Associations Between RBP4, Fshb and Egf Gene Polymorphisms and Reproductive Traits in Pigs

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Posted Date: 21 June 2023

doi: 10.20944/preprints202306.1526.v1

Keywords: pigs; RBP4; FSHB; EGF; DNA polymorphism; reproduction traits

Article

Associations between *RBP4*, *FSHB* and *EGF* gene polymorphisms and reproductive traits in pigs

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Simple Summary: The aim of this study was to determine associations between polymorphism located in *RBP4*, *FSHB* and *EGF* genes and reproduction traits in sows. Investigation covered population (n=483) that consist crossbred and purebred pigs. Results showed that selected variants were related to total number born and number born alive traits (P≤0.05 or P≤0.01).

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 paper 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) and Yorkshire (n=195) sows. Individual genotypes were determined by means of PCR-RFLP (single nucleotide polymorphism in *RBP4*, *FSHB*) or PCR (insertion/deletion in *EGF*) methods. Obtained result showed that *RBP4* variants were related to total number born (TNB) and number born alive (NBA) in 1st, 2nd and 5th parities, *FSHB* to TNB and NBA in first two parities, however *EGF* with both traits in all parities (P≤0.05 or P≤0.01). In case of *RBP4* gene, heterozygous genotype (*AB*) was favorable for analyzed traits, however in case of *FSHB* and *EGF* homozygous genotype, *AA* and *BB* respectively. Obtained results indicate that polymorphism in three analyzed genes is associated with important reproductive traits of pigs kept in Poland.

Keywords: pigs; *RBP4*; *FSHB*; *EGF*; polymorphism; reproduction traits

1. Introduction

The 21st century appears to be the age of genetics [1]. A lot of studies have been published in the past two decades reporting various genes and their associations with production traits in farm animals [2-4]. Around 36% of the global human population consumes pork meat [5], the cost of its production depending largely on the number of piglets per litter [6-7]. 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 [8].

Porcine litter size ranges from 2 to 20 piglets, which makes an average of 10 piglets per litter [9]. 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 [10-12]. 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

 doi:10.20944/preprints202306.1526.v1

genes have been pinpointed to date, using genome-wide genotyping (GWG) and genome-wide association studies (GWAS) [13-14].

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 [15-20]

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

2. Material and Methods

2.1. 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 Danish Large White (Yorkshire) (DLW) sows from a farm located in the West Pomeranian (zachodniopomorskie) province, Poland. All the animals were kept in identical environmental conditions. 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.

2.2. 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_3EDTA). DNA isolation was done with the salting-out method using MasterPureTM kit (Epicentre Biotechnologies®, USA).

Polymorphic variants in the genes under study were detected by PCR-RFLP and PCR-ID. The examined gene fragments were amplified using FastGene® Taq ReadyMix (2X) 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 the selected DNA fragments are shown in Table 1.

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

Gene	Primer sequences	T_a	RE	Source
RBP4	F 5′-GAGCAAGATGGAATGGGTT-3′ R 5′-CTCGGTGTCTGTAAAGGTG-3′	56	*Msp*I	[21]
FSHB	F 5′-AGTTCTGAAATGATTTTTCGGG-3′ R 5′-TTTGCCATTGACTGTCTTAAAGG-3′	58	*Hae*III	[22]
EGF	F 5′-GAAACAATTCCCGTGTTCTCTA-3′ R 5′-TCACTTCCACACCTGTAACATCT 3′	58	indel	[23]

Ta – primer annealing temperature; RE – restriction enzyme, indel - insertion/deletion polymorphism

PCRs were performed in a TGradient thermal cycler (Biometra), and the amplification products, assessed for efficiency and specificity, were digested with restriction enzymes (10 µl) at optimum temperatures following the manufacturer's instructions (MBI Fermentas). Digestion products were separated 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) and archived.

 doi:10.20944/preprints202306.1526.v1

2.3. Statistical analysis

The study populations were tested for genetic equilibrium with the Hardy-Weinberg test using the Gene-Calc tool [24]. 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); μ – 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.

3. 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			χ^2 (p-value)
			AA	*AB*	*BB*	
RBP4	**PLW x PLR**	**288**	0.27 (n=77)	0.35 (n=102)	0.38 (n=109)	13.458 (0.001)
	DLW	195	0.34 (n=66)	0.44 (n=86)	0.22 (n=43)	
FSHB	PLW x PLR	288	0.03 (n=8)	0.34 (n=97)	0.63 (n=183)	12.633 (0.002)
	DLW	195	0.09 (n=18)	0.39 (n=76)	0.52 (n=101)	
EGF	PLW x PLR	288	0.07 (n=20)	0.47 (n=135)	0.46 (n=133)	90.826 (0.000)
	DLW	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 DLW population it was genotype BB (0.89). The differences in genotype frequencies between the two populations were statistically significant (χ^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 DLW populations (χ^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 (χ^2= 13.458).

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

Gene	Genotype	PLW x PLR[1]		DLW[2]		HWE	Allele frequencies	
		Obs.	Exp.	Obs.	Exp.	p-value	*A*	*B*
RBP4	***AA***	77	88.89	66	60.93	0.001[1] 0.338[2]	0.444[1] 0.559[2]	0.556[1] 0.441[2]
	AB	102	142.22	86	96.14			
	BB	109	56.89	43	37.93			
FSHB	*AA*	8	11.08	18	16.08	0.515[1] 0.798[2]	0.196[1] 0.287[2]	0.804[1] 0.713[2]
	AB	97	90.83	76	79.84			
	BB	183	186.08	101	99.08			
EGF	*AA*	20	26.58	4	0.87	0.186[1] 0.001[2]	0.304[1] 0.067[2]	0.696[1] 0.933[2]
	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 DLW in the case of the RBP4 gene) (Table 3). In two cases, genetic equilibrium was found to be disturbed (the EGF gene in DLW sows and the RBP4 gene in PLW x PLR sows).

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

Litter	Trait	*RBP4* genotypes					
		AA		*AB*		*BB*	
		LSM	SD	LSM	SD	LSM	SD
1	TNB	11.57 ^{A}	2.46	12.37 ^{Ba}	1.95	11.87 ^{b}	2.58
	NBA	11.18 ^{A}	2.33	11.99 ^{Ba}	2.06	11.62 ^{b}	2.45
2	TNB	12.28 ^{A}	2.95	13.25 ^{Ba}	2.45	12.41 ^{b}	2.42
	NBA	12.02 ^{A}	2.62	12.85 ^{Ba}	2.10	12.19 ^{b}	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 ^{A}	2.23	12.51	1.89	11.88 ^{B}	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^{A}	2.70	12.80^{B}	2.28	12.17^{A}	2.43
	NBA	12.10^{A}	2.51	12.47^{B}	2.14	11.98^{A}	2.26

LSM – least squares mean, standard deviation (SD); values marked with different letters indicate statistically significant differences: P≤0.05 (^{abc}) or P≤0.01 (^{ABC})

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

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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).

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≤0.01 or P≤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 5. Effect of *FSHB* gene polymorphism on litter size in the studied sows.

Litter	Trait	*FSHB* genotypes					
		AA		*AB*		*BB*	
		LSM	SD	LSM	SD	LSM	SD
1	TNB	13.21 ^{A}	2.57	12.08	2.57	11.60 ^{B}	2.20
	NBA	13.00 ^{A}	2.40	11.76	2.19	11.32 ^{B}	2.23
2	TNB	14.56 ^{A}	3.01	13.15 ^{a}	2.52	12.08 ^{Bb}	2.44
	NBA	14.19 ^{Aca}	2.59	12.82 ^{Bd}	2.12	11.90 ^{b}	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 ^{A}	2.59	12.6 ^{AB}	2.41	12.1 ^{B}	2.34
	NBA	13.46 ^{A}	2.40	12.33 ^{AB}	2.26	11.93 ^{B}	2.24

LSM – least squares mean, standard deviation (SD); values marked with different letters indicate statistically significant differences: P≤0.05 (^{abc}) or P≤0.01 (^{ABC})

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≤0.01 or P≤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	*EGF* genotypes					
		AA		*AB*		*BB*	
		LSM	SD	LSM	SD	LSM	SD
1	TNB	11.04 ^{A}	1.68	11.52 ^{a}	2.20	12.50 ^{Bb}	2.45
	NBA	10.88	1.57	11.35 ^{A}	2.12	12.00 ^{B}	2.43
2	TNB	11.76 ^{A}	1.76	11.93 ^{a}	2.15	13.30 ^{Bb}	2.76
	NBA	11.76	1.76	11.81 ^{A}	1.97	12.82 ^{B}	2.46
3	TNB	12.00 ^{A}	1.95	12.01 ^{a}	2.18	13.70 ^{Bb}	2.73
	NBA	12.00 ^{A}	1.95	11.81 ^{a}	2.27	13.27 ^{Bb}	2.21

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4	TNB	11.11 ^{A}	1.97	12.04 ^{a}	2.21	13.26 ^{Bb}	2.84
	NBA	11.11 ^{A}	1.97	12.03 ^{a}	2.18	12.71 ^{Bb}	2.76
5	TNB	11.06 ^{A}	2.04	12.11 ^{a}	2.19	12.76 ^{Bb}	2.01
	NBA	11.06 ^{A}	2.04	12.09	2.11	12.47 ^{B}	1.96
6	TNB	11.00 ^{A}	1.64	11.75 ^{a}	2.02	12.85 ^{Bb}	2.20
	NBA	11.00 ^{A}	1.64	11.74 ^{a}	2.01	12.55 ^{Bb}	2.02
Total	TNB	11.34 ^{Aa}	1.84	11.88 ^{Ab}	2.15	13.07 ^{B}	2.62
	NBA	11.31^{A}	1.83	11.78 ^{A}	2.10	12.63 ^{B}	2.47

LSM – least squares mean, standard deviation (SD); values marked with different letters indicate statistically significant differences: P≤0.05 (^{abc}) or P≤0.01 (^{ABC})

4. 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) [25]. 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 [26]. In the following years, the effects of single nucleotide polymorphisms (SNPs) in a number of other genes were investigated, often making use of quantitative trait loci (QTLs) mapped to various porcine chromosomes [19, 27-29]. 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.

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 [30-31]. One of the retinol-binding proteins is encoded by the *RBP4* gene, which has been mapped to chromosome 14 [32] 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 [21]. 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 [33].

The present study 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 [34], with allele A being associated with a slightly bigger number of piglets per litter [21]. A positive effect of the *RBP4* genotype on litter size in Landrace pigs was reported by Blowe et al. [35], whose results were later confirmed by other authors [36-37]. A favourable association has also been found between genotype *AA* and litter size in Tibetan [38] and Shandong [39] breeds. Other researchers have shown that an increased litter size in Large White x Landrace crossbred pigs is associated with the *RBP4-Msp*I *AA* genotype [40-41].

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 [9, 42-43]. No effect of this genotype on litter size has

 doi:10.20944/preprints202306.1526.v1

been reported in German Large White [37] and Polish Large White, Landrace and commercial line 990 pigs [44] as well as in Chinese [45] and local Indian breeds [46].

However, contrary to the reported lack of association between the *RBP4* SNP and litter size in German Large White [37], Dall'Olio et al. [47] identified the *Msp*I 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 [48]. Identical associations were also observed in crossbred Large White x Landrace [49] and Landrace sows [50]. Moreover, an analysis of the effect of the *RBP4-Msp*I genotype in interaction with the *ESR-Pvu*II genotype revealed a positive combined effect of these two genes on litter size [51].

Associations between the *RBP4* genotype and reproductive traits consistent with those observed in the present study were reported by Laliotis et al. [52], 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.

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 [53]. In humans, *FSHB* gene polymorphisms (SNP, rs11031006) are associated with the occurrence of polycystic ovary syndrome (PCOS) [54-55] and influence ovarian response to hormonal stimulation [56]. In pigs, FSH level can be regulated by miRNA (miR-361-3p), which can suppress the expression of the *FSHB* gene [57].

The porcine *FSHB* gene was mapped to chromosome 2, and the polymorphism detected in it (RFLP-*Hae*III) was proposed by Rohrer et al. [22] 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 [58]. 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 [9].

The current study 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. [59] 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. [60] 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. [45], whereas Wang et al. [43] 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. [61], 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 trains of sows [62-64].

The last candidate gene whose polymorphism was analyzed in the present study was the *EGF* gene coding for the epidermal growth factor (EGF), which plays a crucial role in the reproductive process [65]. *EGF* is expressed in porcine oviducts [66] and plays a role in oocyte maturation [67] and ovulation [68]. It is also present in the amniotic fluid [69]. The *EGF* gene is expressed in the pig embryo [70], its endometrial expression increasing with the advance of gestation [71].

The porcine *EGF* gene was mapped to chromosome 8 [23, 72], 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 [23], which was later analyzed for its associations with reproductive traits in sows. Variation in the porcine *EGF* gene was examined by Linville et al. [9], but due to the observed genotype distribution (no individuals with genotype *AA*) they did not perform an association analysis with reproductive traits.

The current study results show 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. [44] 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. [64], 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. [73]. 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. [74] 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 [46] 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 [75].

5. 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 Danish Large White sows. It appears, however that the decision whether to continue association analyses of genetic markers (mainly SNPs) should be made predominantly on the basis of recently published GWAS results in this field.

Author Contributions: Conceptualization, M.K., A.T.; methodology, M.K.; validation, K. W-M.; formal analysis, M.K.; investigation, M.K.; data curation, K. W-M.; writing - original draft preparation, A.D., D.P., O.Ch.; writing - review and editing, A.D., D.P.; supervision - A.T., D.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been supported by own funds of Department of Genetics, West Pomeranian University of Technology in Szczecin

Institutional Review Board Statement: The bioethic comitee approval of this study is not necessary because experiment was carried out on biological material. This material derived from breeding farms and were collected during routine veterinary check-up

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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