ESTRUS BEHAVIOUR AND REPRODUCTIVE TRAITS OF PULAWSKA GILTS ASSOCIATED WITH SELECTED GENE POLYMORPHISMS*

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Abstract

Searching for the associations between the gene polymorphism and the reproductive traits is essential in defining the genetic native breed specificity, which distinguishes them from the other breeds. The aim of our study was to determine the associations between mutations in the *PRL*, *PRLR*, *PTGS2*, *FUT1* genes and sexual and periparturient activity in native Pulawska gilts. The analysis included 72 animals which gave birth to the first litter. Evaluation of the productive value of gilts accounted for indicators of sexual and periparturient activity as well as reproductive traits. The biological material for molecular analyses was obtained from the hair roots of the gilts. The genotype was verified by PCR RFLP analysis. The primers and PCR conditions were determined on the basis of available literature data. Statistically significant differences ($P \leq 0.05$) were found at the *PRL* locus: gilts of *AA* genotypes (*Ins/Ins*) at the *PRL* locus were characterised by longest farrowing duration compared to gilts of *AB* genotype ($P \leq 0.05$). The analysis of *PRLR* gene showed that gilts of *TT* genotype revealed a tendency for later occurrence of estrus signs (first and second estrus) and for the markedly longest farrowings ($P \leq 0.05$). With regard to *PTGS2* and *FUT1* loci, no significant differences were found in the parameters of sexual and periparturient activity of the gilts. However, gilts of *FUT1 GG* genotype gave birth to and reared the largest first litters ($P \leq 0.05$). The results of the studies expand the knowledge about the genetic structure and productivity of Pulawska gilts.

Key words: native Pulawska gilts, sexual and periparturient activity, *FUT1*, *PRL*, *PRLR*, *PTGS2*

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In conservation breeds of pigs, often unsatisfactory fattening and slaughter traits are compensated for by good reproductive parameters and adaptive capabilities (Traspov et al., 2016; Mujibi et al., 2018). For these reasons, Polish pig breeders and producers use Pulawska pigs as a maternal component due to their high reproductive value (number of piglets born and reared) and biological value of litters, resulting among others from the high milk yield of the mothers (Stasiak et al., 2006; Babicz et al., 2017 b). Many observations have shown that Pulawska sows are characterised by particularly beneficial maternal characteristics such as maternal responsiveness and low incidence of aggression towards offspring, as well as showing strong signs of estrus, which has a positive influence on reproductive indicators (Babicz et al., 2014, 2017 a, b, 2019). Also stressed is the potential resistance of Pulawska pigs to variable environmental conditions and the relatively low reactivity to stress stimuli (Babicz et al., 2016). Breeding progress for productive traits of farm animals can be achieved through selection of animals with high genetic merit. Therefore, research has been done on genes with potential effects on traits important for breeding (Zhang et al., 2014). In the case of reproductive traits, research carried out to date has focused on polymorphisms in genes such as *PRL* (Mazurowski et al., 2013; Babicz et al., 2017 b), *PRLR* (Mihailov et al., 2014; Mencik et al., 2015; Putnova et al., 2002), *RBP4* (Spotter et al., 2009; Terman et al., 2011), *MC4R* (Szyndler-Nędza et al., 2016), *GHI* (Kmieć et al., 2008), *PTGS2* (*COX2*) (Ding et al., 2006; Sironen et al., 2010), *FSHB* (Polasik et al., 2016) *FUT1* and *ESR* (Horak et al., 2005), *GPR54* (Wu et al., 2019) and *RYR1* (Babicz et al., 2012; Szyndler-Nędza et al., 2019).

Few studies address the association between gene polymorphism and sexual activity of young females, which is one of the major traits affecting fertilisation success in gilts (Babicz et al., 2011, 2014, 2019). There are also no studies concerning the effect of gene locus polymorphism on periparturient activity of the sows. Borell et al. (2007) report that parturition and early lactation are the periods of important behavioural and physiological changes in the sow's body. The restrictions applied during that time are a source of frustration leading, among others, to activation of neuroendocrine pathways that control parturition, colostrum and milk production, and litter survival.

In view of the above, research was conducted on genes whose products are involved in reproductive and behavioural processes and in the body's immune response. These include *PRL*, *PRLR*, *PTGS2* and *FUT1*. Prolactin is an important hormone regulating mammary gland function; it is responsible for the establishment and maintenance of lactation, osmoregulation (water and electrolyte balance) and it also influences the behaviour of animals (Freeman et al., 2000; Saleem et al., 2018).

Prostaglandin-endoperoxide G/H synthase-2 (*PTGS2*), also known as cyclooxygenase 2, is involved together with cyclooxygenase 1 in the conversion of arachidonic acid to prostanoids, i.e. prostaglandin H (*PGH2*) (Vane et al., 1998). The prostaglandins synthesised in the presence of *COX* play key roles in the reproductive system, e.g. during ovulation, fertilisation, embryo implantation and parturition, which has caused *PTGS2* to be recognised as a gene with a potentially large impact on reproductive traits in animals (Ding et al., 2006; Sugimoto et al., 2015).

The *FUT1* gene is considered a potential marker for resistance to edema disease in pigs (Bao et al., 2012; Wang et al., 2012) and for reproductive performance traits of sows (Horak et al., 2005). But the results of research for reproductive performance are discrepant.

The aim of the study was to determine the associations between *PRL*, *PRLR*, *PTGS2*, and *FUT1* genes polymorphisms and reproductive traits in native Pulawska gilts.

Material and methods

The analysis included 72 Pulawska gilts, which gave birth to the first litter and were kept in 15 family farms as part of the Conservation Programme for Pulawska Pig Genetic Resources. In each farm, the experimental gilts were mated to one Pulawska boar. The feeding was adjusted to the age and physiological status of the sows, and housing conditions complied with animal welfare requirements (Journal of Laws 2010 no. 56 item 344 as amended). The performance tests, sexual activity, periparturient activity, and reproductive traits of sows included non-invasive and non-painful live testing. The duration and intensity of estrus and farrowing duration were observed using a camera to minimise stress. The sow's hair used in the molecular analyses was taken by the vet and/or farmer and delivered to the laboratory in specially prepared samples.

Measurement of estrus behaviour and reproductive traits in Pulawska gilts

Sexual activity for the first and second estrus was determined based on age (days), body weight (kg), duration (days) and intensity of estrus signs (pts.). The intensity was scored on a 3-point scale based on response to a teaser and duration of the mounting attempt, where 1 = very strong estrus signs, mounting attempt > 30 s; 2 = strong estrus signs, mounting attempt 20–25 s; 3 = weak signs of estrus, mounting attempt < 5 s.

Reproductive traits were characterised by age at first farrowing (days), farrowing duration (min.) and placental weight (kg). The reproductive performance of the sows was evaluated based on first parity data: number of piglets born alive per litter (head), number of piglets on d 21 of age (head), average piglet weight at birth (g) and average piglet weight on d 21 (g).

Analysis of gene polymorphism

The biological material for molecular analyses was obtained from hair roots of the sows. DNA isolation was performed using a Sherlock AX kit (A&A Biotechnology) following the manufacturer's procedure. The genotype was verified by PCR RFLP analysis. The primers of the *PRL*, *PRLR*, *FUT1* genes and PCR conditions were determined on the basis of available literature data (Table 1). For the *PRLR* gene, the primers were designed using Primer-BLAST (NCBI) and OligoCalc v 3.26 programs, and nucleotide databases (GeneBank ENA) (Table 1), although they concern identification of the SNP polymorphism (rs196956224) in pigs reported by Korwin-Kossakowska et al. (2005). The primer sequences were synthesised by

Genomed Sp. z o.o. and Sigma-Aldrich Sp. z o.o. PCR was performed with a thermal cycler (PTC-200 Peltier DNA Engine, MJ Research). Each sample of 15 µl, prepared for PCR, contained: 7.5 µl of RedTaq® ReadyMix™ PCR Reaction Mix (Sigma-Aldrich), 0.2 µl of each primer (5 µM), 5.9 µl of water (SIGMA) and 1.2 µl of DNA (20 ng/µl). The PCR products were digested with restriction enzymes adapted using the NEBcutter V2.0. program (Vincze et al., 2003) (Table 1). The PCR products were digested with 1 µl each of the enzymes *TaaI*^{FD}, *HinfI*^{FD} and *HhaI*^{FD}, and 2 µl of the *NcoI* enzyme (Table 1). The PCR-RFLP methods were designed and endonucleases selected to digest the unique sequences in each PCR product depending on polymorphisms occurrence. Electrophoretic separation of the PCR product for different genes, following digestion with restriction enzymes, was performed in 1.5% agarose gel (Sigma).

Table 1. Primer sequences, PCR thermal conditions and restriction enzyme digestion products of the analysed genes

Gene	Primer sequences (5'→3')				PCR ¹	References	
<i>PRL</i>	F 5'-TTAAGAGGAGATGCGTTACA-3' R 5'-GAATCAAGGAGAATGAGGTC-3'				298	Babicz et al., 2008	
<i>PRLR</i>	F 5'-TCCCATGAACCAAGCTCTCACTGAA-3' R 5'-CGGGGAAGGAAGGGCAACCG-3'				483	Skrzypczak et al., 2015	
<i>PTGS2</i>	F 5'-TCCAAACAAGGGCACTCAAAGACAA-3' R 5'-ACAGCATGATTTCCCTGGGTGGA-3'				655	Korwin-Kossakowska et al., 2005	
<i>FUT1</i>	F 5'-CTTCAGCCAGGGCTCCTTTAAG-3' R 5'-CTTCTGAACGTCTATCAAGACC-3'				421	Meijerink et al., 1997	
Temperature programme depending on PCR phase							
	ID	D	A	E	n	FE	T _{STOP}
<i>PRL</i>	95°C, 5'	95°C, 35"	58°C, 35"	725°C, 35"	36	72°C, 6'	4°C ~
<i>PRLR</i>		95°C, 35"	62°C, 35"	72°C, 40"	36		
<i>PTGS2</i>	95°C, 5'	95°C, 40"	62°C, 35"	72°C, 50"	37	72°C, 6'	4°C ~
<i>FUT1</i>		95°C, 35"	56°C, 35"	72°C, 40"	36		
	Restriction enzyme	Identified sequence		Temp. (°C)	Time	Length of fragments (bp)	
<i>PRL</i>	<i>TaaI</i> ^{FD, 2}	5'-ACN↓GT-3'		37	5 min	ins: 212, 86 del: 289	
<i>PRLR</i>	<i>NcoI</i> ³	5'-C↓CATGG-3'		37	2 h	AA: 365, 118 AT: 483, 365, 118 TT: 483	
<i>PTGS2</i>	<i>HinfI</i> ^{FD, 2}	5'-G↓ANTC-3'		37	5 min	TT: 288, 176, 137, 54 CT: 288, 176, 137, 114, 62, 54 CC: 288, 137, 114, 62, 54	
<i>FUT1</i>	<i>HhaI</i> ^{FD, 2}	5'-GCG↓C-3'		37	5 min	AA: 328, 93 AG: 87, 93, 241, 328 GG: 241, 93, 87	

¹ – length of PCR product denoted in number of base pairs.
ID – initial denaturation; D – denaturation; A – annealing; E – extension; n – number of PCR cycles (each cycle consists of ID, followed by D and A); FE – final extension of amplification products; T_{STOP} – temperature maintained after the end of PCR.
FD – line of fast digestion enzymes (FastDigest).
Enzyme producers: ² – Thermo Fisher Scientific Inc.; ³ – EURx Ltd.

Statistical analysis

Statistical analysis was performed with the GLM procedure and Genetica (SAS Institute, USA). The Hardy-Weinberg equilibrium was assessed by using the Court Lab – HW calculator. The statistical model used to calculate the effect of polymorphism of the analysed gene was:

$$y_{ijklm} = \mu + H_i + PRL_j + PRLR_k + PTGS2_l + FUTI_m + e_{ijklm}$$

where:

- y_{ijklm} – trait phenotypic value,
- μ – population average,
- H_i – fixed effect of the herd (absorption of effect),
- PRL_j – constant genotype effect in the examined locus *PRL* ($j = 1, 2, 3$),
- $PRLR_k$ – constant genotype effect in the examined locus *PRLR* ($k = 1, 2, 3$),
- $PTGS2_l$ – constant genotype effect in the examined locus *PTGS2* ($l = 1, 2, 3$),
- $FUTI_m$ – constant genotype effect in the examined locus *FUTI* ($m = 1, 2, 3$),
- e_{ijklm} – random error.

Absorption of the effect of the herd was used in the calculations. The model did not take into account the effect of the season and year. The study was carried out in one year at a similar time.

Statistically non-significant effects were removed from the linear model and the calculations performed again.

The calculations were made with the SAS statistical package. The level of the analysed traits was presented as least square means (LSM) while giving the standard errors (SE). The procedure used Tukey-Kramer adjustment for multiple comparisons. Differences between traits were analysed at $P \leq 0.01$ and $P \leq 0.05$ levels of significance.

Results

Genetic variation of *PRL*, *PRLR*, *PTGS2* and *FUTI* loci in Pulawska gilts

Polymorphism of the prolactin gene (*PRL*; rs 789846153), associated with the presence (insertion) or absence (deletion) of 11 nucleotide DNA fragment in the 3'UTR region of exon 5 was identified with the *TaaI* enzyme. As a result of digestion, three genotypes were determined in the studied population of gilts: AA (*Ins/Ins*), AB (*Ins/Del*) and BB (*Del/Del*) (Babicz et al., 2008).

Non-synonymous substitution (SNP: rs45435438) in exon 8 of the prolactin receptor gene (*PRLR*) was identified with the *NcoI* enzyme. As a result of digestion, AA, AT and TT genotypes were identified. Substitution at position 1528 (c.1528A>T) of the gene results in the presence of methionine in place of leucine, at position 510 (p.Met510Leu) of the amino acid chain of the receptor protein (Skrzypczak et al., 2015).

The polymorphism in exon 2 of the *PTGS2* gene (SNP: rs196956224) was examined using the *HinfI* enzyme, and the digestion revealed 2 gilt genotypes: *AG* and *GG* (Korwin-Kossakowska et al., 2005).

Non-synonymous substitution in exon 2 of the *FUT1* gene (SNP: rs335979375) at position 307 (NM_214068.2: c.307A>G), associated with sequence alteration in the protein amino acid chain (p.Thr103Ala) was identified with the *HhaI* enzyme. As a result of digestion, 3 genotypes were identified: *AA*, *AG* and *GG* (Meijerink et al., 1997).

Polymorphism in selected gene loci was determined in the studied population of Pulawska gilts (Table 2). Two genotypes, *AG* and *GG*, were identified at the *PTGS2* locus. Three genetic forms were identified at the locus of other genes. The highest frequency was observed for alleles *PRL B*, *PRLR A*, *PTGS2 G* and *FUT1 A*.

Among genotypes, frequency was highest for heterozygotes *AG* (*PTGS2* and *FUT1*) and lowest for homozygotes *GG* (*FUT1*). Analysis of the frequency of genotypes of these polymorphisms – in accordance with the Hardy-Weinberg law – showed that breeds were not at genetic equilibrium.

Table 2. Analysis of the distribution of alleles and genotypes in the analysed population of animals in accordance with the Hardy-Weinberg law

Gene	No. of sows	Genotype	Frequency			HWE P-value
			genotype	allele		
<i>PRL</i> ¹	15	<i>AA</i>	0.208	<i>A</i>	0.389	0.0163
	26	<i>AB</i>	0.361			
	31	<i>BB</i>	0.431	<i>B</i>	0.611	
<i>PRLR</i>	20	<i>AA</i>	0.278	<i>A</i>	0.583	0.0102
	44	<i>AT</i>	0.611			
	8	<i>TT</i>	0.111	<i>T</i>	0.417	
<i>PTGS2</i>	0	<i>AA</i>	0.000	<i>A</i>	0.319	0.000
	46	<i>AG</i>	0.639			
	26	<i>GG</i>	0.361	<i>G</i>	0.681	
<i>FUT1</i>	22	<i>AA</i>	0.306	<i>A</i>	0.604	0.0129
	43	<i>AG</i>	0.597			
	7	<i>GG</i>	0.097	<i>G</i>	0.396	

¹ – alleles *A* and *B* in the prolactin gene correspond with *Ins/Del* type polymorphism (*AA*: *Ins/Ins*, *AB*: *Ins/Del*; *BB*: *Del/Del*).

HWE – Hardy-Weinberg equilibrium.

Associations of gene polymorphisms with the values of analysed traits *PRL* gene polymorphism

The age and body weight of the gilts from the different genetic groups during first and second estrus were uniform (Table 3). Distinct but statistically non-significant differences were observed in the intensity of estrus signs; in the sows of *AA* genotype this intensity was by far the lowest during both the first and second estrus. There

were no significant differences in the age of the sows at first farrowing and in the weight of expelled placenta. In turn, the analysis of farrowing duration showed that the sows of genotype *AA* took 84 minutes longer to farrow on average compared to heterozygotes *AB*, with a statistically significant difference ($P \leq 0.05$). The number of live born piglets, calculated for the first reproductive cycle, as well as the mean body weight of piglets at birth remained at a similar level regardless of genotype group.

Table 3. Least square means (LSM) and standard errors (SE) for sexual activity parameters, farrowing indicators of the gilts and reproductive performance traits of first- and second-parity sows depending on *PRL* locus genotype (rs 789846153)

Trait	<i>AA</i>	<i>AB</i>	<i>BB</i>
	LSM±SE	LSM±SE	LSM±SE
Sexual activity parameters of first estrus			
Age (d)	203.56±2.92	205.44±2.11	205.94±2.06
Body weight (kg)	86.46±1.98	85.74±1.17	84.12±1.40
Duration (d)	2.00±0.20	2.44±0.16	2.28±0.14
Intensity (pts.)	1.56±0.23	2.11±0.19	2.17±0.16
Sexual activity parameters of second estrus			
Age (d)	224.33±2.94	226.00±2.13	226.33±2.08
Body weight (kg)	101.33±2.19	100.00±1.18	99.11±1.55
Duration (d)	2.78±0.20	2.67±0.15	2.39±0.14
Intensity (pts.)	2.11±0.26	2.44±0.22	2.28±0.19
Farrowing indicators			
Age at first farrowing (d)	325.78±10.55	336.56±8.47	328.83±7.46
Farrowing duration (min)	291.56 a±22.48	208.33 b±12.33	233.06±15.89
Placental weight (kg)	3.11±0.23	3.10±0.12	3.24±0.16
Reproductive traits of first parity			
Number of live born piglets (head)	10.67±0.42	10.56±0.36	10.94±0.29
Number of piglets at 21 d (head)	9.33±0.38	9.44±0.21	9.83±0.27
Mean weight of piglet at birth (g)	1284.44±59.81	1244.44±49.72	1286.11±42.29
Mean weight of piglet at 21 d (g)	5484.44±268.22	5391.11±206.44	5361.11±189.66

a, b – means in rows with different letters differ significantly at $P \leq 0.05$.

***PRLR* gene polymorphism**

Significant differences ($P \leq 0.05$) were found in the age of the gilts during first and second estrus (Table 4), and when the signs of estrus were observed, the gilts of *TT* genotype were older than *AA* gilts by 15 d on average. No statistically significant differences were found in the values of the other indicators. The analysis of farrowing duration showed that the sows of *TT* genotype took by far the longest to farrow. Farrowing time of *TT* sows was on average 122 min longer than that of *AT* heterozygotes, with a statistically significant difference ($P \leq 0.05$). The analysis of

reproductive performance depending on *PRLR* locus genotype demonstrated that the differences between traits of analysed litter, were statistically non-significant.

Table 4. Least square means (LSM) and standard errors (SE) for sexual activity parameters, farrowing indicators of the gilts and reproductive performance traits of first parity sows depending on *PRLR* locus genotype (rs 45435438)

Trait	<i>AA</i>	<i>AT</i>	<i>TT</i>
	LSM±SE	LSM±SE	LSM±SE
Sexual activity parameters of first estrus			
Age (d)	200.45 a±2.38	206.61±1.65	215.50 b±5.58
Body weight (kg)	82.08±1.67	86.04±1.16	91.05±3.92
Duration (d)	2.27±0.19	2.26±0.13	2.00±0.44
Intensity (pts.)	2.00±0.22	2.04±0.15	1.50±0.51
Sexual activity parameters of second estrus			
Age (d)	221.18 a±2.40	227±1.66	236.50 b±5.64
Body weight (kg)	97.45±1.94	100.83±1.34	102.5±4.54
Duration (d)	2.64±0.18	2.48±0.13	3.00±0.43
Intensity (pts.)	1.91±0.23	2.43±0.16	2.50±0.54
Farrowing indicators			
Age at first farrowing (d)	324.27±9.28	335.04±6.42	303.50±21.76
Farrowing duration (min)	268.18±19.98	219.96 a±13.82	342.50 b±46.85
Placental weight (kg)	3.18±0.21	3.18±0.14	2.99±0.48
Reproductive traits of first parity			
Number of live born piglets (head)	10.82±0.37	10.87±0.25	9.50±0.86
Number of piglets at 21 d (head)	9.55±0.34	9.74±0.23	8.50±0.79
Mean weight of piglet at birth (g)	1246.36±53.80	1293.91±37.20	1220.00±126.17
Mean weight of piglet at 21 d (g)	5458.18±230.08	5460.87±159.11	4370.00±539.58

a, b – means in rows with different letters differ significantly at $P \leq 0.05$.

***PTGS2* gene polymorphism**

Sexual activity parameters of the gilts (Table 5) were at a comparable level in both genotype groups under analysis. The analysis of farrowing duration showed that it was shorter in *AG* heterozygotes, despite the fact that these sows farrowed and reared larger litters with lower birth weight. However, the observed differences in the values of the traits were not significant.

***FUT1* gene polymorphism**

No significant differences were noted in the sexual activity indicators and in the farrowing parameters of the gilts (Table 6). The analysis of reproductive performance traits showed that the sows of *GG* genotype ($P \leq 0.05$) farrowed and reared more nu-

merous litters. Furthermore, piglets born to *GG* homozygotes had the highest weight gains and achieved at 21 d of age the highest average body weight, despite the fact that they were characterised by the lowest birth weight.

Table 5. Least square means (LSM) and standard errors (SE) for sexual activity parameters, farrowing indicators of the gilts and reproductive performance traits of first parity sows depending on *PTGS2* locus genotype (rs196956224)

Trait	<i>AG</i>	<i>GG</i>
	LSM±SE	LSM±SE
Sexual activity parameters of first estrus		
Age (d)	204.04±1.78	207.31±2.36
Body weight (kg)	85.29±1.24	84.79±1.65
Duration (d)	2.26±0.13	2.23±0.17
Intensity (pts.)	2.00±0.15	2.00±0.20
Sexual activity parameters of second estrus		
Age (d)	224.52±1.79	227.92±2.38
Body weight (kg)	100.57±1.35	98.69±1.80
Duration (d)	2.57±0.13	2.54±0.17
Intensity (pts.)	2.30±0.16	2.23±0.22
Farrowing indicators		
Age at first farrowing (d)	332.30±6.52	325.92±8.67
Farrowing duration (min)	233.22±15.15	256.15±20.15
Placental weight (kg)	3.24±0.14	3.05±0.19
Reproductive traits of first parity		
Number of live born piglets (head)	10.96±0.25	10.46±0.34
Number of piglets at 21 d (head)	9.74±0.24	9.38±0.31
Mean weight of piglet at birth (g)	1252.17±36.47	1316.15±48.50
Mean weight of piglet at 21 d (g)	5333.04±164.57	5516.92±218.90

Table 6. Least square means (LSM) and standard errors (SE) for sexual activity parameters, farrowing indicators of the gilts and reproductive performance traits of first parity sows depending on *FUT1* locus genotype (rs335979375)

Trait	<i>AA</i>	<i>AG</i>	<i>GG</i>
	LSM±SE	LSM±SE	LSM±SE
1	2	3	4
Sexual activity parameters of first estrus			
Age (d)	204.36±2.61	206.13±1.80	199.50±6.11
Body weight (kg)	83.31±1.78	85.93±1.23	85.50±4.18
Duration (d)	2.18±0.19	2.30±0.13	2.00±0.44
Intensity (pts.)	2.27±0.22	1.87±0.15	2.00±0.50

Table 6 – contd.

1	2	3	4
Sexual activity parameters of second estrus			
Age (d)	225.00±2.62	226.65±1.81	219.50±6.14
Body weight (kg)	98.36±1.97	100.74±1.36	98.50±4.63
Duration (d)	2.73±0.18	2.48±0.13	2.50±0.43
Intensity (pts.)	2.09±0.24	2.35±0.16	2.50±0.56
Farrowing indicators			
Age at first farrowing (d)	325.18±9.00	328.48±6.23	374.00±21.11
Farrowing duration (min)	246.73±21.19	247.61±14.66	142.50±49.70
Placental weight (kg)	3.09±0.20	3.16±0.14	3.76±0.47
Reproductive traits of first parity			
Number of live born piglets (head)	11.36 a±0.32	10.35 a±0.22	12.50 b±0.76
Number of piglets at 21 d (head)	9.73±0.32	9.39 a±0.22	11.50 b±0.74
Mean weight of piglet at birth (g)	1280.91±54.04	1279.57±37.37	1195.00±126.74
Mean weight of piglet at 21 d (g)	5196.36±239.26	5479.13±165.46	5600.00±561.11

a, b – means in rows with different letters differ significantly at $P \leq 0.05$.

Discussion

The risk of the native Pulawska pigs becoming extinct has caused this breed to be included in the genetic resources conservation programme. The main objective of conservation breeding is to increase populations to the level that makes them stable and biologically sustainable, while maintaining genetic distinctiveness and within-breed variation (Babicz et al., 2017 a, b). In this context, it is important to determine the breed's genetic structure and to analyse associations between specific genetic markers and reproduction traits of pigs (Wu et al., 2019; Onteru et al., 2009).

The lack of genetic equilibrium in the analysed gene polymorphisms, revealed in the population under study, suggests the possible effect of selective breeding which, according to the principles of the conservation programme for the Pulawska breed, aims to minimise population inbreeding and improve reproductive traits while maintaining fattening and slaughter traits at a constant level. Because the reproductive performance traits of sows show a low coefficient of heritability (Tyra and Różycki, 2004), it takes a relatively long time to improve them using traditional methods. In this respect, identification of the associations between the polymorphism of genes and reproductive performance traits of the sows may accelerate breeding progress.

The *Ins/Del* type polymorphism, identified by Babicz et al. (2008) in the prolactin gene (rs 789846153), was used to search for associations with the reproductive characteristics of highly productive maternal breeds of pigs (Mazurowski et al., 2013; Milczewska et al., 2011). The authors of these studies showed this polymor-

phism had no significant effect on age at first farrowing in Polish Large White and Polish Landrace gilts, and on the number of piglets born and reared. Likewise, our study showed no statistically significant effect of the polymorphism of this gene on age at first farrowing in Pulawska gilts, and on indicators of sexual activity during first and second estrus. Neither were there any differences in the number of piglets in the first litter. Significant differences ($P \leq 0.05$) were noted in the duration of farrowing (Table 3), with *AA* sows having the longest farrowing time and *AB* heterozygotes the shortest. This information could be used in productive herds of Pulawska pigs that produce fatteners. Reproductive use of *PRL AB* heterozygous gilts will help to improve farrowing management and reduce handling costs. For the prolactin receptor, Van Rens and Van der Lende (2002) reported that homozygous *BB* (*PRLR/AluI*) gilts were significantly ($P \leq 0.05$) younger than *AA* gilts, by an average of 41 d at first estrus and by 49 d at first insemination. No significant differences were found in gestation length and placental weight between the analysed sow genotypes, although *BB* homozygotes had higher mean placental weight but these sows gave birth to the smallest litters ($P \leq 0.05$).

In our study, differences in the values of traits between the genotypes analysed at the *PRLR/NcoI* locus (rs45435438) were only shown for the age of sows during estrus and for the duration of first farrowing (Table 4). At first and second estrus, the oldest sows among all the genotype groups were *TT* homozygotes, with a significant difference between the *TT* and *AA* sows ($P \leq 0.05$). *TT* sows gave birth to first offspring much earlier (by an average of 21 d compared to *AA* homozygotes and by 32 d compared to *AT* heterozygotes) (this difference was not significant), but at the same time the *TT* sows had longer ($P \leq 0.05$) farrowing duration (345 min in *TT* sows and 220 min in *AT* sows). We did not show an unambiguously favourable effect of genotype at the *PRLR* locus on the indicators of litter size and piglet weight, although the highest values were typical for heterozygotes and the lowest values for *TT* homozygotes. Omelka et al. (2008) showed a significantly higher ($P \leq 0.01$) total number of piglets born in *AA* homozygous (*PRLR/AluI*) sows of Slovak Landrace, Large White and White Meaty breeds. Mihailov et al. (2014) showed that *BB* homozygotes (*PRLR/AluI*) gave birth to markedly more piglets ($P \leq 0.01$), regardless of breed. A similar relationship for the total number of piglets born and number of piglets born alive, but only in the first and second litter, was reported by Mencik et al. (2015). The analysis of third and fourth parities and all parities (1–4) revealed the advantage of sows with *AA* genotype over the others.

Our study of the association between *PTGS2* locus polymorphism (rs196956224) and the level of analysed indicators showed no significant relationships for sexual and periparturient activity of the gilts, or for traits related to litter size and weight. Sironen et al. (2010) – based on the results from the second and subsequent litters – demonstrated that the identified allele 1 (notation of the discussed polymorphism used by the authors) at the *COX2* locus (*PTGS2*) was significantly related to a greater number of piglets born when compared to allele 2 (an increase of 0.31; additive effect found). Nevertheless, as in our study, this relationship was not observed for first litters. Horak et al. (2005), who analysed parities 1–6 in a population of Prestice Black-Pied sows, showed higher values of reproductive parameters in sows of *FUT1*

GG genotype compared to those of *AA* genotype. Different results were obtained by Zhu et al. (2014) when analysing the reproductive performance of Yorkshire sows. The authors showed that in parities 3–5, sows of *AA* genotype produced significantly the largest number of piglets. Our results (Table 6) agree with those of Horak et al. (2005). Pulawska sows of *FUT1 GG* genotype (rs335979375) gave birth to and reared significantly the largest number of piglets in the first litter ($P \leq 0.05$), compared to sows of *AG* and *AA* genotype. The lowest value of this trait was obtained for the sows of *FUT1 AG* genotype.

Summing up and generalising the results of our research, it can be stated that gilts of *AA* genotypes (*Ins/Ins*) at the *PRL* locus are characterised by longest farrowing duration compared to gilts of *AB* genotype. The analysis of the polymorphic associations of the *PRLR* gene variants did not show statistically significant differences for the performance test indicators in gilts, whereas in *TT* homozygotes it revealed a tendency for later occurrence of estrus signs (first and second estrus) and for the markedly longest farrowings ($P \leq 0.05$). With regard to *PTGS2* and *FUT1* loci, no statistically significant differences were observed in the indicators related to the level of sexual and periparturient activity in gilts. It was found, however, that females of *FUT1 GG* genotype produced and reared the largest first litters ($P \leq 0.05$).

Conclusions

The results of the studies presented above expand knowledge about the genetic structure and productivity of Pulawska sows. Searching for the associations between the gene polymorphism and the productive traits is essential to defining the genetically determined breed specificity of these animals which distinguishes them from other breeds.

Our study suggests the association between analysed gene polymorphism and productive traits. Polymorphism in the *PRL* locus affects farrowing duration. The analysis of the polymorphic associations of the *PRLR* gene variants revealed a tendency for later occurrence of estrus signs (first and second estrus) and for the markedly longest farrowings. In the case of the *FUT1* gene, it was found that polymorphism of the *FUT1* locus influences the size of the first litters.

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