

Monika KOWALEWSKA , Arkadiusz TERMAN , Oliwia CHOMCZYŃSKA ,  
Katarzyna WOJDAK-MAKSYMIEC , Daniel POLASIK , Andrzej DYBUS 

## ASSOCIATIONS BETWEEN *RBP4*, *FSHB* AND *EGF* GENE POLYMORPHISMS AND REPRODUCTIVE TRAITS IN PIGS

Department of Genetics, Faculty of Biotechnology and Animal Breeding, West Pomeranian University of Technology, Szczecin, Poland

**Abstract.** Genes encoding retinol binding protein 4 (*RBP4*), follicle stimulating hormone subunit beta (*FSHB*) and epidermal growth factor (*EGF*) have been proposed as candidate genes for reproduction traits in pigs. The study presented in this work aimed to find associations between variants of these genes and reproduction traits in pigs reared in Poland. Investigation included Polish Large White x Polish Landrace (n = 288) crossbreed and Yorkshire (n = 195) breed sows. Insertion-deletion polymorphism (indel) in *EGF* gene was determined by means of PCR (polymerase chain reaction) method, however single nucleotide polymorphism (SNP) in *RBP4* and *FSHB* genes by means of PCR-RFLP (restriction fragment length polymorphism) method. Obtained results showed that total number born (TNB) and number born alive (NBA) in 1st, 2nd and 5th parities were associated with *RBP4* gene variants, TNB and NBA in first two parities with *FSHB* variants, however both traits in all parities with *EGF* variants ( $p \leq 0.05$  or  $p \leq 0.01$ ). In case of *RBP4* gene, heterozygous genotype (AB) was favorable for analyzed traits, however in relation to *FSHB* and *EGF* genes homozygous genotypes, AA and BB respectively. Obtained results indicate that polymorphism in three analyzed genes is associated with important reproductive traits of pigs kept in Poland.

**Key words:** pigs, *RBP4*, *FSHB*, *EGF*, polymorphism, reproduction traits.

## INTRODUCTION

The 21st century appears to be the age of genetics (Roberts and Middleton 2017). A lot of studies have been published in the past two decades reporting various genes and their associations with production traits in farm animals (Ibeagha-Awemu et al. 2008; Bello et al. 2022; Knol et al. 2022). Around 36% of the global human population consumes pork meat (OECD 2022), the cost of its production depending largely on the number of piglets per litter (Rothschild 2004; Spötter and Distl 2006). In the case of pigs, traditional reproductive traits improvement methods have proved to be of insufficient effectiveness and hence searching for genetic markers for reproductive traits, including litter size, seems to be a more promising alternative (Kumalska and Terman 2017).

---

Corresponding author: Arkadiusz Terman, Department of Genetics, Faculty of Biotechnology and Animal Breeding, West Pomeranian University of Technology in Szczecin, Piastów 45, 70-311 Szczecin, Poland, e-mail: arkadiusz.terman@zut.edu.pl.

Porcine litter size ranges from 2 to 20 piglets, which makes an average of 10 piglets per litter (Linville et al. 2001). The key factors affecting the final number of piglets born include ovulation rate, uterus volume, embryo survival rate and number of teats in the sow (Rohrer et al. 1999; Vallet and Christenson 2004; Bidanel et al. 2008). Inheritance of reproductive traits is a rather complex process. The heritability coefficients for various reproductive traits are generally low to medium. Understanding the physiological mechanisms behind the expression of reproductive traits makes it possible to identify genes involved in regulating these traits. A number of candidate genes have been pinpointed to date, using genome-wide genotyping (GWG) and genome-wide association studies (GWAS) (Wijesena et al. 2020; Nonneman and Lents 2023).

Although a lot of quantitative trait *loci* (QTLs) have been found for reproductive traits, only a limited number of genes (e.g. *PRLR*, *FSHB*, *RBP4*, *PRKD1*, *THRB* and *PGR*) have been shown to be significantly associated with these traits (Von Steinheuer et al. 2003; Kątska-Książkiewicz et al. 2006; Onteru et al. 2009; Ding et al. 2021; Sell-Kubiak et al. 2022; Wu et al. 2022).

A lot of studies have shown that in order for gestation to proceed normally, the developing foetus must be provided with adequate nutrients. In pigs, of vital importance are retinol-binding proteins, which play a key role in transporting vitamin A to the developing embryo. Importantly, supplementing the diet fed to gestating sows with vitamin A results in significantly larger litter sizes (Brief and Chew 1985; Harney et al. 1993). One of the retinol-binding proteins is encoded by the *RBP4* gene, which has been mapped to chromosome 14 (Messer et al. 1996) and is believed to be a strong candidate gene for litter size in pigs. The most studied polymorphism in the *RBP4* gene is the SNP (DQ344026: g.447G>C) located in intron 4 (Rothschild et al. 2000). Other genomic studies have shown that litter size can also be determined by a polymorphism in a gene coding for another protein of the retinol-binding family – RBP7. Two SNPs in the *RBP7* gene have been reported to be associated with the total number of piglets born alive in a litter: rs81320475 in region 5' and rs81285644 in the intron (Suwannasing et al. 2018).

Follicle-stimulating hormone (FSH) is of crucial importance in reproduction. Its subunit beta, also known as follitropin subunit beta, is a component of a biologically functional heterodimer and is involved in follicle development (egg cell maturation) and spermatogenesis (UniProt P01228). A study carried out on goats showed a link between FSH level and litter size. A positive correlation was observed between the expression (mRNA) of the *FSHB* gene and the number of kids born (Cui et al. 2009). In humans, *FSHB* gene polymorphisms (SNP, rs11031006) are associated with the occurrence of polycystic ovary syndrome (PCOS) (Hayes et al. 2015; Tian et al. 2016) and influence ovarian response to hormonal stimulation (Riccetti et al. 2017). In pigs, FSH level can be regulated by miRNA (miR-361-3p), which can suppress the expression of the *FSHB* gene (Ye et al. 2017).

The porcine *FSHB* gene was mapped to chromosome 2, and the polymorphism detected in it (RFLP-*Hae*III) was proposed by Rohrer et al. (1994) as a potential marker for reproductive performance in pigs, including litter size. An additional allele in this gene was later identified in Chinese swine breeds (Li et al. 2000). However, direct selection for the favourable variant *B* of the *FSHB* gene does not always lead to an increased litter size in all pig populations (Linville et al. 2001).

Epidermal growth factor (EGF) is responsible for stimulation of cell growth and differentiation and also influences the reproductive process (Kim et al. 2002). *EGF* is expressed in porcine oviducts (Swanchara et al. 1995) and plays a role in oocyte maturation (Downs et al. 1988) and ovulation (Shimada et al. 2016). It is also present in the amniotic fluid (Brig-

stock et al. 1996). The *EGF* gene is expressed in the pig embryo (Vaughan et al. 1992), its endometrial expression increasing with the advance of gestation (Wollenhaupt et al. 1999).

The porcine *EGF* gene was mapped to chromosome 8 (Mendez et al. 1999; Kim et al. 2001), where a QTL for uterine capacity was detected (Rohrer et al. 1999). An indel polymorphism (*A* – insertion, *B* – deletion) was found in the *EGF* gene (Mendez et al. 1999), which was later analyzed for its associations with reproductive traits in sows. Variation in the porcine *EGF* gene was examined by Linville et al. (2001), but due to the observed genotype distribution (no individuals with genotype *AA*) they did not perform an association analysis with reproductive traits.

The aim of this study was to search for associations between *RBP4*, *FSHB* and *EGF* gene polymorphisms and selected reproductive traits in pigs.

## MATERIAL AND METHODS

### Animals

The study included 288 crossbred Polish Large White (♀) x Polish Landrace (♂) sows (PLW x PLR) from a farm located in the Kuyavian-Pomeranian (kujawsko-pomorskie) province and 195 purebred Yorkshire sows from a farm located in the West Pomeranian (zachodniopomorskie) province, Poland. All the animals were kept in similar rearing conditions including microclimate (temperature, humidity), area of pigpen and area of floor per sow. Because slightly different ways of feeding between farms, their effect ( $k_j$  in the linear model) was taken into consideration during association study. Data on the sows' reproductive performance was obtained from the breeding records maintained for the two herds. Total number of piglets born (TNB), number of piglets born alive (NBA) and number of litters were analyzed.

### Polymorphism analysis

The biological material used for genetic analyses consisted of whole blood samples (2 ml) drawn from the external jugular vein into vacuum test tubes containing anticoagulant ( $K_3$ EDTA). DNA isolation was done with the salting-out method using MasterPure™ kit (Epicentre Biotechnologies®, USA).

Polymorphic variants in the genes under study were detected by PCR-RFLP and PCR. The examined gene fragments were amplified using FastGene® Taq ReadyMix (2X) (Nippon Genetics, Japan) complete reagent kit with the addition of appropriate primer sequences, template DNA and water to fill up to 15 µl. Details of the methods applied to detect polymorphisms in selected genes are shown in Table 1.

Table 1. Identification of polymorphic variants in the genes under study

Gene	Primer sequences	Ta	RE	Source
<i>RBP4</i>	F 5'-GAGCAAGATGGAATGGGTT-3' R 5'-CTCGGTGTCTGTAAAGGTG-3'	56	<i>MspI</i>	Korwin-Kossakowska et al. 2003
<i>FSHB</i>	F 5'-AGTTCTGAAATGATTTTTTCGGG-3' R 5'-TTTGCCATTGACTGTCTTAAAGG-3'	58	<i>HaeIII</i>	Korwin-Kossakowska et al. 2005
<i>EGF</i>	F 5'-GAAACAATTCCCGTGTCTCTA-3' R 5'-TCACTTCCACACCTGTAACATCT 3'	58	–	Kumalska and Terman 2017

Ta – primer annealing temperature, RE – restriction enzyme.

PCRs were performed in a TGradient thermal cycler (Biometra, Germany). 5 µl of the amplification products were assessed for efficiency and specificity, then remaining 10 µl were digested with 3U of restriction enzyme (MBI Fermentas, USA) at 37°C overnight. Digestion products without thermal inactivation of enzymes were separated directly on 3% agarose gels (Bio Standard, Prona) except for the *EGF* gene, where undigested PCR products (15 µl) were separated on 1.5% agarose gels. The separated DNA fragments were visualized in UV light (Vilber Lourmat, France) and archived.

### Statistical analysis

The study populations were tested for genetic equilibrium with the Hardy–Weinberg test using the Gene-Calc tool (Bińkowski and Miks 2018). Differences in genotype frequencies between the two breeds under study were analyzed with the  $\chi^2$  test (Gene-Calc).

The analyzed polymorphisms were examined for their associations with selected reproductive traits using the general linear model (GLM) module available in the Statistica package (v. 13, PL). Significance of differences between the means in the two genotypic groups was verified with the Bonferroni test. Separate analyses were performed for each litter, the 6th and subsequent litters being grouped in a single category due to their decreasing sizes.

When analyzing all the sows and all the litters in total, a multi-factor ANOVA model was applied in the calculations. Apart from genotype, pig farm and parity, the model also included fixed environmental effect, that is the sow effect, as the studied sows were recorded to have had a few litters. This effect was nested in the analyzed genotypes. The model can be written as:

$$y_{ijnp} = \mu + g_i + k_j + l_n + m_p(g_i) + e_{ijnp}$$

where:

- $y_{ijnp}$  – value of analyzed trait (TNB, NBA),
- $\mu$  – mean value of analyzed trait (TNB, NBA),
- $g_i$  – genotype under analysis ( $i = 1, 2, 3$ ),
- $k_j$  – pig farm/genetic group ( $j = 1, 2$ ),
- $l_n$  – parity ( $k = 1, 2, 3, 4, 5, 6$ ),
- $m_p(g_i)$  – sow (effect nested in genotype),
- $e_{ijnp}$  – error.

A similar model was used to analyze each litter separately, the only modification being that the parity effect was excluded.

## RESULTS

As a result of PCR product digestion with restriction enzymes (in the case of the *RBP4* and *FSHB* genes) followed by electrophoretic separation of the DNA fragments obtained, the genotypes of the studied animals were determined. In the case of the *EGF* gene (insertion-deletion polymorphism), PCR products were separated without being treated with a restriction enzyme. The identified genotypes and their frequencies are presented in Table 2.

Table 2. Genotype frequencies in the sow populations under study

Gene	Breed	n	Genotypes			$\chi^2$ (p-value)
			AA	AB	BB	
<i>RBP4</i>	PLW x PLR	288	0.27 (n = 77)	0.35 (n = 102)	0.38 (n = 109)	13.458 (0.001)
	Yorkshire	195	0.34 (n = 66)	0.44 (n = 86)	0.22 (n = 43)	
<i>FSHB</i>	PLW x PLR	288	0.03 (n = 8)	0.34 (n = 97)	0.63 (n = 183)	12.633 (0.002)
	Yorkshire	195	0.09 (n = 18)	0.39 (n = 76)	0.52 (n = 101)	
<i>EGF</i>	PLW x PLR	288	0.07 (n = 20)	0.47 (n = 135)	0.46 (n = 133)	90.826 (0.000)
	Yorkshire	195	0.02 (n = 4)	0.09 (n = 18)	0.89 (n = 173)	

It was found that, in the case of the *EGF* gene, the most frequent genotype in the studied PLW x PLR population was genotype *AB* (0.47), while in the Yorkshire population it was genotype *BB* (0.89). The differences in genotype frequencies between the two populations were statistically significant ( $\chi^2 = 90.826$ ). As for the *FSHB* gene, genotype *BB* was the most prevalent in both breeds, followed by genotype *AB*, whereas genotype *AA* was the least frequent. However, statistically significant differences were found in genotype distribution between the PLW x PLR and Yorkshire populations ( $\chi^2 = 12.633$ ). A fairly even distribution of genotypes was observed for the *RBP4* gene (from 0.22 to 0.44), the differences in their frequencies between the two breeds being statistically significant ( $\chi^2 = 13.458$ ).

Table 3. Genetic equilibrium test results for the sow populations under study

Gene	Genotype	PLW x PLR <sup>1</sup>		Yorkshire <sup>2</sup>		HWE p-value	Allele frequencies	
		obs.	exp.	obs.	exp.		A	B
<i>RBP4</i>	AA	77	88.89	66	60.93	0.001 <sup>1</sup> 0.338 <sup>2</sup>	0.44 <sup>1</sup> 0.56 <sup>2</sup>	0.56 <sup>1</sup> 0.44 <sup>2</sup>
	AB	102	142.22	86	96.14			
	BB	109	56.89	43	37.93			
<i>FSHB</i>	AA	8	11.08	18	16.08	0.515 <sup>1</sup> 0.798 <sup>2</sup>	0.20 <sup>1</sup> 0.29 <sup>2</sup>	0.80 <sup>1</sup> 0.71 <sup>2</sup>
	AB	97	90.83	76	79.84			
	BB	183	186.08	101	99.08			
<i>EGF</i>	AA	20	26.58	4	0.87	0.186 <sup>1</sup> 0.001 <sup>2</sup>	0.30 <sup>1</sup> 0.07 <sup>2</sup>	0.70 <sup>1</sup> 0.93 <sup>2</sup>
	AB	135	121.83	18	24.27			
	BB	133	139.58	173	169.87			

The Hardy–Weinberg equilibrium (HWE) test showed that in most cases the observed genotype distribution corresponded to the expected distribution (in both breeds in the case of the *FSHB* gene, in the PLW x PLR breed in the case of the *EGF* gene, and in Yorkshire in the case of the *RBP4* gene) (Table 3). In two cases, genetic equilibrium was found to be disturbed (the *EGF* gene in Yorkshire sows and the *RBP4* gene in PLW x PLR sows).

An analysis of the effect of the *RBP4* gene polymorphism on the number of piglets per litter in the studied sows revealed statistically significant differences between the genotype groups.

Heterosis was observed in the 1st and 2nd litter, i.e. heterozygous sows had larger litters than those with genotypes *AA* and *BB*, the differences being statistically significant (Table 4).

Table 4. Effect of *RBP4* gene polymorphism on litter size in the studied sows

Litter	Trait	<i>RBP4</i> genotypes					
		<i>AA</i>		<i>AB</i>		<i>BB</i>	
		LSM	SD	LSM	SD	LSM	SD
1	TNB	11.57 <sup>A</sup>	2.46	12.37 <sup>Ba</sup>	1.95	11.87 <sup>b</sup>	2.58
	NBA	11.18 <sup>A</sup>	2.33	11.99 <sup>Ba</sup>	2.06	11.62 <sup>b</sup>	2.45
2	TNB	12.28 <sup>A</sup>	2.95	13.25 <sup>Ba</sup>	2.45	12.41 <sup>b</sup>	2.42
	NBA	12.02 <sup>A</sup>	2.62	12.85 <sup>Ba</sup>	2.10	12.19 <sup>b</sup>	2.15
3	TNB	12.88	3.01	13.21	2.40	12.59	2.45
	NBA	12.62	2.87	12.86	2.15	12.42	2.21
4	TNB	12.65	2.80	12.85	2.56	12.27	2.63
	NBA	12.39	2.49	12.48	2.51	12.08	2.36
5	TNB	12.64 <sup>A</sup>	2.23	12.51	1.89	11.88 <sup>B</sup>	2.15
	NBA	13.80	2.68	12.14	2.04	12.15	2.03
6	TNB	12.56	2.28	12.39	2.13	11.91	2.05
	NBA	12.89	2.07	12.16	1.98	11.73	2.01
Total	TNB	12.37 <sup>A</sup>	2.70	12.80 <sup>B</sup>	2.28	12.17 <sup>A</sup>	2.43
	NBA	12.10 <sup>A</sup>	2.51	12.47 <sup>B</sup>	2.14	11.98 <sup>A</sup>	2.26

LSM – least squares mean, standard deviation (SD, values marked with different letters indicate statistically significant differences:  $p \leq 0.05$  (<sup>abc</sup>) or  $p \leq 0.01$  (<sup>ABC</sup>).

Table 5. Effect of *FSHB* gene polymorphism on litter size in the studied sows

Litter	Trait	<i>FSHB</i> genotypes					
		<i>AA</i>		<i>AB</i>		<i>BB</i>	
		LSM	SD	LSM	SD	LSM	SD
1	TNB	13.21 <sup>A</sup>	2.57	12.08	2.57	11.60 <sup>B</sup>	2.20
	NBA	13.00 <sup>A</sup>	2.40	11.76	2.19	11.32 <sup>B</sup>	2.23
2	TNB	14.56 <sup>A</sup>	3.01	13.15 <sup>a</sup>	2.52	12.08 <sup>Bb</sup>	2.44
	NBA	14.19 <sup>Aca</sup>	2.59	12.82 <sup>Bd</sup>	2.12	11.90 <sup>b</sup>	2.26
3	TNB	13.80	1.81	12.91	2.47	12.49	2.48
	NBA	13.60	1.91	12.57	2.29	12.30	2.31
4	TNB	12.83	2.93	12.91	2.63	12.26	2.46
	NBA	12.83	2.93	12.48	2.73	12.06	2.34
5	TNB	13.80	2.68	12.26	2.16	12.25	2.09
	NBA	13.80	2.68	12.14	2.04	12.15	2.03
6	TNB	12.67	2.08	12.20	1.99	12.20	2.20
	NBA	12.67	2.08	12.15	1.98	12.01	2.04
Total	TNB	13.70 <sup>A</sup>	2.59	12.6 <sup>AB</sup>	2.41	12.1 <sup>B</sup>	2.34
	NBA	13.46 <sup>A</sup>	2.40	12.33 <sup>AB</sup>	2.26	11.93 <sup>B</sup>	2.24

LSM – least squares mean, standard deviation (SD); values marked with different letters indicate statistically significant differences:  $p \leq 0.05$  (<sup>abc</sup>) or  $p \leq 0.01$  (<sup>ABC</sup>).

As far as the polymorphism in the *FSHB* gene is concerned, sows with phenotype *AA* were found to produce larger litters than sows carrying the other genotypes, but the differences with regard to the analyzed reproductive traits were statistically significant for the 1st and 2nd litter only ( $p \leq 0.01$  or  $p \leq 0.05$ ). The lowest number of piglets was observed in sows with genotype *BB*. In some litters, the difference in the number of piglets between sows with homozygous genotypes was over two piglets per litter (Table 5).

Table 6 shows the effect of the *EGF* gene polymorphism on the reproductive performance of the sows under study. As can be seen, sows with genotype *BB* had the highest number of piglets in all of the analyzed litters, and the differences in relation to the sows with other genotypes were found to be statistically significant ( $p \leq 0.01$  or  $p \leq 0.05$ ). The following relationships were revealed between litter size and the genotypes under study: the largest litters were farrowed by sows carrying genotype *BB*, females with genotype *AB* had medium-sized litters, and the smallest litters were obtained from sows with genotype *AA*.

Table 6. Effect of *EGF* gene polymorphism on litter size in the studied sows

Litter	Trait	<i>EGF</i> genotypes					
		<i>AA</i>		<i>AB</i>		<i>BB</i>	
		LSM	SD	LSM	SD	LSM	SD
1	TNB	11.04 <sup>A</sup>	1.68	11.52 <sup>a</sup>	2.20	12.50 <sup>Bb</sup>	2.45
	NBA	10.88	1.57	11.35 <sup>A</sup>	2.12	12.00 <sup>B</sup>	2.43
2	TNB	11.76 <sup>A</sup>	1.76	11.93 <sup>a</sup>	2.15	13.30 <sup>Bb</sup>	2.76
	NBA	11.76	1.76	11.81 <sup>A</sup>	1.97	12.82 <sup>B</sup>	2.46
3	TNB	12.00 <sup>A</sup>	1.95	12.01 <sup>a</sup>	2.18	13.70 <sup>Bb</sup>	2.73
	NBA	12.00 <sup>A</sup>	1.95	11.81 <sup>a</sup>	2.27	13.27 <sup>Bb</sup>	2.21
4	TNB	11.11 <sup>A</sup>	1.97	12.04 <sup>a</sup>	2.21	13.26 <sup>Bb</sup>	2.84
	NBA	11.11 <sup>A</sup>	1.97	12.03 <sup>a</sup>	2.18	12.71 <sup>Bb</sup>	2.76
5	TNB	11.06 <sup>A</sup>	2.04	12.11 <sup>a</sup>	2.19	12.76 <sup>Bb</sup>	2.01
	NBA	11.06 <sup>A</sup>	2.04	12.09	2.11	12.47 <sup>B</sup>	1.96
6	TNB	11.00 <sup>A</sup>	1.64	11.75 <sup>a</sup>	2.02	12.85 <sup>Bb</sup>	2.20
	NBA	11.00 <sup>A</sup>	1.64	11.74 <sup>a</sup>	2.01	12.55 <sup>Bb</sup>	2.02
Total	TNB	11.34 <sup>Aa</sup>	1.84	11.88 <sup>Ab</sup>	2.15	13.07 <sup>B</sup>	2.62
	NBA	11.31 <sup>A</sup>	1.83	11.78 <sup>A</sup>	2.10	12.63 <sup>B</sup>	2.47

LSM – least squares mean, standard deviation (SD), values marked with different letters indicate statistically significant differences:  $p \leq 0.05$  (<sup>abc</sup>) or  $p \leq 0.01$  (<sup>ABC</sup>)

## DISCUSSION

In some instances, a strong focus on improving fattening performance and slaughter value of pigs has had a negative effect on the number of piglets born per litter, which has even started decreasing. Therefore, genetic improvement of litter size is now in the centre of attention of pork producers. Unfortunately, due to low heritability of this reproductive trait, improvement of litter size using traditional methods is difficult and long-lasting. An alternative strategy is to search for genetic markers by identifying so-called candidate genes or, more recently, by genomic analyses (microarray genotyping, genomic sequencing and genome-wide association

studies (GWAS) (Vaishnav et al. 2023). In practice, marker-assisted selection for increased litter size based on candidate genes started to be used in pig breeding after the results of a study on the effect of *ESR* gene polymorphism on this trait were published (Rothschild 1996). In the following years, the effects of single nucleotide polymorphisms in a number of other genes were investigated, often making use of quantitative trait *loci* (QTLs) mapped to various porcine chromosomes (Ma et al. 2018; Wu et al. 2018; Bakoev et al. 2020; Sell-Kubiak et al. 2022). The *RBP4*, *FSHB* and *EGF* genes analyzed in this study belong to a group of candidate genes that have been studied for years as potential markers of reproductive performance.

The present study regarding *RBP4* gene has shown that the largest litters are farrowed by sows with genotype *AB* (in the 1st and 2nd litter), both in terms of TNB and NBA. Earlier studies in this area found that litter size in sows might be positively influenced by a variation in the *RBP4* gene sequence (Ollivier et al. 1997), with allele *A* being associated with a slightly bigger number of piglets per litter (Rothschild et al. 2000). A positive effect of the *RBP4* genotype on litter size in Landrace pigs was reported by Blowe et al. (2006), whose results were later confirmed by other authors (Omelka et al. 2008; Spötter et al. 2009). A favourable association has also been found between genotype *AA* and litter size in Tibetan (Niu et al. 2008) and Shandong (Sun et al. 2009) breeds. Other researchers have shown that an increased litter size in Large White x Landrace crossbred pigs is associated with the *RBP4-MspI AA* genotype (Marantidis et al. 2016; Mencik et al. 2019).

By contrast, other studies did not show any associations between the SNP in the *RBP4* gene (g.447G>C) and the number of piglets per litter (Drogemuller et al. 2001; Linville et al. 2001; Wang et al. 2006). No effect of this genotype on litter size has been reported in German Large White (Spötter et al. 2009) and Polish Large White, Landrace and commercial line 990 pigs (Korwin-Kossakowska et al. 2005) as well as in Chinese (Liu et al. 2010) and local Indian breeds (Vashi et al. 2021).

However, contrary to the reported lack of association between the *RBP4* SNP and litter size in German Large White (Spötter et al. 2009), Dall'Olio et al. (2010) identified the *MspI* polymorphism in the *RBP4* gene as a potential marker for reproductive performance in Italian Large White sows. Similar results had been reported earlier by Polish authors, who showed that Large White sows with genotype *BB* farrowed significantly more piglets per litter than sows carrying other genotypes (Terman et al. 2007). Identical associations were also observed in crossbred Large White x Landrace (Terman et al. 2011) and Landrace sows (Vinh et al. 2019). Moreover, an analysis of the effect of the *RBP4-MspI* genotype in interaction with the *ESR-PvuII* genotype revealed a positive combined effect of these two genes on litter size (Muñoz et al. 2010).

Associations between the *RBP4* genotype and reproductive traits consistent with those observed in the present study were reported by Laliotis et al. (2017), who found that sows with genotype *AB* had larger litters (in terms of both TNB and NBA) compared with homozygous individuals (*AA* and *BB*,  $p < 0.001$ ).

In the light of the above results, it can be concluded that there is no single variant of the *RBP4* gene that might be linked to an increased number of piglets per litter in all pig breeds or commercial synthetic lines. This might make a practical application of this gene in selection programmes difficult, particularly in cross-breed hybrids (e.g. Large White x Landrace), which are commonly used in porker production as the maternal component.

The current study results concerning *FSHB* gene has shown that the highest numbers of piglets in the first two litters were born from sows with genotype *AA* ( $p < 0.01$  or  $p < 0.05$ ) compared with sows carrying the other genotypes. A similar association was observed by



Luoreng et al. (2007) whose study revealed a positive effect of allele *A* on the reproductive traits of sows. In the first litter, the TNB recorded in females with genotype *AA* was on average 0.96 and 1.85 higher than in sows with genotypes *AB* and *BB*, respectively. A similar tendency was observed for NBA.

Different results were published by other authors for Landrace and Yorkshire pigs. Zhao et al. (1998) reported that allele *B* of the *FSHB* gene increased (on average) the number of piglets per litter by 1.5 (in all the analyzed litters), and accordingly they proposed that *FSHB* gene polymorphism could be used in marker-assisted selection (MAS). Similarly, a positive effect of allele *B* on the litter size in a Tibetan breed of swine was observed by Liu et al. (2010), whereas Wang et al. (2006) found that Large White sows with genotype *BB* had an average of 1.13 piglets more (in the 2nd litter) than females with genotype *AB*. The result was confirmed in a more recent study by Pang et al. (2019), who estimated the additive effect of allele *B* of the *FSHB* gene on the litter size in Large White sows at 0.48 (for TNB) and 0.65 (for NBA) piglets per litter ( $p < 0.01$ ).

However, other authors studying Polish and Hungarian swine populations did not find any associations between the *FSHB* gene polymorphism and the reproductive traits of sows (Korwin-Kossakowska et al. 2003; Hunyadi-Bagi et al. 2016; Polasik et al. 2016).

The last candidate gene (*EGF*) analysis shows that sows with genotype *BB* farrowed significantly more piglets (TNB and NBA) in all litters than sows with the other genotypes (Table 4). By comparison, Korwin-Kossakowska et al. (2005) reported a positive effect of the *EGF* gene polymorphism on TNB ( $p \leq 0.01$ ) and NBA ( $p \leq 0.05$ ) in Polish Large White, Landrace and line 990 pigs, with the highest reproductive performance recorded in sows with genotype *AA*. Identical associations were found in a population of Hungarian Large White swine studied by Hunyadi-Bagi et al. (2016), who recorded a higher number of piglets born (TNB and NBA,  $p \leq 0.01$ ) in sows with genotype *AA* compared with *AB* and *BB* sows.

A different tendency in, among others, Large White and Landrace breeds was observed by Mucha et al. (2013). Their study showed that females with genotype *BB* had a higher NBA ( $p < 0.01$  and  $p < 0.05$ ) and a higher number of piglets at day 21 of rearing (N21) compared with sows with the other genotypes. Sato et al. (2016) also reported an association between *EGF* gene variation and the number of piglets born per litter (TNB and NBA) and the average weight of piglet at weaning (AWW) in second parity Large White sows. A more recent study (Vashi et al. 2021) showed a positive effect of *EGF* genotypes *AB* and *BB* (in the 1st litter) on litter size at weaning (LSW) and litter weight at weaning (LWW) compared with sows with genotype *AA*. On the other hand, no statistically significant associations were found between sow reproductive traits and the *EGF* gene polymorphism in a study of 13 Chinese swine breeds (Zhou et al. 2010).

## CONCLUSIONS

For the past two decades, the so-called candidate gene approach has been commonly used in studies searching for genetic markers for production traits in farm animals, including reproductive traits in sows. Conducted analysis showed that *RBP4*, *FSHB* and *EGF* variants are associated with TNB and NBA in population of Polish Large White x Polish Landrace crossbreed and Yorkshire sows. It appears, however that it is hard to find universal marker which can be applied in most of the analyzed pig breeds as well as their crossbreeds, because different genotype-trait tendency or lack of association. Investigations should be

continued including new genes and newly discovered variants in candidate genes, taking into consideration individual characteristics of analyzed pig breed/crossbreed.

## REFERENCES

- Bakoev S., Getmantseva L., Bakoev F., Kolosova M., Gabova V., Kolosov A., Kostyunina O.** 2020. Survey of SNPs associated with total number born and total number born alive in pig. *Genes* 11(5), 491. DOI: 10.3390/genes11050491.
- Bello S.F., Adeola A.C., Nie Q.** 2022. The study of candidate genes in the improvement of egg production in ducks – a review. *Poult Sci.* 101, 101850. DOI: 10.1016/j.psj.2022.101850.
- Bidanel J.P., Rosendo A., Iannuccelli N., Riquet J., Gilbert H., Caritez J.C., Billon Y., Amigues Y., Prunier A., Milan D.** 2008. Detection of quantitative trait loci for teat number and female reproductive traits in Meishan × Large White F2 pigs. *Animal* 2(6), 813–820. DOI: 10.1017/S1751731108002097.
- Bińkowski J., Miks S.** 2018. Gene-calc [computer software]. Available from: [www.gene-calc.pl](http://www.gene-calc.pl), access: 01.08.2018.
- Blowe C.D., Boyette K.E., Ashwell M.S., Eisen E.J., Robison O.W., Cassady J.P.** 2006. Characterization of a line of pigs previously selected for increased litter size for RBP4 and follistatin. *J. Anim. Breed. Genet.* 123, 389–395. DOI: 10.1111/j.1439-0388.2006.00620.x.
- Brief S., Chew B.P.** 1985. Effects of vitamin A and beta-carotene on reproductive performance in gilts. *J. Anim. Sci.* 60(4), 998–1004. DOI: 10.2527/jas1985.604998x.
- Brigstock D.R., Kim G.Y., Steffen C.L., Liu A., Vegunta R.K., Ismail N.H.** 1996. High molecular mass forms of epidermal growth factor in pig uterine secretions. *J. Reprod. Fertil.* 108(2), 313–320. DOI: 10.1530/jrf.0.1080313.
- Cui H.X., Zhao S.M., Cheng M.L., Guo L., Ye R.Q., Liu W.Q., Gao S.Z.** 2009. Cloning and expression levels of genes relating to the ovulation rate of the Yunling black goat. *Biol. Reprod.* 80(2), 219–226. DOI: 10.1095/biolreprod.108.069021.
- Dall’Olio S., Fontanesi L., Tognazzi L., Russo V.** 2010. Genetic structure of candidate genes for litter size in Italian Large White pigs. *Vet. Res. Commun.* 34, 203–206. DOI: 10.1007/s11259-010-9380-7.
- Ding R., Qiu Y., Zhuang Z., Ruan D., Wu J., Zhou S., Ye J., Cao L., Hong L., Xu Z., Zheng E., Li Z., Wu Z., Yang J.** 2021. Genome-wide association studies reveals polygenic genetic architecture of litter traits in Duroc pigs. *Theriogenology* 173, 269–278. DOI: 10.1016/j.theriogenology.2021.08.012.
- Downs S.M., Daniel S.A., Eppig J.J.** 1988. Induction of maturation in cumulus cell-enclosed mouse oocytes by follicle-stimulating hormone and epidermal growth factor: evidence for a positive stimulus of somatic cell origin. *J. Exp. Zool.* 245, 86–96. DOI: 10.1002/jez.1402450113.
- Drogemuller C., Hamann H., Distl O.** 2001. Candidate gene markers for litter size in different German pig lines. *J. Anim. Sci.* 79, 2565–2570. DOI: 10.2527/2001.79102565x.
- Harney J.P., Ott T.L., Geisert R.D., Bazer F.W.** 1993. Retinol-binding protein gene expression in cyclic and pregnant endometrium of pigs, sheep, and cattle. *Biol. Reprod.* 49, 1066–1073. DOI: 10.1095/biolreprod49.5.1066.
- Hayes M.G., Urbanek M., Ehrmann D.A., Armstrong L.L., Lee J.Y., Sisk R., Karaderi T., Barber T.M., McCarthy M.I., Franks S., Lindgren C.M., Welt C.K., Diamanti-Kandarakis E., Panidis D., Goodarzi M.O., Azziz R., Zhang Y., James R.G., Olivier M., Kissebah A.H., Stener-Victorin E., Legro R.S., Dunaif A.** 2015. Genome-wide association of

- polycystic ovary syndrome implicates alterations in gonadotropin secretion in European ancestry populations. *Nat. Commun.* 6, 7502. DOI: 10.1038/ncomms8502.
- Hunyadi-Bagi Á., Balogh P., Nagy K., Kusza S.** 2016. Association and polymorphism study of seven candidate genes with reproductive traits in three pig breeds in Hungary. *Acta Biochim. Pol.* 63(2), 359–364. DOI: 10.18388/abp.2015\_1188.
- Ibeagha-Awemu E.M., Kgwatalala P., Zhao X.** 2008. A critical analysis of production-associated DNA polymorphisms in the genes of cattle, goat, sheep, and pig. *Mamm. Genome* 19(9), 591–617. DOI: 10.1007/s00335-008-9141-x.
- Kątska-Książkiewicz L., Lechniak-Cieślak D., Korwin-Kossakowska A., Alm H., Ryńska B., Warzych E., Sosnowski J., Sender G.** 2006. Genetical and biotechnological methods of utilization of female reproductive potential in mammals. *Reprod. Biol.* 6, 21–36.
- Kim J.G., Vallet J.L., Christenson R.K.** 2001. Characterization of uterine epidermal growth factor during early pregnancy in pigs. *Domest. Anim. Endocrinol.* 20(4), 253–265. DOI: 10.1016/S0739-7240(01)00097-2.
- Kim J.G., Vallet J.L., Rohrer G.A., Christenson R.K.** 2002. Mapping of the porcine AREG and EGF genes to SSC8. *Anim. Genet.* 33(4), 314–315. DOI: 10.1046/j.1365-2052.2002.t01-2-00886.x.
- Knol E.F., van der Spek D., Zak L.J.** 2022. Genetic aspects of piglet survival and related traits: a review. *J. Anim. Sci.* 100(6), skac190. DOI: 10.1093/jas/skac190.
- Korwin-Kossakowska A., Kamyczek M., Cieślak D., Pierzchała M., Kuryl J.** 2003. Candidate gene markers for reproductive traits in polish 990 pig line. *J. Anim. Breed. Genet.* 120, 181–191. DOI: 10.1046/j.1439-0388.2003.00386.x.
- Korwin-Kossakowska A., Kapelański W., Bocian M., Kamyczek M., Sender G.** 2005. Preliminary study of the RBP4, EGF and PTGS2 genes polymorphism in pigs and its association with reproduction traits of sows. *Anim. Sci. Pap. Rep.* 23, 95–105.
- Kumalska M., Terman A.** 2017. Genetic associations of reproductive traits in pigs. *Ann. Wars. Univ. Life Sci.-SGGW, Anim. Sci.* 56, 265–275. DOI: 10.22630/AAS.2017.56.2.28.
- Laliotis G.P., Marantidis A., Avdi M.** 2017. Association of BF, RBP4, and ESR2 genotypes with litter size in an autochthonous pig population. *Anim. Biotechnol.* 28, 138–143. DOI: 10.1080/10495398.2016.1242490.
- Li M.D., Rohrer G.A., Wise T.H., Ford J.J.** 2000. Identification and characterization of a new allele for the beta subunit of follicle-stimulating hormone in Chinese pig breeds. *Anim. Genet.* 31, 28–30. DOI: 10.1046/j.1365-2052.2000.00581.x.
- Linville R.C., Pomp D., Johnson R.K., Rothschild M.F.** 2001. Candidate gene analysis for loci affecting litter size and ovulation rate in swine. *J. Anim. Sci.* 79, 60–67. DOI: 10.2527/2001.79160x.
- Liu X., Chamba Y., Wang Q., Ling Y., Gu X.D., Wu K.L., Zhang H.** 2010. [Effects of multi-genes for reproductive traits in Tibet pig]. *Yi Chuan* 32, 480–485 [in Chinese]. DOI: 10.3724/SP.J.1005.2010.00480.
- Luoreng Z., Wang L.X., Sun S.D.** 2007. [Genetic polymorphism of FSH b subunit gene and correlation with reproductive traits in Beijing Black Pig]. *Yi Chuan* 29, 1497–1503 [in Chinese].
- Ma X., Li P.H., Zhu M.X., He L.C., Sui S.P., Gao S., Su G.S., Ding N.S., Huang Y., Lu Z.Q., Huang X.G., Huang R.H.** 2018. Genome-wide association analysis reveals genomic regions on Chromosome 13 affecting litter size and candidate genes for uterine horn length in Erhualian pigs. *Animal* 12, 2453–2461. DOI: 10.1017/S1751731118000332.
- Marantidis A., Laliotis G.P., Avdi M.** 2016. Association of RBP4 genotype with phenotypic reproductive traits of sows. *Genet. Res. Int.* 4940532. DOI: 10.1155/2016/4940532.

- Mencik S., Vukovic V., Spehar M., Modric M., Ostovic M., Ekert Kabalin A.** 2019. Association between ESR1 and RBP4 genes and litter size traits in a hyperprolific line of Landrace × Large White cross sows. *Vet. Med.* 64, 109–117. DOI: 10.17221/87/2018-VETMED.
- Mendez E.A., Messer L.A., Larsen N.J., Robic A., Rothschild M.F.** 1999. Epidermal growth factor maps to pig chromosome 8. *J. Anim. Sci.* 77, 494–495. DOI: 10.2527/1999.772494x.
- Messer L.A., Wang L., Yelich J., Pomp D., Geisert R.D., Rothschild M.F.** 1996. Linkage mapping of the retinol-binding protein 4 (RBP4) gene to porcine chromosome 14. *Mamm. Genome* 7, 396. DOI: 10.1007/s003359900117.
- Mucha A., Ropka-Molik K., Piórkowska K., Tyra M., Oczkiewicz M.** 2013. Effect of EGF, AREG and LIF genes polymorphisms on reproductive traits in pigs. *Anim. Reprod. Sci.* 137, 88–92. DOI: 10.1016/j.anireprosci.2012.12.009.
- Muñoz M., Fernández A.I., Ovilo C., Muñoz G., Rodríguez C., Fernández A., Alves E., Silió L.** 2010. Non-additive effects of RBP4, ESR1 and IGF2 polymorphisms on litter size at different parities in a Chinese-European porcine line. *Genet. Sel. Evol.* 42, 23. DOI: 10.1186/1297-9686-42-23.
- Niu S.Y., Wang X.P., Hao F.G., Zhao R.X.** 2008. Effect of the polymorphism of RBP4 and OPN genes on litter size in Tibet pigs. *Acta. Agric. Scand. Sect. A* 58, 10–13. DOI: 10.1080/09064700802054170.
- Nonneman D.J., Lents C.A.** 2023. Functional genomics of reproduction in pigs: Are we there yet? *Mol. Reprod. Dev.* 90(7), 436–444. DOI: 10.1002/mrd.23625.
- OECD.** 2022. Meat consumption (indicator), accessed on 02 November 2022. DOI: 10.1787/fa290fd0-en
- Ollivier L., Messer L.A., Rothschild M.F., Legault C.** 1997. The use of selection experiments for detecting quantitative trait loci. *Genet. Res.* 69(3), 227–232. DOI: 10.1017/S0016672397002802.
- Omelka R., Martiniaková M., Peškovičová D., Bauerová M.** 2008. Associations between RBP4/Mspl polymorphism and reproductive traits in pigs: an application of animal model. *J. Agrobiol.* 25(1), 77–80.
- Onteru S.K., Ross J.W., Rothschild M.F.** 2009. The role of gene discovery. QTL analyses and gene expression in reproductive traits in the pig. *Soc. Reprod. Fertil. Suppl.* 66, 87–102. DOI: 10.2527/jas.2011-4581.
- Pang P., Li Z., Hu H., Wang L., Sun H., Mei S., Li F.** 2019. Genetic effect and combined genotype effect of ESR, FSH $\beta$ , CTNNAL1 and miR-27a loci on litter size in a Large White population. *Anim. Biotechnol.* 30, 287–292. DOI: 10.1080/10495398.2018.1486322.
- Polasik D., Kumalska M., Źak G., Tyra M., Urbański P., Terman A.** 2016. Analysis of FSHB gene polymorphism in Polish landrace and Polish large white x Polish landrace sows. *EJPAU.* 19, 1–6.
- Riccetti L., De Pascali F., Gilioli L., Santi D., Brigante G., Simoni M., Casarini L.** 2017. Genetics of gonadotropins and their receptors as markers of ovarian reserve and response in controlled ovarian stimulation. *Best Pract. Res. Clin. Obstet. Gynaecol.* 44, 15–25. DOI: 10.1016/j.bpobgyn.2017.04.002.
- Roberts J., Middleton A.** 2017. Genetics in the 21st Century: Implications for patients, consumers and citizens. *F1000Res.* 6, 2020. DOI: 10.12688/f1000research.12850.2.
- Rohrer G.A., Alexander L.J., Beattie C.W.** 1994. Mapping the beta subunit of follicle stimulating hormone (FSHB) in the porcine genome. *Mamm. Genome* 5, 315–317. DOI: 10.1007/BF00389548.

- Rohrer G.A., Ford J.J., Wise T.H., Vallet J.L., Christenson R.K.** 1999. Identification of quantitative trait loci affecting female reproductive traits in a multigeneration Meishan-White composite swine population. *J. Anim. Sci.* 77, 1385–1391. DOI: 10.2527/1999.7761385x.
- Rothschild M.F., Messer L., Day A., Wales R., Short T., Southwood O., Plastow G.** 2000. Investigation of the retinol-binding protein 4 (RBP4) gene as a candidate gene for increased litter size in pigs. *Mamm. Genome* 11, 75–77. DOI: 10.1007/s003350010015.
- Rothschild M.F.** 1996. Genetics and reproduction in the pig. *Anim. Reprod. Sci.* 42, 143–151. DOI: 10.1016/0378-4320(96)01486-8.
- Rothschild M.F.** 2004. Porcine genomics delivers new tools and results: This little piggy did more than just go to market. *Genet. Res.* 83, 1–6. DOI: 10.1017/s0016672303006621.
- Sato S., Kikuchi T., Uemoto Y., Mikawa S., Suzuki K.** 2016. Effect of candidate gene polymorphisms on reproductive traits in a Large White pig population. *Anim. Sci. J.* 87, 1455–1463. DOI: 10.1111/asj.12580.
- Sell-Kubiak E., Dobrzanski J., Derks M.F.L., Lopes M.S., Szwaczkowski T.** 2022. Meta-analysis of SNPs determining litter traits in pigs. *Genes* 13, 1730. DOI: 10.3390/genes13101730.
- Shimada M., Umehara T., Hoshino Y.** 2016. Roles of epidermal growth factor (EGF)-like factor in the ovulation process. *Reprod. Med. Biol.* 15, 201–216. DOI: 10.1007/s12522-016-0236-x.
- Spötter A., Distl O.** 2006. Genetic approaches to the improvement of fertility traits in the pig. *Vet. J.* 172, 234–247. DOI: 10.1016/j.tvjl.2005.11.013.
- Spötter A., Müller S., Hamann H., Distl O.** 2009. Effect of polymorphisms in the genes for LIF and RBP4 on litter size in two German pig lines. *Reprod. Domest. Anim.* 44, 100–105. DOI: 10.1111/j.1439-0531.2007.01004.x.
- Sun Y.X., Zeng Y.Q., Tang H., Fan X.Z., Chen Q.M., Li H., Qian Y., Song Y.P.** 2009. [Relationship of genetic polymorphism of PRLR and RBP4 genes with litter size traits in pig]. *Yi Chuan* 31, 63–86 [in Chinese]. DOI: 10.3724/SP.J.1005.2009.00063.
- Suwannasing R., Duangjinda M., Boonkum W., Taharnklaew R., Tuangsithtanon K.** 2018. The identification of novel regions for reproduction trait in Landrace and Large White pigs using a single step genome-wide association study. *Asian-Australas J. Anim. Sci.* 31, 1852–1862. DOI: 10.5713/ajas.18.0072.
- Swanchara K.W., Henricks D.M., Birrenkott G.P., Bodine A.B., Richardson M.E.** 1995. Expression of epidermal growth factor (EGF) and the EGF receptor in the porcine oviduct. *Biol. Reprod.* 53, 911–922. DOI: 10.1095/biolreprod53.4.911.
- Terman A., Kmiec M., Polasik D., Pradziadowicz K.** 2007. Retinol binding protein 4 gene and reproductive traits in pigs. *Arch. Anim. Breed.* 50, 181–185.
- Terman A., Kmiec M., Polasik D., Rybarczyk A.** 2011. Association between RBP4 gene polymorphism and reproductive traits in Polish sows. *J. Anim. Vet. Adv.* 10, 2639–2641.
- Tian Y., Zhao H., Chen H., Peng Y., Cui L., Du Y., Wang Z., Xu J., Chen Z.J.** 2016. Variants in FSHB are associated with polycystic ovary syndrome and luteinizing hormone level in Han Chinese women. *J. Clin. Endocrinol. Metab.* 101, 2178–2184. DOI: 10.1210/jc.2015-3776.
- Vaishnav S., Chauhan A., Ajay A., Saini B.L., Kumar S., Kumar A., Bhushan B., Gaur G.K.** 2023. Allelic to genome wide perspectives of swine genetic variation to litter size and its component traits. *Mol. Biol. Rep.* 50, 3705–3721. DOI: 10.1007/s11033-022-08168-5.
- Vallet J.L., Christenson R.K.** 2004. Effect of progesterone, mifepristone, and estrogen treatment during early pregnancy on conceptus development and uterine capacity in Swine. *Biol. Reprod.* 70, 92–98. DOI: 10.1095/biolreprod.103.020214.

- Vashi Y., Magotra A., Kalita D., Banik S., Sahoo N.R., Gupta S.K., Naskar S.** 2021. Evaluation of candidate genes related to litter traits in Indian pig breeds. *Reprod Domest. Anim.* 56, 577–585. DOI: 10.1111/rda.13895.
- Vaughan T.J., James P.S., Pascall J.C., Brown K.D.** 1992. Expression of the genes for TGF alpha, EGF and the EGF receptor during early pig development. *Development* 116(3), 663–669. DOI: 10.1242/dev.116.3.663.
- Vinh N.T., Luc D.D., Thinh N.H., Bo H.X., Mai H.N., Phuong N.T., Farnir F., Ton V.D.** 2019. Additive genetic effects of RNF4, RBP4, and IGF2 polymorphisms on litter size in Landrace and Yorkshire sows. *VJAS* 2, 314–320. DOI: 10.31817/vjas.2019.2.1.02.
- Von Steinheuer R., Drögemüller C., Hamann H., Götz K-U., Distl O.** 2003. Einfluss von Kandidatengeneffekten auf die Anzahl lebend geborener und aufgezogener Ferkel bei Besamungsebern der Deutschen Landrasse. *Züchtungskunde* 75, 204–213.
- Wang X., Wang A., Fu J., Lin H.** 2006. Effects of ESR1, FSH $\beta$  and RBP4 genes on litter size in a large white and a landrace herd. *Arch. Anim. Breed.* 49, 64–70. DOI: 10.5194/aab-49-64-2006.
- Wijesena H.R., Kachman S.D., Lents C.A., Riethoven J.J., Trenhaile-Grannemann M.D., Safranski T.J., Spangler M.L., Ciobanu D.C.** 2020. Fine mapping genetic variants associated with age at puberty and sow fertility using SowPro90 genotyping array. *J. Anim. Sci.* 98, skaa293. DOI: 10.1093/jas/skaa293.
- Wollenhaupt K., Einspanier R., Gabler C., Schneider F., Kanitz W., Brüßow K.P.** 1999. Identification of the EGF/EGF-R system in the oviduct and endometrium of pigs in early stages of pregnancy and early conceptus. *Exp. Clin. Endocrinol. Diabetes.* 107, 530–508. DOI: 10.1055/s-0029-1232562.
- Wu P., Wang K., Yang Q., Zhou J., Chen D., Ma J., Tang Q., Jin L., Xiao W., Jiang A., Jiang Y., Zhu L., Li M., Li X., Tang G.** 2018. Identifying SNPs and candidate genes for three litter traits using single-step GWAS across six parities in Landrace and Large White pigs. *Physiol. Genomics* 50, 1026-1035. DOI: 10.1152/physiolgenomics.00071.2018.
- Wu Z.C., Wang Y., Huang X., Wu S., Bao W.** 2022. A genome-wide association study of important reproduction traits in large white pigs. *Gene* 838, 146702. DOI: 10.1016/j.gene.2022.146702.
- Ye R.S., Li M., Li C.Y., Qi Q.E., Chen T., Cheng X., Wang S.B., Shu G., Wang L.N., Zhu X.T., Jiang Q.Y., Xi Q.Y., Zhang Y.L.** 2017. miR-361-3p regulates FSH by targeting FSHB in a porcine anterior pituitary cell model. *Reproduction* 153, 341–349. DOI: 10.1530/REP-16-0373.
- Zhao Y., Li N., Xiao L., Cao G., Chen Y., Zhang S., Chen Y., Wu C., Zhang J., Sun S., Xu X.** 1998. FSHB subunit gene is associated with major gene controlling litter size in commercial pig breeds. *Sci. China C. Life Sci.* 41, 664–668. DOI: 10.1007/BF02882910.
- Zhou L., Xiao S., Yang G., Chen C., Ding N., Gao J.** 2010. Genetic variation of the porcine epidermal growth factor gene and its association with litter size. *China Anim. Husb. Vet. Med.* 37, 147–150.

## ZALEŻNOŚCI POMIĘDZY POLIMORFIZMAMI GENÓW *RBP4*, *FSHB* I *EGF* A CECHAMI ROZRODCZYMI U ŚWIŃ

**Streszczenie.** Geny kodujące białko wiążące retinol typu 4 (*RBP4*), podjednostkę beta hormonu folikulotropowego (*FSHB*) i nabłonkowy czynnik wzrostu (*EGF*) zostały zaproponowane jako geny kandydujące dla cech rozrodczych u świń. Badania przedstawione w niniejszej pracy miały na celu znalezienie zależności pomiędzy wariantami tych genów a cechami rozrodczymi u świń hodowanych w Polsce. Badaniami objęto lochy krzyżówki międzyrasowej wielka biała polska x polska biała zwistoucha ( $n = 288$ ) i rasy yorkshire ( $n = 195$ ). Polimorfizm insercyjno-delecyjny (indel) genu *EGF* określono metodą PCR (reakcja łańcuchowa polimerazy), natomiast polimorfizm pojedynczego nukleotydu (SNP) w genach *RBP4* i *FSHB* metodą PCR-RFLP (polimorfizm długości fragmentów restrykcyjnych). Uzyskane wyniki wykazały, że liczba prosiąt urodzonych (TNB) i liczbą prosiąt żywo urodzonych (NBA) w pierwszym, drugim i piątym miocie były powiązane z wariantami genu *RBP4*, TNB i NBA w pierwszych dwóch miotach z wariantami *FSHB*, natomiast obie cechy we wszystkich miotach z wariantami genu *EGF* ( $p \leq 0,05$  lub  $p \leq 0,01$ ). W przypadku genu *RBP4* korzystny dla analizowanych cech był genotyp heterozygotyczny (*AB*), natomiast w odniesieniu do genów *FSHB* i *EGF* genotypy homozygotyczne odpowiednio *AA* i *BB*. Uzyskane wyniki wskazują, że polimorfizm w trzech analizowanych genach jest powiązany z ważnymi cechami rozrodczymi świń utrzymywanych w Polsce.

**Słowa kluczowe:** *RBP4*, *FSHB*, *EGF*, polimorfizm, cechy rozrodcze.