with low heritability, it is essential to utilize genetic markers both within Quantitative Trait Loci and across the entire genome, which form the basis of marker-assisted and genomic selection, respectively (Boichard et al., 2016; Vashchenko et al., 2022; Saienko et al., 2023).

Pig breeding remains a leading sector of agro-industrial production. From the perspective of biodiversity conservation and expanded breeding opportunities, an important aspect is the study of local pig breeds characteristic of specific regions (Vashchenko et al., 2019; Krupa et al., 2021). In Ukraine, pig breeds with small populations include the Myrhorod and Poltava meat breeds (Shostya & Sarnavska, 2023; Voitenko, 2024). These breeds are well adapted to local rearing conditions and production systems. Additionally, they exhibit superior meat quality compared to widely used commercial breeds such as Landrace, Large White, and other universal or meat-type pig breeds. While the Myrhorod and Poltava meat breeds demonstrate satisfactory meat productivity, their most valuable characteristic is the superior organoleptic properties of their meat products (Voitenko, 2012; Ruban et al., 2015; Voitenko, 2024). A significant drawback of these breeds, however, is their relatively low reproductive performance. This contrasts with global trends in pig breeding, which aim to increase litter sizes from 10 to 20 live-born piglets per farrowing (Peltoniemi et al., 2021; Lee et al., 2024). Preliminary data indicate that the Myrhorod breed has a litter size of 9.6–10.7 piglets, while the Poltava meat breed averages 10.0 piglets per farrowing (Voitenko, 2024). In comparison, the Welsh pig breed, also classified as a meat-type breed, produces approximately 11.93–13.64 piglets per farrow, depending on the genotype by the RYR1 polymorphism (Zhukorskyi et al., 2022).

Reproductive traits, particularly litter size, are among the most critical economic traits in pig breeding (Khalak et al., 2022; Vargovic et al., 2022; Bortolozzo et al., 2023). Advances in animal genetics have facilitated the identification of genomic loci associated with litter size and enabled the genotyping of animals using molecular genetic markers. According to Distl (2007), more than 50 QTLs have been mapped, and over 12 candidate genes have demonstrated associations with litter size. The genotyping of these loci allows the prediction of reproductive potential, thereby informing selection decisions.

One of the key genes associated with litter size in pigs is the estrogen receptor 1 (ESR1) gene (Short, 1997; Rahman et al., 2021). This gene has known allelic variants characterized by single nucleotide polymorphisms at restriction sites for the endonucleases PvuII, AvaI, and MspA1I (Drogemüller et al., 1997; Kaminski et al., 2003). Several studies have demonstrated that sows with the BB genotype at the PvuII polymorphic site outperform those with the AB and AA genotypes in terms of litter size, with differences ranging from 0.60 (Isler, 2002) to 3.58 (Chen et al., 2000) additional piglets per litter. However, the strength of this association may vary among pig breeds, genetic lines, and populations (Balatsky et al., 2012; Gibson et al., 2012; Wu et al., 2023).

The prolactin receptor (PRLR) gene also plays a crucial role in regulating reproductive traits in pigs, particularly litter size and milk production in sows, as well as sperm quality in boars (Putnová et al., 2002). The PRLR gene is located on chromosome 16, and the AluI polymorphism within this gene has been associated with increased litter size and the number of live-born piglets. The A allele and AA genotype of this polymorphism have been identified as desirable for improving reproductive performance (Terman, 2005; Kmiec & Terman, 2006; Hong et al., 2020).

Thus, genotypes of the ESR1 and PRLR genes can serve as genetic markers for selecting sows with enhanced reproductive potential. In this study, we conduct an association analysis of ESR1 and PRLR gene polymorphisms in three small-population pig breeds: Myrhorod, Poltava meat bred, and Welsh. Enhancing the reproductive traits of Ukrainian local pig breeds (Myrhorod and Poltava meat) will contribute to the expansion of their populations and increase their economic attractiveness for breeding and commercial use. Furthermore, monitoring reproductive gene polymorphisms in the Welsh breed will help maintain high litter size levels and enable the selection of highly prolific individuals for further breeding.

Materials and methods

The experimental protocol was approved by the Scientific Council of the Institute of Pig Breeding and Agroindustrial Production of the National Academy of Agrarian Sciences of Ukraine. All animal handling procedures complied with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985). In order to reduce barriers to the animals' physiological and ethological demands being met, the animals were housed in a dedicated shed in individual pens that were large enough (5.5 m² per animal) to guarantee adequate housing circumstances. The pens have concrete floors. The pigs were given an underlay composed of wheat and barley straw at a rate of 2.5 kg per animal per day. Use of supply valves in the walls ensured that the shed's ventilation occurred naturally. The greatest measured air movement speed in the pigpen during the experiment was 1.0 m/s, the ammonia content was 18.2 mg/m³, and the carbon dioxide concentration was 0.25%.

The study was conducted on 20, 20, and 21 sows of the Myrhorod, Poltava meat, and Welsh breeds, respectively. According to the main guidelines of research organization, groups were formed and the animals were evaluated (Ibatullin & Zhukorskiy, 2017). During the studies, the experimental pigs received the same diet and were kept under the same technological conditions.

Biomaterial samples (bristles) were collected from pigs maintained at the experimental facility of the Institute of Pig Breeding and Agroindustrial Production of the National Academy of Agrarian Sciences of Ukraine. Reproductive characteristics of the sows were evaluated, considering data from two to four farrowings, along with certain indicators of individual productivity. We considered the number of piglets that were born alive (n), the number of piglets that were weaned (n) and their weight at the time of weaning (kg), which was conducted at 28 days after birth. Prestarter feed, which had 15.4 MJ/kg of metabolic energy, 231 g of crude protein, and 11.1 g of lysine per 1 kilogram of dry matter, was given to the nursing piglets. The SIRQS index (selection index of reproductive characteristics of sows) was used to conduct a thorough assessment of the reproductive potential of the sows. The index's value was determined in accordance with Zhukorskyi et al. (2022).

Fig. 1. Experimental sows of the Myrhorod, Poltava meat and Welsh breeds during the farrowing period (photo by P. A. Vashchenko)

DNA was isolated using the Chelex 100 reagent (Walsh, 2013), and the extracted DNA samples were stored at –20 °C. Genotyping of ESR1 and PRLR gene polymorphisms was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique (Waters & Shapter, 2014; Dai & Long, 2015). PCR was carried out in a Tercyk-2 thermocycler using 0.5 mL Eppendorf microcentrifuge tubes (Eppendorf, Germany) in a total reaction volume of 25 µL. Restriction digestion was performed using the PvuII and AluI restriction enzymes to identify ESR1 and PRLR polymorphisms, respectively (Short et al., 1997; Hong et al., 2020).

The oligonucleotide primer sequences for PCR, amplicon sizes, melting temperatures, and restriction fragment sizes are presented in Table 1.

Table 1
Parameters of PCR-RFLP

Genes	Oligonucleotide primers' structure	PCR product size	T_m, °C	Fragments after restriction
ESR1	F: 5'-CCTGTTTTTACAGTGACTTTTTACAGAG-3' R: 5'-CACTTCGAGGGTCAGTCCAATTAG-3'	120	156	*PvuII*: allele A: 120 bp; allele B: 65 + 55 bp
RPRL	F: 5'-CGTGGCTCCGTTTGAAGAACC-3' R: 5'-CTGAAAGGAGTGCATAAAGCC-3'	163	55	*AluI*: allele A: 85+59+19 bp; allele B: 104+59 bp

The restriction fragment mixture was analysed by electrophoresis in an 8% polyacrylamide gel. The sizes of the restriction fragments were estimated by comparison with the molecular weight marker pBR322 DNA-MspI Digest (New England Biolabs, USA) after staining with ethidium bromide (Galindo-Murillo & Cheatham, 2021).

The statistical analysis aimed to evaluate differences in the reproductive performance of sows, first by comparing different breeds and then by examining animals within the same breed that carried different genotypes for polymorphisms in ESR1 and PRLR. Data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. For each group the mean ($\bar{x}$), standard deviation (SD), median, and interquartile range (IQR) were calculated for the studied parameters. Since the distribution of many productive traits deviated from normality, non-parametric tests were used to compare groups. Based on genotype distribution for a given polymorphism, three groups were formed: two groups consisting of homozygous animals with different alleles and one group of heterozygous animals. Comparisons between groups were conducted only when each group contained at least three animals. If some genotype group included fewer than three animals, comparisons were made between the two other genotype groups. For comparisons of a specific trait within two groups, the Mann-Whitney U test was applied. When three groups were compared, the Kruskal-Wallis test was used, followed by Dunn's post hoc test with Bonferroni correction. Statistical significance was set at $P < 0.05$. The Spearman correlation was also assessed between the number of piglets and the mass of a single piglet at two time points (at birth, and at weaning).

Results

First, the differences between the three pig breeds (Myrhorod, Poltava Meat, and Welsh) were assessed. The results obtained (Table 2) show statistically significant differences between the three breeds, with all indicators – except for mass of a single newborn piglet – reaching a significance level of $P < 0.001$. These significant differences in the analysis of the three breeds can be explained by the fact that the Welsh breed is characterized by a higher number of piglets born alive and, consequently, a higher number of piglets at weaning, greater weaning litter weight, and higher SIRQS than the Myrhorod and Poltava Meat breeds.

At farrowing, the Poltava Meat breed exhibits a slightly higher number of piglets born alive than the Myrhorod breed, though this difference is not statistically significant. In contrast, the Welsh breed has a statistically significantly higher number of piglets born alive (Table 2, Fig. 2a). At the same time, the birth weight of an individual piglet is greater in the Poltava Meat breed compared to the Welsh breed, while the Myrhorod breed occupies an intermediate position in this indicator. Thus, the increase in the number of piglets in the Welsh breed occurs concurrently with a slight decrease in the birth weight of individual piglets (Table 2, Fig. 2b).

At weaning, the number of piglets was statistically significantly different among all three breeds, which may indicate differences in development and survival rates of piglets from different breeds (Fig. 2c). It is also noteworthy that the Myrhorod breed is characterized by the lowest number of piglets at weaning. However, the weaning weight of an individual Myrhorod piglet is statistically greater than that of Poltava Meat and Welsh piglets (Fig. 2d).

The correlation between the number of piglets and the mass of a single piglet at both birth and weaning was assessed for all breeds. The corresponding scatterplots are shown in Figure 3. Results of the Spearman correlation analysis indicate a significant moderate negative relationship between the number of piglets born alive and the mass of a single newborn piglet ($R_s = –0.3452$, $P = 0.0064$). Additionally, a significant strong negative relationship was observed between the number of piglets at weaning and the mass of a single piglet at weaning ($R_s = –0.5106$, $P = 2.62 \times 10^{–5}$). Thus, as litter size increases, the mass of an individual live piglet decreases, with this effect being more pronounced at weaning than at birth.

Next, differences between pigs with different genotypes within the studied breeds were assessed. Typical electropherograms obtained in our study, illustrating the separation of restriction fragments in polyacrylamide gel based on ESR1 and PRLR genotypes, are shown in Figures 4 and 5. The reproductive performance of sows with homozygous (AA or BB) and heterozygous (AB) genotypes for the PvuII polymorphic site of the ESR1 gene and the AluI polymorphic site of the PRLR gene is presented in Tables 3–8.

In the Myrhorod breed, no statistically significant differences were observed between animals with genotypes AA and AB for the ESR1 polymorphism. However, it is noteworthy that the SIRQS value was lower in the small BB genotype group (n = 2) compared to other genotypes for this polymorphism (Table 3). Regarding the PRLR polymorphism, animals with genotype AB had a significantly greater birth weight per piglet than those with genotype AA (Table 4).

In the Poltava Meat breed, statistically significant associations were found between ESR1 polymorphism and total number of piglets, number of piglets born alive, number of piglets at weaning, mass of a single piglet at weaning, and SIRQS. Sows with the homozygous AA genotype had the lowest number of piglets born alive and piglets at weaning but were statistically significantly superior to heterozygous (AB) animals in terms of the weight of a single piglet at weaning (Table 5). Regarding PRLR polymorphism, homozygous BB animals had a statistically greater newborn piglet weight than homozygous AA animals (Table 6).

Several associative relationships were found in the micropopulation of Welsh pigs. Statistically significant associations of the ESR1 gene with the number of piglets born alive and at weaning were observed. As in the Poltava meat breed, pigs with the AA genotype had fewer piglets. These same homozygous animals also had the lowest SIRQS scores (Table 7). According to the polymorphism in the PRLR gene, animals of the Welsh breed with the BB genotype had a significantly greater weaning litter weight than those with the AB genotype (Table 8).

Discussion

Modern animal breeding aims to preserve existing breeds of farm animals (Berry et al., 2014; Liang et al., 2023), enhance their adaptation to environmental conditions, and improve their productive traits. Among the primary objectives of pig breeding, improving reproductive potential holds a central position, particularly in increasing the number of piglets born alive and at weaning, litter weight at weaning, and the mass of individual piglets.

Table 2
Differences in reproductive parameters of Myrhorod, Poltava Meat and Welsh breed sows

Productivity traits	Myrhorod breed (n = 20)			Poltava meat breed (n = 20)			Welsh breed (n = 21)			P-value
	x̄ ± SD	median	IQR	x̄ ± SD	median	IQR	x̄ ± SD	median	IQR	
Total number of piglets (average per sow and per 2–4 farrowings), animals	10.65 ± 1.42[a]	11.00	9.50–12.00	12.00 ± 1.72[a]	11.50	11.00–13.50	14.71 ± 2.67[b]	14.00	12.50–16.00	1.26×10^{-6}
Number of piglets born alive (average per sow and per 2–4 farrowings), animals	9.95 ± 1.50[a]	10.00	9.00–11.00	11.15 ± 1.39[a]	11.00	10.00–12.00	13.57 ± 1.94[b]	13.00	12.00–14.00	2.28×10^{-7}
Mass of a single newborn piglet (average per sow), kg	0.96 ± 0.16[ab]	1.00	0.80–1.00	1.04 ± 0.13[a]	1.00	1.00–1.10	0.91 ± 0.09[b]	0.90	0.80–1.00	0.013
Number of piglets at weaning (average per sow), animals	8.95 ± 1.05[a]	9.00	8.00–10.00	10.35 ± 0.99[b]	10.00	10.00–11.00	12.52 ± 1.33[c]	12.00	11.50–13.50	5.58×10^{-10}
Weaning litter weight (average per sow), kg	73.75 ± 9.56[a]	75.50	66.0–81.0	75.50 ± 7.61[a]	73.50	70.50–81.50	93.38 ± 7.92[b]	91.00	88.00–99.50	1.89×10^{-8}
Mass of a single piglet at weaning (average per sow), kg	8.243 ± 0.497[a]	8.26	7.95–8.59	7.315±0.598[b]	7.185	7.000–7.710	7.482±0.431[b]	7.540	7.205–7.795	5.76×10^{-6}
SIRQS (average per sow), units	79.38 ± 11.22[a]	80.25	71.6–87.5	87.05 ± 9.64[a]	86.15	80.40–92.10	106.4 ± 13.2[b]	104.20	96.2–111.5	4.75×10^{-8}

Note: statistical significance was determined between animals of different breeds using the Kruskal-Wallis test followed by Dunn's test with Bonferroni correction. Different superscript letters ([a], [b], [c]) indicate statistically significant differences between groups based on Dunn's test; groups sharing the same letter are not significantly different from each other.

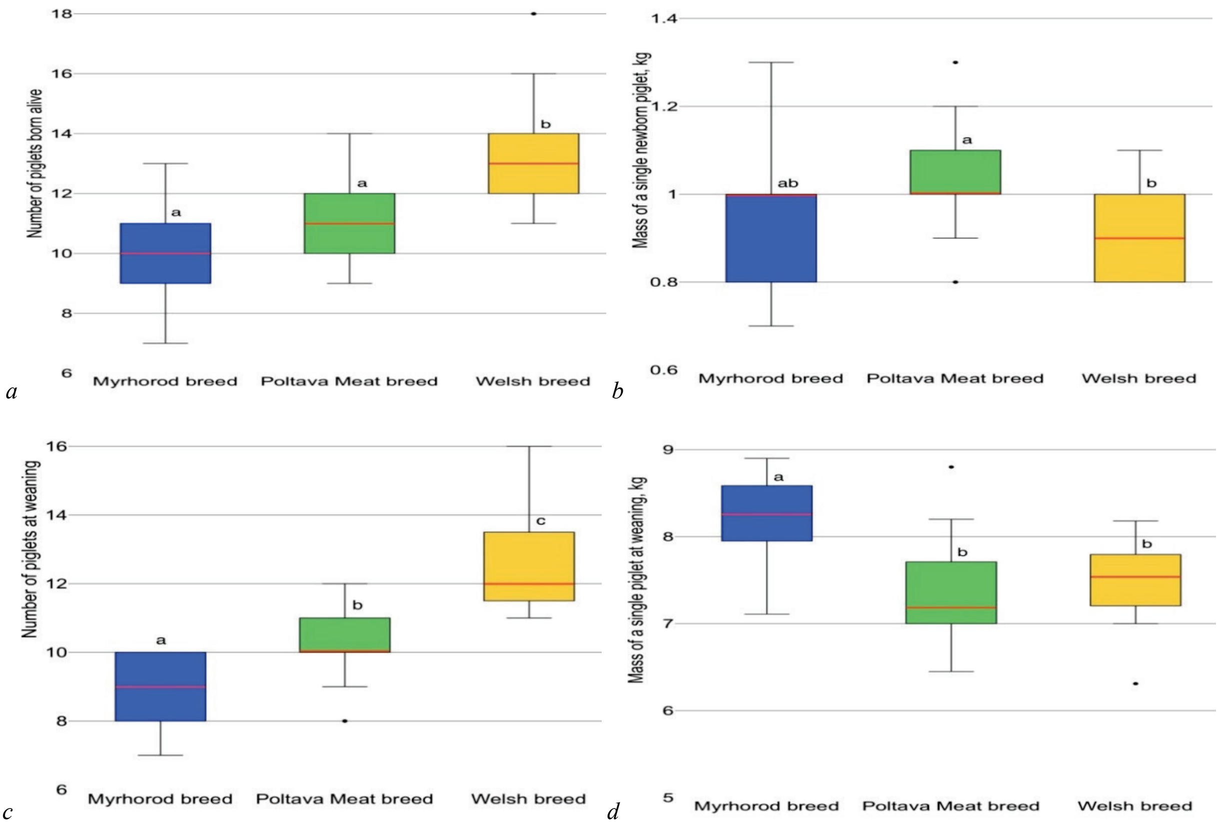

Fig. 2. Boxplot of the number of piglets and their mass distribution (median is shown with red line): *a* – number of piglets born alive, *b* – mass of a single newborn piglet, *c* – number of piglets at weaning, *d* – mass of a single piglet at weaning; statistical significance was determined between animals of different breeds using the Kruskal-Wallis test followed by Dunn's test with Bonferroni correction; different superscript letters (^{a}, ^{b}, ^{c}) indicate statistically significant differences between groups based on Dunn's test; groups sharing the same letter are not significantly different from each other

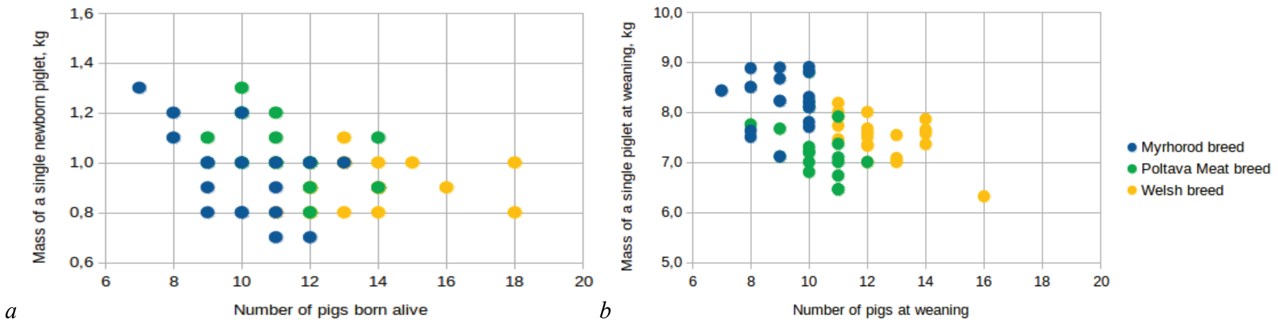

Fig. 3. Scatterplots showing the distribution of the mass of a single piglet depending on the number of piglets: *a* – distribution at birth, *b* – distribution at weaning

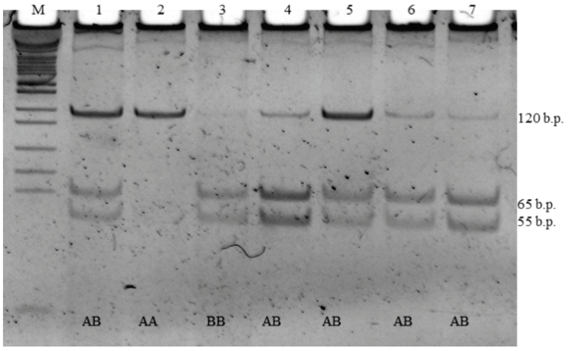

Fig. 4. Electrophoregram of products by PvuII – polymorphic site of the estrogen receptor gene in 8% polyacrylamide gel: 1–7 – samples under study; corresponding genotypes of sows are shown as AA, AB and BB; M is the molecular weight marker of pBR322 DNA-MspI

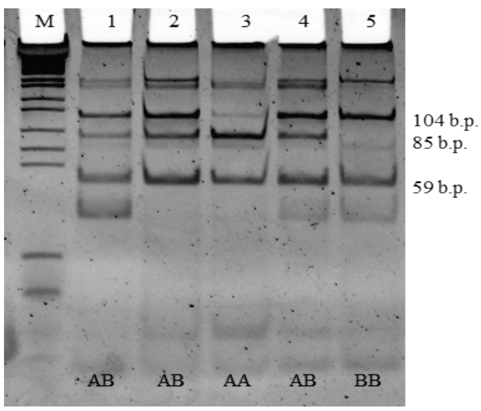

Fig. 5. Electrophoregram of products at AluI – polymorphic site of prolactin receptor gene in 8% polyacrylamide gel: 1–5 – samples under study; corresponding genotypes of sows are shown as AA, AB and BB. M is the molecular weight marker of pBR322 DNA-MspI

Table 3
Relationship of genotypes by polymorphism in the ESR1 gene with reproductive traits in the Myrhorod pig breed (n = 20)

Productivity traits	*ESR1* AA (n = 12)			*ESR1* AB (n = 6)			*ESR1* BB (n = 2)	P-value
	x̄ ± SD	median	IQR	x̄ ± SD	median	IQR	x̄	
Total number of piglets (average per sow and per 2-4 farrowings), animals	10.17 ± 1.40	10.00	9.00–11.00	11.33 ± 1.37	11.50	11.00–12.00	11.50	0.116
Number of piglets born alive (average per sow and per 2-4 farrowings), animals	9.42 ± 1.56	9.00	8.50–10.00	10.50 ± 1.05	10.50	10.00–11.00	11.50	0.093
Mass of a single newborn piglet (average per sow), kg	0.99 ± 0.18	1.00	0.80–1.15	0.93 ± 0.12	1.00	0.90–1.00	0.85	0.627
Number of piglets at weaning (average per sow), animals	8.50 ± 1.09	8.00	8.00–9.50	9.50 ± 0.55	9.50	9.00–10.00	10.00	0.064
Weaning litter weight (average per sow), kg	71.42 ± 10.94	69.50	60.50–80.00	76.67 ± 6.92	79.00	74.00–81.00	79.00	0.281
Mass of a single piglet at weaning (average per sow), kg	8.387 ± 0.448	8.465	8.210–8.735	8.070 ± 0.590	8.160	7.800–8.300	7.900	0.223
SIRQS (average per sow), units	75.56 ± 11.84	73.30	68.20–81.35	83.46 ± 8.01	84.05	79.70–87.60	44.73	0.159

Note: statistical significance was determined between animals with AA and AB genotypes determined using the Mann-Whitney U test.

Table 4
Relationship of genotypes by polymorphism in the PRLR gene with reproductive traits in the Myrhorod pig breed (n = 20)

Productivity traits	*PRLR* AA (n = 13)			*PRLR* AB (n = 6)			*PRLR* BB (n = 1)	P-value
	x̄ ± SD	median	IQR	x̄ ± SD	median	IQR	x	
Total number of piglets (average per sow and per 2-4 farrowing), animals	10.46 ± 1.20	11.00	9.00–11.50	10.83 ± 1.94	10.50	10.00–13.00	12.00	0.687
Number of piglets born alive (average per sow and per 2-4 farrowings), animals	9.77 ± 1.30	10.00	9.00–11.00	10.17 ± 2.04	10.00	8.00–12.00	11.00	0.788
Mass of a single newborn piglet (average per sow), kg	0.91 ± 0.17[a]	0.90	0.80–1.00	1.08 ± 0.10[b]	1.05	1.00–1.20	0.90	0.022
Number of piglets at weaning (average per sow), animals	8.85 ± 0.99	9.00	8.00–10.00	9.00 ± 1.26	9.50	8.00–10.00	10.00	0.714
Weaning litter weight (average per sow), kg	72.23 ± 9.35	74.00	62.50–80.50	75.83 ± 10.76	78.00	68.00–83.00	81.00	0.455
Mass of a single piglet at weaning (average per sow), kg	8.167 ± 0.554	8.220	7.665–8.650	8.433 ± 0.373	8.465	8.30–8.67	8.100	0.236
SIRQS (average per sow), units	77.89 ± 9.81	76.00	71.60–86.90	81.24 ± 14.98	80.80	66.10–94.10	87.60	0.693

Note: statistical significance was determined between animals with AA and AB genotypes determined using the Mann-Whitney U test; different superscript letters ([a], [b]) indicate statistically significant differences between groups based on the Mann-Whitney U test; groups sharing the same letter are not significantly different from each other.

Table 5
Relationship of genotypes by polymorphism in the ESR1 gene with reproductive traits in the Poltava Meat pig breed (n = 20)

Productivity traits	*ESR1* AA (n = 5)			*ESR1* AB (n = 13)			*ESR1* BB (n = 2)	P-value
	x̄ ± SD	median	IQR	x̄ ± SD	median	IQR	x̄	
Total number of piglets (average per sow and per 2-4 farrowings), animals	10.40 ± 0.55[a]	10.00	10.00–11.00	12.31 ± 1.65[b]	12.00	11.00–13.50	14.00	0.013
Number of piglets born alive (average per sow and per 2-4 farrowings), animals	9.60 ± 0.55[a]	10.00	9.00–10.00	11.46 ± 1.05[b]	11.00	11.00–12.00	13.00	0.003
Mass of a single newborn piglet (average per sow), kg	1.12 ± 0.13	1.10	1.00–1.25	1.00 ± 0.13	1.00	0.90–1.10	1.05	0.126
Number of piglets at weaning (average per sow), animals	9.40 ± 0.89[a]	10.00	8.50–10.00	10.62 ± 0.77[b]	11.00	10.00–11.00	11.00	0.018
Weaning litter weight (average per sow), kg	73.20 ± 8.41	72.00	65.50–81.50	74.62 ± 6.54	73.00	70.70–79.50	87.00	0.767
Mass of a single piglet at weaning (average per sow), kg	7.783 ± 0.397[a]	7.750	7.435–8.150	7.032 ± 0.388[b]	7.00	6.765–7.25	7.983	0.009
SIRQS (average per sow), units	77.13 ± 5.31[a]	79.2 0	71.45–87.75	88.68 ± 7.42[b]	89.20	84.95–92.10	101.22	0.010

Note: see note to Table 4.

Table 6
Relationship of genotypes by polymorphism in the PRLR gene with reproductive traits in the Poltava Meat pig breed (n = 20)

Productivity traits	PRLR AA (n = 7)			PRLR AB (n = 10)			PRLR BB (n = 3)			P-value
	x̄ ± SD	median	IQR	x̄ ± SD	median	IQR	x̄ ± SD	median	min-max	
Total number of piglets (average per sow and per 2-4 farrowings), animals	12.57 ± 2.30	13.00	10.00–14.00	11.90 ± 1.45	12.00	11.00–13.00	11.00 ± 0.00	11.00	11.00–11.00	0.572
Number of piglets born alive (average per sow and per 2-4 farrowings), animals	11.29 ± 1.70	12.00	10.00–12.00	11.20 ± 1.40	11.00	10.00–12.00	10.67 ± 0.58	11.00	10.00–11.00	0.773
Mass of a single newborn piglet (average per sow), kg	0.96 ± 0.14[a]	1.00	0.80–1.00	1.04 ± 0.08[ab]	1.00	1.00–1.10	1.20 ± 0.10[b]	1.02	1.10–1.30	0.030
Number of piglets at weaning (average per sow), animals	10.14 ± 1.35	10.00	9.00–11.00	10.50 ± 0.85	10.50	10.00–11.00	10.33 ± 0.58	10.00	10.00–11.00	0.853
Weaning litter weight (average per sow), kg	75.00 ± 10.05	74.00	64.00–84.00	74.5 ± 5.84	72.50	70.00–78.00	80.0 ± 7.55	81.00	72.00–87.00	0.505
Mass of a single piglet at weaning (average per sow), kg	7.435 ± 0.847	7.110	6.730–8.20	7.104±0.329	7.130	7.000–7.300	7.736 ± 0.474	7.910	7.200–8.100	0.236
SIRQS (average per sow), units	87.73 ± 12.10	90.900	77.10–95.50	87.08 ± 9.70	86.15	79.20–92.50	85.35 ± 3.80	85.20	81.60–89.20	0.8886

Note: statistical significance was determined between animals with AA, AB and BB genotypes using the Kruskal-Wallis test followed by Dunn's test with Bonferroni correction.

Table 7
Relationship of genotypes by polymorphism in the ESR1 gene with reproductive traits in the Welsh pig breed (n = 21)

Productivity traits	ESR1 ^{AA} (n = 4)			ESR1 ^{AB} (n = 12)			ESR1 ^{BB} (n = 5)			P-value
	x̄ ± SD	median	IQR	x̄ ± SD	median	IQR	x̄ ± SD	median	IQR	
Total number of piglets (average per sow and per 2-4 farrowings), animals	11.80 ± 0.50^{a}	12.00	11.50–12.00	14.40 ± 1.51^{ab}	14.00	13.50–16.00	17.80 ± 2.86^{b}	18.00	15.00–20.50	0.002
Number of piglets born alive (average per sow and per 2–4 farrowings), animals	11.50 ± 0.58^{a}	11.50	11.00–12.00	13.50 ± 1.09^{ab}	13.50	13.00–14.00	15.40 ± 2.61^{b}	15.00	13.00–18.00	0.008
Mass of a single newborn piglet (average per sow), kg	0.93 ± 0.05	0.95	0.85–1.00	0.92 ± 0.09	0.90	0.85–1.00	0.90 ± 0.10	0.90	0.80–1.00	0.914
Number of piglets at weaning (average per sow), animals	11.25 ± 0.50^{a}	11.00	11.00–11.50	12.50 ± 1.00^{ab}	12.50	12.00–13.00	13.60 ± 1.67^{b}	14.00	12.00–15.00	0.028
Weaning litter weight (average per sow), kg	87.00 ± 3.74	87.50	84.50–89.50	93.25 ± 7.48	91.50	89.00–97.00	98.80 ± 8.47	101.00	90.0–106.5	0.067
Mass of a single piglet at weaning (average per sow), kg	7.737 ± 0.260	7.745	7.515–7.955	7.471 ± 0.404	7.430	7.080–7.795	7.305 ± 0.570	7.570	6.820–7.655	0.375
SIRQS (average per sow), units	92.22 ± 3.45^{a}	91.70	89.35–95.10	105.88 ± 7.84^{b}	105.85	101.2–109.1	118.8 ± 17.3^{b}	118.30	102.1–135.8	0.008

Note: statistical significance was determined between animals with AA, AB and BB genotypes using the Kruskal-Wallis test followed by Dunn's test with Bonferroni correction; different superscript letters (^{a}, ^{b}) indicate statistically significant differences between groups based on Dunn's test; groups sharing the same letter are not significantly different from each other.

Table 8
Relationship of genotypes by polymorphism in the PRLR gene with reproductive traits in the Welsh pig breed (n = 21)

Productivity traits	*PRLR* ^{AA} (n = 1)	*PRLR* ^{AB} (n = 16)			*PRLR* ^{BB} (n = 4)			P-value
	x	x̄ ± SD	median	IQR	x̄ ± SD	median	IQR	
Total number of piglets (average per sow and per 2-4 farrowings), in animals	20.00	14.19 ± 2.43	14.00	12.5–15.0	15.50 ± 2.52	16.00	14.00–17.00	0.288
Number of piglets born alive (average per sow and per 2-4 farrowings), in animals	18.00	13.30 ± 1.82	13.00	12.00–14.00	13.50 ± 1.29	13.50	12.50–14.50	0.663
Mass of a single newborn piglet (average per sow), in kg	0.80	0.90 ± 0.08	0.90	0.80–1.00	1.00 ± 0.08	1.00	0.95–1.05	0.066
Number of piglets at weaning (average per sow), in animals	16.00	12.13 ± 1.02	12.00	11.00–13.00	13.25 ± 0.96	13.50	12.50–14.00	0.077
Weaning litter weight (average per sow), in kg	101.00	90.94 ± 6.53^{a}	90.00	87.50–92.00	101.25 ± 8.46^{b}	102.00	94.50–108.00	0.037
Mass of a single piglet at weaning (average per sow), in kg	6.313	7.516 ± 0.380	7.475	7.205–7.820	7.638 ± 0.148	7.575	7.555–7.720	0.539
SIRQS (average per sow)	134.95	104.14 ± 12.36	102.30	95.35–108.45	108.02 ± 9.77	108.80	100.25–115.85	0.369

Note: see note to Table 4.

This study examines reproductive traits in three pig breeds selected for meat production: Myrhorod, Poltava Meat, and Welsh. The first two are rare Ukrainian breeds, with the Myrhorod breed, in particular, on the verge of extinction due to the African swine fever outbreak, which led to the near-total loss of this unique breed (Vashchenko et al., 2019). Currently, efforts are underway to restore the Myrhorod breed from a small surviving population, a process that inevitably results in genetic drift and "bottleneck effect", whereby allele frequencies at different loci shift over generations (Kirkpatrick & Jarne, 2000; Choudhuri, 2014). Consequently, genotyping these pigs for polymorphisms in genes associated with economically valuable traits is of paramount importance. The Poltava Meat breed, closely related to the Myrhorod breed, plays a role in its restoration, making their comparison particularly relevant.

This study demonstrates that the Myrhorod and Poltava Meat breeds exhibit lower reproductive performance than the Welsh breed in most indicators related to fertility. This reduced fertility makes them less attractive for commercial breeding, impacting their prevalence, the number of breeding populations, and ultimately threatening their survival. Thus, selection to enhance the fertility of these breeds is a crucial area for further research.

Genetic selection based on markers associated with reproductive traits may offer a solution for improving the Myrhorod and Poltava Meat breeds. Numerous studies have identified polymorphisms in genes linked to litter size in pigs (Distl, 2007), including estrogen receptor 1 (ESR1) (Rothschild et al., 1996; Short et al., 1997), estrogen receptor 2 (ESR2) (Muñoz et al., 2004), erythropoietin receptor (EPOR) (Fahrenkrug et al., 2000; Vallet et al., 2005a), leptin (LEP) and leptin receptor (LEPR) (Chen et al., 2004a; Chen et al., 2004b), prolactin receptor (PRLR) (Vincent et al., 2007), follicle-stimulating hormone β (FSHB) (Mellink et al., 1995), properdin (BF) (Buske et al., 2006), gonadotropin-releasing hormone receptor (GNRHR) (Jiang et al., 2001), epidermal growth factor (EGF) (Mendez et al., 1999), prostaglandin-endoperoxide synthase 2 (PTGS2), secreted folate-binding protein (sFBP) (Vallet et al., 2005b), retinol-binding protein 4(RBP4) (Rothschild et al., 2000), leukemia inhibitory factor (LIF) (Spötter et al., 2005), and fucosyltransferase 1 (FUT1) (Horák et al., 2005).

In this study, we focused on polymorphisms in ESR1 and PRLR. Analysis of the PvuII and AluI polymorphic sites in these genes revealed associations between specific quantitative trait loci and reproductive traits in the three studied breeds. Notably, pigs with the AA genotype had smaller litter sizes.

Furthermore, the calculated selection index of reproductive qualities of sows (SIRQS) significantly differed among animals with different ESR1 genotypes in all three breeds, with the BB genotype associated with the highest SIRQS values. This index provides a comprehensive measure of breeding value, facilitating its transmission to offspring and enabling a gradual increase in reproductive performance within the population. Previous studies have similarly demonstrated associations between ESR1 and PRLR genotypes and reproductive traits, with BB genotypes in ESR1 (Short et al., 1997) and AA genotypes in PRLR (Hoang et al., 2020) linked to higher fertility and increased numbers of live-born piglets.

In this study, statistically significant differences were observed for all reproductive traits when comparing pigs of different breeds. However, within the same breed, statistically significant differences between genotypes were found for only some of the studied traits. This may indicate that reproductive potential is a polygenic trait, shaped by the interaction of multiple genes (Ma & Zhou, 2021). As a result, the contribution of each individual polymorphism to reproductive potential is relatively small, making it more challenging to isolate its specific effect. In contrast, different breeds exhibit variations in multiple polymorphisms simultaneously, collectively shaping the breed's reproductive profile. This highlights the importance of further research using modern whole-genome analysis methods, such as next-generation sequencing (Sharma et al., 2017) or microarray analysis (Ramos et al., 2009; Piórkowska & Ropka-Molik, 2021), which enable the simultaneous detection of thousands of polymorphisms and their combined effects on the phenotype.

Conclusions

This associative study examined differences in the reproductive traits of sows from three meat-production breeds: Myrhorod, Poltava Meat, and Welsh, the first two of which are rare Ukrainian breeds. The results indicate that the Welsh breed significantly outperforms the Myrhorod and Poltava Meat breeds in terms of the number of piglets born alive and at weaning, litter weight at weaning, and the selection index of reproductive qualities of sows (SIRQS). Additionally, the study investigated associations between genotypes at the PvuII and AluI polymorphic sites of the ESR1 and PRLR genes accordingly, and reproductive traits within herds of each breed. Based on the genotyping data, selecting sows with favorable ESR1 and PRLR genotypes may enhance reproductive performance and overall breeding value, which is particularly important for the conservation and genetic improvement of the Myrhorod and Poltava Meat breeds.

The authors declare no conflicts of interest.

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