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Association between *ESR1* and *RBP4* genes and litter size traits in a hyperprolific line of Landrace × Large White cross sows

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Abstract: This study was aimed at analysing single-nucleotide polymorphisms in the oestrogen receptor 1 (*ESR1*) and retinol-binding protein 4 (*RBP4*) genes in a hyperprolific line of Landrace × Large White (Topigs 20) cross sows (n = 101). The following litter size traits were analysed: total number born, number of born alive and number of weaned piglets. *ESR1* and *RBP4* genotypes determined on the basis of single-nucleotide polymorphisms were analysed using the least square method with the GLM procedure in SAS with eight effects. The REG procedure was used to calculate the effects of the additive and dominance components. The second parity sows with *ESR1* BB genotype had a significantly higher ($P < 0.05$) number of weaned piglets compared to AB, with a tendency towards difference ($P < 0.1$) between homozygotes for number of born alive and number of weaned piglets. In the case of the *RBP4* gene, the first parity sows of the AA genotype had a significantly higher total number born ($P < 0.05$) compared with the BB genotype, with a tendency towards difference ($P < 0.1$) between AA and heterozygotes for total number born, and homozygotes for number of born alive. The BB genotype showed a tendency for higher number of weaned piglets ($P < 0.1$) as compared with the AA genotype in the third parity sows for the *RBP4* gene. In all parities, significant effects ($P < 0.05$) of parity were recorded for total number born, number of born alive and number of weaned piglets, season of farrowing for total number born, and the *ESR1* and *RBP4* interaction for number of born alive. In the first parity sows, significant effects ($P < 0.05$) on total number born were determined for gene interaction and gestation length, the latter also being recorded in the second parity sows. The additive (a) effect of single-nucleotide polymorphisms in *RBP4* was significant ($P < 0.05$) for total number born in all parities as well as in the first parity sows, and dominance effect (d) ($P < 0.05$) of single-nucleotide polymorphisms in *ESR1* for number of weaned piglets in the third parity sows. The obtained results regarding the investigated genes could help to provide a better understanding of the effect of single-nucleotide polymorphisms on litter size and thus promote genetic progress in pig reproduction management.

Keywords: candidate gene; pigs; reproductive trait; SNPs

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New genetic trends in pig breeding over the last twenty years have employed different combinations of crosses resulting in hyperprolific sow lines (Bergfelder-Druing et al. 2015), i.e. in sows with an increased number of pigs per litter (Krupa and Wolf 2013). As fertility is a polygenic trait with low heritability and is by nature sex-limited, genetic progress in litter size (LS) traits is determined by numerous factors, such as specific genetic lines of sows, selection procedure and environmental factors (Rothschild et al. 2000).

Identification of candidate genes that influence the reproductive characteristics of sows opens new possibilities for their use in procedures related to selection and productivity improvement (Rempel et al. 2010). However, different studies have reported variable data on the presence and prevalence of particular gene variants along with frequently contradictory results related to their effect on fertility and productivity of various pig breeds and their crosses in intensive production. The current state-of-the-art in candidate genes and their specific single nucleotide polymorphisms (SNPs) suggests that they may have major effects on pig biological function by encoding important hormones and receptors (Distl 2007), including LS polygenic traits (Wang et al. 2006). The first important research on the effect of candidate genes and their SNPs on the indicators of LS traits in pigs referred to the group of nucleotide and retinoid receptors (Munoz et al. 2010), of which the oestrogen receptor gene (ESRs) and retinol-binding proteins (RBPs) are most widely investigated, especially in the Western and European breeds of pigs such as Large White (LW) and Landrace (L) as typical pure breeds used in commercial production and their cross-bred lines (LW × L or L × LW) (Bergfelder-Druing et al. 2015). Yet, previous studies have shown that significant differences exist in LS traits and have described heterogeneity in gene frequencies of the polymorphic variants of the *ESR1* and *RBP4* genes between pure pig breeds such as LW and L and their cross-breeds, and hyperprolific sow lines (Alfonso 2005; Kapelanski et al. 2013).

In this study we aimed to: (1) assess the presence of SNPs within exon 3 (c.669T>C) in the *ESR1* gene and the presence of SNPs in the intronic region (c.249-63G>C) of the *RBP4* gene; (2) assess the presence of particular alleles according to genotype frequencies in hyperprolific sow lines crosses of L and LW (Topigs 20 line) and analyse LS traits according to genotype within the population relative to order of parity (OP); (3) estimate the sources of variability effects for LS traits included in the model of calculation with single gene analyses; (4) estimate genetic variation according to the genotype interaction with additive (*a*) and dominance (*d*) effects.

MATERIAL AND METHODS

Animals, traits and farm management. Genotyping was performed in 101 sows of the Topigs 20 line with 461 litters from a commercial farm. Reproductive data included OP, date of sow birth with dates of farrowing, age at first farrowing, length of service period, gestation length, breed of the sire (S), lactation length and analysed traits as follows: total number born (TNB), number of born alive (NBA) and number of weaned (NW) piglets. According to the farm management, the sows were transferred to the farrowing unit with a controlled microclimate five to seven days before the expected farrowing. Farrowing was supervised during working hours, between 06.00 h and 20.00 h.

Blood sampling and laboratory methods. Blood sampling was performed in the service unit in accordance with the Decree on the Measures of Animal Protection from Infectious and Parasitic Diseases, issued by the Ministry of Agriculture, Fisheries and Rural Development (Official Gazette 135/2006), and DNA isolation was performed as described previously by Mencik et al. (2016). Amplification of *ESR1* gene fragments was performed using PCR on a Mastercycler Personal 5332 (Eppendorf AG, Hamburg, Germany) as described by Short et al. (1997). The procedure of *ESR1* gene amplification and the final composition of the reaction mixture for determination of SNPs were according to the PCR-RFLP (restriction fragment length polymorphism) digestion procedure method using the restriction enzyme *Pvu*II. The electrophoresis conditions and identification of the gene fragments according to the obtained genotype were performed as described by Mencik et al. (2016).

PCR amplification of *RBP4* gene fragments was performed according to Rothschild et al. (2000) in reactions with final volumes of 25 µl. The final composition of the reaction mixture for *RBP4* amplification was 30–50 ng/µl genomic DNA 1× PCR buffer, 1.5 mM $MgCl_2$, 2 mM of each dNTP, 4 µM of

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each primer and 1 IU of *Taq* polymerase (Promega, Madison, USA). Initial *RBP4* denaturation was performed at a temperature of 94°C for 4 min, followed by 30 cycles of primer coupling at 55°C, elongation for 1 min at 72°C and final extension at 72°C for 8 min with final cooling at 4°C. The amplified PCR products were digested with the restriction enzyme *Msp*I. Ten microlitres of each amplified PCR product were digested with 5 IU *Msp*I (10 IU/µl), 0.2 µl of bovine serum albumin (10 mg/ml), 2 µl of buffer for restriction enzyme (10×), with the addition of ultrapure water for molecular analyses up to a final volume of 20 µl (Promega, Madison, USA). The mixture was incubated at a temperature of 37°C for 4 hours. Based on the specific site of the *Msp*I restriction enzyme cleavage (5'C/CGG'3) fragment lengths were determined at 2.5% (80 V, 60 minutes) and the sow *RBP4* genotype was identified.

Statistical analysis. Deviation of the observed frequencies from the theoretically expected frequencies, i.e. harmonization with the principles of the Hardy-Weinberg law on the gene and genotype equilibrium in the population was tested by use of the χ^2-test according to the method described by Rodriguez et al. (2009). Variance analysis was performed using the general linear model procedure based on least squares method in the Statistical Analysis Software v. 9.4 (SAS Institute Inc., 2013). Least squares means (LSM ± SE) of analysed traits were computed for each of the significant effects in the model with the following linear equation:

$$Y_{ijklmnop} = \mu + OP_i + ESR1_j + RBP4_k + (ESR1 \times RBP4)_{jk} + SF_l + SP_m + S_n + GL_o + LL_p + e_{ijklmnop} \quad (1)$$

where:

Y – litter size traits (TNB, NBA, NW);

µ – intercept;

OP_i – effect of the i^{th} order of parity (i = 1, 2, 3 and all from 1st to 7th), the effect of *OP* was excluded from the calculation model in the single parity analysis between the 1st and the 3rd parity data;

$ESR1_j$ – effect of the j^{th} genotype (j = AA, AB, BB);

$RBP4_k$ – effect of the k^{th} genotype (k = AA, AB, BB);

$(ESR1 \times RBP4)_{jk}$ – interaction between jk^{th} *ESR1* and *RBP4* gene;

SF_l – effect of the l^{th} season of farrowing (l = 1, 2, 3, 4); four farrowing seasons were defined: from March to May (spring), from June to August (summer), from September to November (autumn) and from December to February as the winter period;

SP_m – effect of m^{th} service period (m = 1, 2, 3) divided into three levels of postweaning: first (≤ 32 days), second (33–54 days) and third (≥ 55 days); the effect of SP_m was excluded only from the first parity data analysis;

S_n – effect of the sire breed used for sow fertilisation (n = 1, 2, 3);

GL_o – effect of the o^{th} gestation length (o = 1, 2, 3) divided into three levels: first (≤ 112 days), second (113–115 days) and third (≥ 116 days);

LL_p – effect of the p^{th} lactation length (p = 1, 2, 3) divided in three levels, up to 27, 28 and 29 days, respectively;

$e_{ijklmnop}$ – residual error, order of parity as an effect was not included in the model for single parity analysis.

The regression procedure was used to calculate additive (a) and dominance (d) effects for SNPs of the investigated candidate genes. Effects of the (a) components were estimated as –1, 0 and 1 for AA, AB and BB genotypes and dominance (d) as 1 for each homozygote genotype (AA and BB) and –1 for heterozygote (AB) genotype, respectively. Values lower than 0.05 were considered significant, while value with probabilities between 0.051 and 0.10 tended to be significantly different.

RESULTS

Frequencies of alleles and their genotypes in the analysed population of sows for the presence of SNPs in *ESR1* and *RBP4* are shown in Table 1, revealing that deviation from the Hardy-Weinberg equilibrium was significant for each polymorphic marker ($P < 0.05$). The effects of genotype on LS traits for *ESR1* and *RBP4* genes are shown in Table 2. Pooled farrowing analysis showed that TNB and NBA tended to be higher ($P < 0.1$) in the BB-*ESR1* genotype compared to AA-*ESR1*, whereas no significant differences between genotypes were observed in the case of the *RBP4* gene. There were no significant genotype differences in the *ESR1*

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Table 1. Allele and genotype frequencies of *ESR1-Pvu*II and *RBP4-Msp*I polymorphisms in the population of the Topigs 20 line of sows

Candidate gene	Genotype frequencies (*n*/*f*)			Allele frequencies		χ^2	*P*-value
	AA	AB	BB	allele A	allele B		
ESR1	33/0.3267	59/0.5842	9/0.0891	0.619	0.381	5.73	< 0.05
RBP4	29/0.2871	32/0.3168	40/0.3961	0.445	0.555	13	< 0.05

ESR1 = oestrogen receptor 1 gene; *f* = genotype frequency; *n* = number of sows; *RBP4* = retinol-binding protein 4 gene

gene for the first parity sows either, whereas the *RBP4* gene sows with AA genotype had significantly higher TNB ($P < 0.05$) than those with the BB genotype, with a tendency towards difference between *RBP4* AA and AB genotypes ($P < 0.1$). In the first parity sows, a tendency towards difference between homozygotes ($P < 0.1$) was calculated for NBA. Second parity sows with the BB allele of the *ESR1* gene tended ($P < 0.1$) to have higher NBA and NW compared to AA, while the BB genotype had

Table 2. Litter size traits (least squares means ± SE) in different genotypes of Topigs 20 sows according to order of parity

Order of parity	Candidate gene	Genotype	Litter size traits		
			TNB	NBA	NW
All parities (*n* = 461)	*ESR1*	AA (*n* = 154)	13.76 ± 0.57^{b}	12.73 ± 0.52^{b}	11.19 ± 0.25
		AB (*n* = 269)	14.04 ± 0.54	12.91 ± 0.49	11.20 ± 0.23
		BB (*n* = 38)	15.09 ± 0.69^{b}	13.90 ± 0.63^{b}	11.39 ± 0.30
	RBP4	AA (*n* = 145)	14.46 ± 0.52	13.06 ± 0.47	11.29 ± 0.23
		AB (*n* = 138)	14.33 ± 0.57	13.40 ± 0.52	11.22 ± 0.25
		BB (*n* = 178)	14.10 ± 0.58	13.08 ± 0.53	11.28 ± 0.25
First parity (*n* = 101)	*ESR1*	AA (*n* = 33)	13.60 ± 1.16	12.13 ± 1.15	12.14 ± 0.64
		AB (*n* = 59)	13.04 ± 1.03	11.54 ± 1.02	11.87 ± 0.56
		BB (*n* = 9)	13.89 ± 1.33	12.10 ± 1.32	11.67 ± 0.73
	RBP4	AA (*n* = 29)	15.07 ± 1.07^{a,b}	13.82 ± 1.06^{b}	12.10 ± 0.59
		AB (*n* = 32)	13.03 ± 1.19^{b}	12.95 ± 1.18	11.43 ± 0.65
		BB (*n* = 40)	12.42 ± 1.26^{a}	12.00 ± 1.25^{b}	12.15 ± 0.69
Second parity (*n* = 97)	*ESR1*	AA (*n* = 32)	11.57 ± 1.47	11.09 ± 1.31^{b}	11.27 ± 0.52^{b}
		AB (*n* = 56)	12.79 ± 1.27	12.29 ± 1.13	11.25 ± 0.44^{a}
		BB (*n* = 9)	15.19 ± 2.02	14.37 ± 1.60^{b}	12.81 ± 0.71^{a,b}
	RBP4	AA (*n* = 28)	12.55 ± 1.42	11.74 ± 1.26	11.77 ± 0.50
		AB (*n* = 30)	13.35 ± 1.38	12.98 ± 1.23	11.90 ± 0.48
		BB (*n* = 39)	13.64 ± 1.55	13.03 ± 1.39	11.67 ± 0.55
Third parity (*n* = 91)	*ESR1*	AA (*n* = 30)	14.70 ± 1.32	13.45 ± 1.25	11.77 ± 0.53
		AB (*n* = 52)	14.59 ± 1.30	13.34 ± 1.23	12.09 ± 0.53
		BB (*n* = 9)	14.04 ± 1.40	13.32 ± 1.33	11.27 ± 0.57
	RBP4	AA (*n* = 28)	14.07 ± 1.13	13.29 ± 1.07	11.33 ± 0.46^{b}
		AB (*n* = 28)	14.18 ± 1.20	12.96 ± 1.14	11.78 ± 0.49
		BB (*n* = 35)	15.09 ± 1.39	13.87 ± 1.32	12.03 ± 0.56^{b}

ESR1 = oestrogen receptor 1 gene; NBA = number of piglets born alive; NW = number of weaned piglets; *RBP4* = retinol-binding protein 4 gene; TNB = total number of piglets born

^{a,b}Values in the same column with the same letters differ significantly between particular genotypes of the respective candidate gene in the same parity at the level $^{a,a}P < 0.05$; with tendency $^{b,b}P < 0.1$

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significantly higher NW ($P < 0.05$) as compared to the AB genotype. There were no significant genotype differences in the second parity sows for the *RBP4* gene, as well as in the third parity sows for the *ESR1* gene in any of the litter size traits examined. There was a tendency ($P < 0.1$) of higher NW in third parity sows of *RBP4* gene BB genotype in comparison to the AA genotype. The proportion of phenotypic variation (R^2) explained by the fixed effects (Table 3) was the highest in the third parity for analysed traits (from 29.0% to 47.6%). A lower proportion was obtained for analysed traits in the joint analyses (9.2% to 17.1%). The pooled farrowing analysis revealed a significant effect of OP and SF ($P < 0.05$) on TNB, with a tendency ($P < 0.1$) for the genes to interact (*ESR1* × *RBP4*). Order of parity and interaction of analysed genes had significant effect ($P < 0.05$) on NBA in all parities analysed. In addition, a significant effect ($P < 0.05$) of the OP was recorded on the NW. In the first parity sows, gene interaction and gestation length had a significant effect ($P < 0.05$) on TNB, with a tendency ($P < 0.1$) towards significant interaction for *RBP4* and SF. In the same parity sows, NBA tended ($P < 0.1$) to be influenced by gene interaction. In the second parity sows, gestation length had a significant effect ($P < 0.05$) on TNB, with a tendency ($P < 0.1$) for NBA. In sows at the same parity, NW tended ($P < 0.1$) to be influenced by *ESR1* and lactation length. No significant effects were observed in the third parity sows.

Assessment of the *a* and *d* effects (Table 4) in sows of all parities revealed a significant effect of *a-RBP4* ($P < 0.05$) on TNB, with *d-RBP4* tendency ($P < 0.1$). A significant effect ($P < 0.05$) of *a-RBP4* on TNB was recorded in the first parity sows, and a tendency ($P < 0.1$) of *a-ESR1* effect in the second parity sows. In the third parity sows, there was a significant ($P < 0.05$) effect of *d-ESR1* on NW.

DISCUSSION

Considering the pivotal role of ESRs and RBPs in mammalian reproduction, i.e. in the regulation of hormonal activities, growth and development of organs relevant for reproduction, as well as of the embryo and foetus, it is quite understandable why SNPs of the *ESR1* gene and *RBP4* gene have been extensively investigated in many pig breeds with special reference to LS (Dever et al. 2010; Bondesson et al. 2015). Previous studies in various populations of Western breeds of pigs such as LW, L and their cross-breeds have reported different al-

Table 3. Coefficient of determination (R^2) of the models and significance (*P*-values) of the effects fitted in the model by order of parity in Topigs 20 sows

		Traits analysed per parity											
		all parities (n = 461)			first parity (n = 101)			second parity (n = 97)			third parity (n = 91)		
		TNB	NBA	NW	TNB	NBA	NW	TNB	NBA	NW	TNB	NBA	NW
R^2 (%)		17.1	14.3	9.2	28.6	22.6	16.3	26.2	25.7	30.0	29.0	47.6	33.2
Effect[#]	OP	*	*	*	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	ESR1	0.22	0.26	0.83	0.60	0.64	0.73	0.23	0.20	**	0.91	0.98	0.22
	RBP4	0.77	0.72	0.95	**	0.29	0.44	0.72	0.50	0.89	0.54	0.62	0.20
	ESR1 × *RBP4*	**	*	0.68	*	**	0.74	0.74	0.51	0.36	0.48	0.88	0.25
	SF	*	0.16	0.11	**	0.51	0.97	0.38	0.39	0.33	0.26	0.48	0.69
	S	0.75	0.79	0.18	0.30	0.43	0.87	0.84	0.88	0.32	0.79	0.79	0.90
	SP	0.31	0.22	0.14	0.39	0.76	0.83	0.99	0.90	0.90	0.57	0.97	0.38
	LL	0.61	0.52	0.23	0.49	0.58	0.71	0.71	0.85	**	0.17	0.54	0.42
	GL	0.16	0.49	0.88	*	0.27	0.37	*	**	0.49	0.63	0.72	0.38

ESR1 = oestrogen receptor 1 gene; *ESR1* × *RBP4* = interaction of oestrogen receptor 1 gene and retinol-binding protein 4 gene; GL = gestation length; LL = lactation length; n.i. = not included in the model of calculation; NBA = number of piglets born alive; NW = number of weaned piglets; OP = order of parity; *RBP4* = retinol-binding protein 4 gene; S = sire; SF = season of farrowing; SP = service period; TNB = total number of piglets born

*$P < 0.05$; **$P < 0.1$

[#]Estimated value of the variability variance included in the model of calculation

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Table 4. Estimation of additive (*a*) and dominance (*d*) effects with regression coefficient (± SEM) of genotype on litter size traits according to order of parity (OP) for *ESR1* and *RBP4*

OP	Effect	TNB	NBA	NW
All parities	*a-ESR1*	0.30 ± 0.25	0.20 ± 0.23	0.01 ± 0.10
	d-ESR1	0.09 ± 0.17	0.07 ± 0.16	–0.02 ± 0.07
	a-RBP4	–0.36 ± 0.18[a]	–0.22 ± 0.16	0.03 ± 0.07
	d-RBP4	0.26 ± 0.16[b]	0.11 ± 0.15	0.04 ± 0.07
First parity	*a-ESR1*	–0.35 ± 0.51	–0.49 ± 0.46	0.05 ± 0.06
	d-ESR1	0.29 ± 0.35	0.27 ± 0.31	0.02 ± 0.04
	a-RBP4	–0.80 ± 0.36[a]	–0.51 ± 0.33	0.09 ± 0.08
	d-RBP4	0.02 ± 0.032	–0.05 ± 0.29	–0.03 ± 0.02
Second parity	*a-ESR1*	1.02 ± 0.60[b]	0.72 ± 0.53	0.17 ± 0.23
	d-ESR1	0.14 ± 0.41	–0.03 ± 0.37	0.07 ± 0.15
	a-RBP4	0.28 ± 0.44	0.34 ± 0.39	0.01 ± 0.16
	d-RBP4	0.07 ± 0.40	–0.11 ± 0.35	0.04 ± 0.15
Third parity	*a-ESR1*	0.01 ± 0.44	0.29 ± 0.39	–0.03 ± 0.18
	d-ESR1	–0.15 ± 0.30	–0.09 ± 0.27	–0.26 ± 0.12[a]
	a-RBP4	0.11 ± 0.32	–0.03 ± 0.29	0.01 ± 0.02
	d-RBP4	0.21 ± 0.29	0.24 ± 0.26	–0.01 ± 0.02

ESR1 = oestrogen receptor 1 gene; NBA = number of piglets born alive; NW = number of weaned piglets; *RBP4* = retinol-binding protein 4 gene; TNB = total number of piglets born [a,b]Value of the regression coefficients differ significantly from 0, [a]differ significantly at the level of $P < 0.05$, [b]with tendency $P < 0.1$

lele and genotype frequencies for LS traits defined by SNPs in the *ESR1* and *RBP4* candidate genes.

Deviations in allele and genotype frequencies for the *ESR1* gene were significant in genotyped populations of pigs of L (Kmiec et al. 2002; Wang et al. 2006) and LW (Chvojkova and Hraska 2008) breeds, which is consistent with the results of this study. A high frequency of the B allele was recorded in a Chinese population of L breed pigs (Wu et al. 2006) and a European population of LW breed (Humpolicek et al. 2009). Liu et al. (2002) reported that a high presence of the B allele in the Chinese populations of LW and L pigs may be due to the influence of different breeding schemes with primary selection aimed at improving fertility and increasing piglet postnatal survival rate per sow. The predominant effect of the B allele on sow fertility has been recorded in a PIC synthetic line with LW origin (Short et al. 1997) and in different populations of LW pigs (Horogh et al. 2005; Wang et al. 2006; Vasconcellos Goncalves et al. 2008; Humpolicek et al. 2009). In the present study, a lower frequency of the B allele of the *ESR1* gene was recorded, as also previously reported by Short et al. (1997) in a population of PIC LW synthetic line pigs and by Chvojkova and Hraska (2008) and Dall' Olio et al. (2011) in the LW breed. An even lower frequency of the B-*ESR1* gene has been reported in a population of LW pigs (Matousek et al. 2003; Aparecida Santana et al. 2006; Omelka et al. 2008; Humpolicek et al. 2009; Kernerova et al. 2009). The frequencies of the B allele of the *ESR1* gene in a European population of L pigs were considerably lower as compared with LW sows (Noguera et al. 2003) and their crosses (Drogemuller et al. 2001; Kmiec et al. 2002; Wang et al. 2006).

The frequencies of the BB-*ESR1* genotype recorded in this study were consistent with previous literature which reported the proportion of BB homozygotes to be lower in comparison to the other two genotypes. This could be due to the higher frequency of the A allele of the *ESR1* gene in the European population of LW pigs, which can be ascribed to the effect of the highly prolific selected lines of sires: European populations of highly prolific sow lines have been coupled with sires of LW and L breeds, which are often present in crosses intended for the production of maternal lines (Clop et al. 2000).

The results obtained for TNB and NBA in joint parity analysis were consistent with the results of the higher fertility of BB genotype sows in pig populations of LW origin (Short et al. 1997; Matousek et al. 2003). The sows of LW (Goliasova and Wolf 2004; Chvojkova and Hraska 2008) and L (Noguera et al. 2003) breeds harbouring the B allele exhibited better fertility, which may have been attributed to the effect of selection procedures undertaken during the study period. According to the results reported by Isler et al. (2002), sows with the *ESR1* B allele were more prolific at third parity, which may have been influenced by genetic progress and selection procedures targeting reproductive traits/low hereditary traits. A favourable effect of the A allele was observed in a Brazilian pig population where sows with the presence of the A allele showed better fertility results as compared with BB homozygotes (Aparecida Santana et al. 2006).

Sows with the homozygous BB allele of *ESR1* gene in our study showed the highest value for TNB and NBA, similar to the findings in a population

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of LW pigs (Wang et al. 2006). Similar genotype frequencies defined by SNPs detected in the *ESR1* and *RBP4* genes recorded in our study have been reported by Hernandez Lopez et al. (2006), whereas lower genic frequencies for the *RBP4* gene in pure LW and L breeds and their crosses have been reported by Drogemuller et al. (2001), Korwin-Kossakowska et al. (2005), Wang et al. (2006), Omelka et al. (2008), Spotter et al. (2009), Munoz et al. (2010) and Terman et al. (2011). Previous studies suggested that the A allele of *RBP4* gene was associated with better LS traits (Rothschild et al. 2000). A favourable effect of the A allele of the *RBP4* gene has been described in sows of PIC commercial lines of LW and L origin, a synthetic line consisting of Duroc and LW breeds (Rothschild et al. 2000) and a Brazilian population of different pig lines and their crosses (Hernandez Lopez et al. 2006). The variable frequency of *RBP4* genotypes in different pig lines can be a consequence of mutations, substitutions or the replacement of single nucleotides within particular genetic groups. It is also considered that differences among particular populations of pure breeds and/or cross-breeds occur as a possible consequence of the low frequency of the B allele in certain pig breeds, in particular sires of the L breed (Rothschild et al. 2000).

Study results for joint parity analysed for the *RBP4* gene showed NBA to be higher in sows with the A allele, which is consistent with reproductive data in L breed populations (Vasconcellos Goncalves et al. 2008; Spotter et al. 2009; Munoz et al. 2010) and crosses of LW and L breeds (Marantidis et al. 2016). Terman et al. (2007; Terman et al. 2011) found primiparous sows of the LW breed with the BB-*RBP4* genotype to have the highest TNB and NBA values, with significant difference between homozygous genotypes. Wang et al. (2006) found TNB and NBA to be lowest in AA homozygotes of the *RBP4* gene in LW and L populations. Completely different results were recorded in the present study, with higher TNB and NBA in genotypes with the A allele, as also confirmed by Spotter et al. (2009), who found NBA to be highest in AA homozygotes of the L population. Third parity sows with the BB *RBP4* genotype showed a tendency for higher NW compared with the AA genotype. A similar finding has been reported by Terman et al. (2007; Terman et al. 2011). Differences in pig LS traits according to genotypes among various breeds can be attributed to the effect of selection procedures in different genetic lines of sows and boars and to the presence of so-called Asian alleles in European populations of LW and L pig breeds (Clop et al. 2000).

Statistical analysis with a linear equation and components of the predictors included in the calculation model indicated a significant effect of particular factors on the values observed. The effect of SF was significant for TNB in all parities, with a tendency towards significance in primiparous sows, which is consistent with the results reported by Wang et al. (2006). Also, in this study the LS traits were also influenced by OP, *ESR1* and *RBP4*, and their interaction, SF, lactation length and gestation length.

The additive and dominant components of variance of the SNPs tested can point to the favourable effect of polymorphism on TNB, NBA and NW; i.e. this method of calculation enables more exact assessment of production characteristics in the interactive gene analysis. According to Nagy et al. (2014), knowing the estimation of genetic effects, such as (*a*) and (*d*) components of variance, is important in animal breeding, especially in the prediction of genetic merit. Considering the additive component of *ESR1* gene variance in the present study, genotype superiority favoured the additive effect of the B allele for TNB, especially in the second parity sows. According to Vitezica et al. (2016), the higher values obtained on additive component assessment may be a consequence of the genotype itself, or of the interaction between the genotype and environmental factors. The additive component had a greater effect on TNB and NBA as compared with NW with both candidate genes, and the lower NW could be explained as a consequence of farm management during the period of lactation. The additive component of variance observed for the gene SNPs indicated a more reliable genotype assessment according to LS traits.

In conclusion, the presence of SNPs in the *ESR1* and *RBP4* genes pointed to higher frequency of the A allele in the *ESR1* gene and a relatively uniform frequency of *RBP4* gene genotypes in comparison with L and LW breeds and their crosses. A better effect on LS traits in joint parity was recorded with the B allele of *ESR1* and the A allele of *RBP4*. Individual analysis of the investigated SNPs indicated a relatively small proportion of genetic variability in LS traits, whereas the analysis of gene interaction pointed to their significant effect/ten-

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dency on TNB and NBA. The results obtained in the genotyped sows can contribute to the knowledge of the effect of SNPs on pig reproduction and thus promote genetic progress in pig production.

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