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NEW POLYMORPHIC CHANGES IN THE *WNT7A* GENE AND THEIR EFFECT ON REPRODUCTIVE TRAITS IN PIGS*

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Abstract

For many years, research has been undertaken to identify genetic markers associated with reproductive traits in pigs, but these issues are still open to research. *WNT7A* expression was found in endometrium during embryo implantation as well as in early pregnancy in humans and in different species of animals. Our study was designed to identify polymorphic changes in the *WNT7A* gene and their effect on reproductive traits in 480 Polish Large White (PLW) and Polish Landrace (PL) pigs in 4 successive litters. As a result, 3 mutations were identified: two transitions in exon 3 g.6270G>A (rs326274214) and in intron 4 g.36251G>A (rs321204163) and deletion/insertion in intron 4 g.36220delinsAT (rs338143418). Frequency analysis of the genotypes of these polymorphisms in the *WNT7A* gene showed departure from Hardy-Weinberg equilibrium for PLW at locus g.36220delinsAT and for total breeds and PLW at locus g.36251G>A. Generally, the largest litters were found in sows with *AA* genotypes for mutations g.6270G>A and g.36251G>A and in sows with *ins/ins* genotype for mutation g.3622delinsAT. However, the sows of these genotypes formed only a small percentage (from 9% to 13%) in the studied group of animals. Statistical analysis showed significant differences only for the number of piglets born and reared in parity 1 (1NBA and 1N21d) in favor of the sows with genotype AA g.6270G>A (P≤0.05) and for the number of piglets born in litter 4 (4NBA) with genotype ins/ins g.3622delinsAT (P≤0.05). The analysed mutations had no effect on the age at first farrowing and the successive farrowing intervals.

Key words: pigs, *WNT7A* gene polymorphisms, reproductive traits

Increasing the number of piglets born per sow per year is an economically important issue in pig breeding and production. However, the improvement of sow fertility through breeding treatments based on phenotypic characteristics is difficult because reproductive traits have low heritability (Engblom et al., 2010; Imboonta et al., 2007; Kapell et al., 2009; Popovac et al., 2012).

Current knowledge and modern laboratory techniques allow us to search for genetic markers for quantitative traits. Their use in selection may increase breeding progress

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compared to conventional methods. One of the methods for identification of genetic markers is to search for candidate genes, the protein products of which are involved in physiological processes that contribute to a given trait. Essential to the improvement of reproductive traits are genes that are expressed in female reproductive organs. In pigs, researchers have looked for a relationship between litter size and the polymorphism of genes such as: *ESR* (estrogen receptor), *RBP4* (retinol binding protein 4), *AREG* (amphiregulin), *LIF* (leukemia inhibitory factor), *IGF2* (insulin-like growth factor), *EGF* (epidermal growth factor), *TCF12* (transcription factor 12), *CTNNAL1* (catenin alpha-like protein 1), *PREI3* (preimplantation protein 3) and *WNT10B* (Wingless-type MMTV integration site family, member 10B) (Hunyadi-Bagi et al., 2016; Jiang et al., 2002; Mucha et al., 2013; Muñoz et al., 2010; Niu et al., 2006; Tao et al., 2013).

Wnt (Wingless-type MMTV integration site family) signaling pathway is essential for the growth and development of humans and animals. Studies with mice and sheep demonstrated that WNT proteins are important regulators of uterine receptivity to implantation (Hayashi et al., 2007; Kobayashi and Behringer, 2003; Mohamed et al., 2005; van Amerongen and Nusse, 2009). Many *WNT* genes are expressed in the uterus of women during the proliferative and secretory phase (Tulac et al., 2003). Moreover, canonical WNT signaling pathway was found to affect embryo development in humans and sheep (Hayashi et al., 2007; Pollheimer et al., 2006).

One of the genes of the Wnt signaling pathway is *WNT7A*. *WNT7A* coordinates a variety of cell and developmental pathways that guide postnatal uterine growth and hormonal responses and disruption of these pathways leads to aberrant cell death (Carta and Sassoon, 2004). A study with macaques detected *Wnt7a* expression in the endometrial luminal epithelium (LE) and upper regenerating glands after menstruation (day 5–6), implicating *Wnt7a* in postmenstrual regeneration and growth of endometrial epithelium in primates (Fan et al., 2012). The same study also showed that *WNT7A* mRNA levels in human and macaque endometrium were higher during the proliferative phase compared to the secretory phase. These findings are not supported by Tulac et al. (2003) and Peng et al. (2012), who showed no clear differences between the proliferative and secretory phases. In early pregnant sheep, Hayashi et al. (2009) detected *WNT7A* mRNA only in the LE, and maximal abundance of *WNT7A* mRNA was observed in the implantation site on day 5. In pigs, Kiewisz et al. (2011) showed the spatial localization of *WNT7A* proteins in porcine endometrium during periimplantation period of pregnancy and indicated significant changes of *WNT7A* gene expression before implantation.

Increased litter size has become one of the main selection objectives in pig breeding programmes. However, litter size depends on many factors, including ovulation rate, embryo survival, and endometrial receptivity, which are determined by many genes. Therefore, it seems appropriate to undertake research aimed at finding genetic markers associated with these traits. *WNT7A* gene and its products are involved in regulating endometrial receptivity during the implantation window, which suggests that mutations in this gene can influence litter size. In addition, the porcine *WNT7A* gene was localized on chromosome 13, where QTLs for litter size were identified (Pig QTLdb). However, the available literature provides no information concerning the effect of the polymorphism of this gene on reproductive traits in pigs.

The aim of the study was to identify polymorphic changes in the *WNT7A* gene and their effect on reproductive traits in Polish Large White and Polish Landrace pigs.

Material and methods

Animal material and DNA samples

The study was conducted with material from 259 Polish Large White (PLW) and 221 Polish Landrace (PL) sows evaluated for reproductive performance at two nucleus farms. On both farms, PLW and PL sows were kept as separate breeding. The following reproductive data were collected: (1, 2, 3, 4)NBA – number of piglets born alive in the first and subsequent parities; (1, 2, 3, 4)N21d – number of piglets at 21st day in the first and subsequent parities; 1-4NBA – average number of live-born piglets from parities 1–4; 1-4NB21d – average number of piglets at 21st day from parities 1–4; AFF – the age of gilt at first farrowing; 12IBTSL – the interval between 1 and 2 successive litters; 23IBTSL – the interval between 2 and 3 successive litters; 34IBTSL – the interval between 3 and 4 successive litters; 1-4IBTSL – average interval between two successive litters from parities 1–4. The number of animals which served as a basis for the analyses in individual litters differed because not all the sows had four litters. Means and standard deviations (SD) of analysed reproductive traits of PLW and PL sows are presented in Table 1. Peripheral blood was collected from the sows into tubes containing EDTA as an anticoagulant. The biological material was collected by other studies (the experimental procedures were approved by the Local Ethics Committee, Poland, No. 666/2009).

Table 1. Means and standard deviations (SD) of analysed reproductive traits

Traits	N	Means	SD
1NBA	480	11.83	1.68
1N21d	480	11.38	1.59
2NBA	463	12.52	1.68
2N21d	463	11.97	1.54
3NBA	387	12.71	1.74
3N21d	387	12.19	1.64
4NBA	265	12.71	1.78
4N21d	265	12.03	1.71
1-4NBA	264	12.28	0.94
1-4N21d	264	11.83	0.88
AFF	480	361.85	36.14
12IBTSL	463	167.88	31.83
23IBTSL	387	160.74	21.47
34IBTSL	265	158.66	19.34
1-4IBTSL	265	159.84	14.09

(1, 2, 3, 4)NBA – number of piglets born alive in the first and subsequent parities; (1, 2, 3, 4)N21d – number of piglets at 21st day in the first and subsequent parities; 1-4NBA – average number of live-born piglets from parities 1–4; 1–4N21d – average number of piglets at 21st day from parities 1–4; AFF – the age of gilt at first farrowing; 12IBTSL – the interval between 1 and 2 successive litters; 23IBTSL – the interval between 2 and 3 successive litters; 34IBTSL – the interval between 3 and 4 successive litters; 1–4IBTSL – average interval between two successive litters from parities 1–4.

Identification of polymorphic changes

DNA was isolated from whole blood using Genomic Wizard Purification Kit (Promega, Madison, USA) according to the attached protocol. New polymorphic changes in the *WNT7A* gene were identified by High Resolution Melting (HRM) screening method using KAPA HRM FAST PCR Kits (KAPA Biosystem, USA) and the Eco Real-Time PCR System (Illumina, USA). Mutation types were identified by Sanger sequencing using the GenomeLab DTCS-Quick Start Kit (Beckman Coulter, USA) on a Beckman Coulter sequencer. For selected fragments of the *WNT7A* gene (covering coding and regulatory sequences), primers were designed which amplified fragments of 250–300 bp. Next, the samples showing melting curve variation compared to the others were sequenced to identify the type of polymorphism. To determine the frequency of the newly identified polymorphic changes in the *WNT7A* gene in the studied populations, restriction enzymes were designed to allow their detection in PCR-RFLP reaction (Table 2). The amplification step was performed using Ampli-Taq Gold 360 master mix (Applied Biosystems, USA).

Table 2. PCR primers and conditions for identification of mutations in *WNT7A* (NW_003611702.1)

Locus	Primers (5'–3')	Annealing temperature (°C)	Restriction enzyme	Genotype pattern (bp)
g.A6270G	F: CCCACCACTACCACCTTGTT R: GGTCTCCCCAGAGCCTAAAA	55	Cac8I	G 155/88/44/33/17 A 188/88/44/17
g.36220delinsAT	F: GACCTGGAAGCACTGGTAGC R: AGACATGAGCCCATCAACCT	55	HaeIII	G 205/163/112/68/58/48/31/30 A163/132/112/73/68/48/31/30
g.A36251G	F: GACCTGGAAGCACTGGTAGC R: AGACATGAGCCCATCAACCT	55	TaqI	A 715 G 439/274

Statistical analysis

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

$$Y_{ijklm} = \mu + d_i + g_j + f_k + h_l + e_{ijklm}$$

where:

y_{ijklm} – ijklmth observation,

μ – overall mean,

d_i – fixed effect of ith breeding (accounts for each breed separately in particular farms),

g_j – fixed effect of jth season (only for traits concerning individual litters; the division into season was based on successful mating or sow insemination date),

f_k – fixed effect of kth genotype mutation in *WNT7A* gene (separately for mutation),

h_l – random effect of the sow,

e_{ijklm} – random error.

Means were calculated by the least-square means method (LSM ± SE). If the analysis showed significant differences, the data were subjected to post-hoc test. Significant differences between genotypes were analysed using the Tukey-Kramer test at 1% and 5%.

Results

As a result of the study, we identified 3 mutations in the *WNT7A* gene, which have been deposited with accession numbers in the NCBI database. The first mutation was identified in exon 3: transition g.6270G>A (rs326274214), and the next two mutations were localized in intron 4: transition g.36251G>A (rs321204163) and deletion/insertion AT at position 36220 (g.36220delinsAT) (rs338143418). In order to detect frequencies in the investigated pig populations the restriction enzymes were matched: *Cac8I*, *HaeII* and *TaqI*, respectively.

Table 3. Genotype and allele frequencies of *WNT7A* mutations at three loci in Polish Large White (PLW) and Polish Landrace (PL) pigs

Locus	Breed	Genotype			Allele		HWE P-value
g.6270G>A		**GG**	**AG**	**AA**	**G**	**A**	
	Total	0.43	0.44	0.13	0.65	0.35	0.495
	PLW	0.40	0.43	0.17	0.61	0.39	0.135
	PL	0.47	0.45	0.08	0.70	0.30	0.290
g.36220delinsAT		**ins/ins**	**ins/del**	**del/del**	**ins**	**del**	
	Total	0.12	0.51	0.37	0.37	0.63	0.069
	PLW	0.13	0.57	0.30	0.42	0.58	**0.009**
	PL	0.10	0.44	0.46	0.32	0.68	0.933
g.36251G>A		**GG**	**AG**	**AA**	**G**	**A**	
	Total	0.40	0.51	0.09	0.65	0.35	**0.004**
	PLW	0.32	0.57	0.11	0.60	0.40	**0.002**
	PL	0.49	0.45	0.06	0.71	0.29	0.204

HWE – Hardy-Weinberg equilibrium.

Genotype and allele frequencies for the analysed mutations are shown in Table 3. Genotypes of 469 animals were identified at locus g.6270G>A, genotypes of 478 animals at locus g.36220delinsAT, and genotypes of 477 animals at locus g.36251G>A. For the analysed mutations of the *WNT7A* gene all of the three genotypes were found to be present. In the analysed group of sows, lower frequency of the A (g.6270G>A), insertion alleles (g.36220delinsAT) and A alleles (g.36251G>A) was observed for all the breeds together and separately. For the mutation at the g.A6270G, the frequency of AG and GG genotypes was similar (from 0.40 to 0.47). In the case of g.36220delinsAT and g.36251G>A for total pigs and PLW, ins/del (0.44 to 0.57) and AG (0.45 to 0.57) heterozygotes were the most frequent, and ins/ins and AA homozygotes the least frequent, respectively. In the same mutations for

PL, del/del (0.46) and GG (0.49) homozygotes were the most frequent. Frequency analysis of the genotypes of these mutations in the *WNT7A* gene – in accordance with the Hardy-Weinberg law – were in most cases in genetic equilibrium except for PLW in mutation g.36220delinsAT as well as total pigs and PLW for g.36251G>A.

Table 4. Effects of the *WNT7A g.6270G>A* genotype on reproductive traits in pigs

Traits	N	GG		AG		AA	
		LSM	SE	LSM	SE	LSM	SE
1NBA	469	11.72 a	0.11	11.80	0.11	12.29 b	0.21
1N21d	469	11.23 a	0.11	11.39	0.11	11.85 b	0.20
2NBA	452	12.57	0.12	12.44	0.12	12.64	0.22
2N21d	452	12.02	0.11	11.86	0.11	12.15	0.20
3NBA	381	12.70	0.14	12.70	0.13	12.97	0.26
3N21d	381	12.21	0.13	12.19	0.13	12.30	0.25
4NBA	261	12.85	0.18	12.55	0.16	12.92	0.30
4N21d	261	12.18	0.17	11.91	0.16	12.17	0.29
1-4NBA	261	12.28	0.09	12.21	0.08	12.57	0.15
1-4N21d	261	11.85	0.08	11.76	0.08	12.07	0.15

Values with the different letters show significant differences in rows P≤0.05.

N – number of animals; LSM – least square means; SE – standard error.

(1, 2, 3, 4)NBA – number of piglets born alive in the first and subsequent parities; (1, 2, 3, 4)N21d – number of piglets at 21st day in the first and subsequent parities; 1-4NBA – average number of live-born piglets from parities 1–4; 1-4N21d – average number of piglets at 21st day from parities 1–4.

Tables 4–6 present the results of analysis of the relationship between polymorphisms of the mutations in the *WNT7A* gene and reproductive traits. For the mutation at the 6270G>A locus, the highest number of piglets born and reared in all analysed parities was found in the sows with AA genotype (except the number of piglets reared to day 21 in parity 4 – 4N21d), and the lowest in sows with GG genotype in the first parity and sows with AG genotype in the other parities (Table 4). However, statistically significant differences were found only between genotypes for number of piglets born (1NBA, P≤0.05) and number of piglets reared to day 21 (1N21d, P≤0.05) in parity 1. In the case of g.36220delinsAT, the highest number of piglets born and reared per litter was found in most parities from ins/ins homozygous sows, except for the number of piglets born in parity 2 and 3, and in all the four parities together (2NBA, 3NBA and 1-4NBA) (Table 5). Statistical differences were only observed between the number of piglets reared to day 21 in parity 4 (4N21d) of the homozygous ins/ins and del/del sows (P≤0.05). Also analysis of the polymorphism of the *WNT7A* gene, g.36251G>A showed the highest values for the number of piglets per litter in sows with AA genotype in parities 1 to 4 and in parities 1–4 together (Table 6). However, no statistically significant differences were found between the analysed traits. No conclusive results were obtained when analysing the age at first farrowing and the successive farrowing intervals for all the three mutations (data not shown in tables).

Table 5. Effects of the *WNT7A g.36220delinsAT* genotype on reproductive traits in pigs

Traits	N	*ins/ins*		*ins/del*		*del/del*	
		LSM	SE	LSM	SE	LSM	SE
1NBA	478	12.14	0.21	11.79	0.10	11.77	0.12
1N21d	478	11.59	0.20	11.36	0.10	11.33	0.12
2NBA	461	12.57	0.22	12.58	0.11	12.42	0.13
2N21d	461	12.07	0.21	11.93	0.10	11.99	0.12
3NBA	385	12.83	0.26	12.49	0.13	12.92	0.14
3N21d	385	12.46	0.25	11.99	0.12	12.36	0.13
4NBA	263	13.33	0.33	12.79	0.16	12.45	0.17
4N21d	263	12.77 a	0.31	12.12	0.15	11.75 b	0.16
1-4NBA	263	12.34	0.17	12.78	0.08	12.26	0.09
1-4N21d	263	12.01	0.17	11.79	0.08	11.82	0.08

Values with the different letters show significant differences in rows P≤0.05.

(1, 2, 3, 4)NBA – number of piglets born alive in the first and subsequent parities; (1, 2, 3, 4)N21d – number of piglets at 21st day in the first and subsequent parities; 1-4NBA – average number of live-born piglets from parities 1–4; 1-4N21d – average number of piglets at 21st day from parities 1–4.

Table 6. Effects of the *WNT7A g.36251G>A* genotype on reproductive traits in pigs

Traits	N	GG		AG		AA	
		LSM	SE	LSM	SE	LSM	SE
1NBA	477	11.79	0.12	11.82	0.10	12.07	0.24
1N21d	477	11.34	0.11	11.38	0.10	11.54	0.24
2NBA	461	12.44	0.12	12.56	0.11	12.73	0.26
2N21d	461	11.97	0.11	11.95	0.10	12.13	0.24
3NBA	385	12.90	0.14	12.48	0.13	12.99	0.30
3N21d	385	12.33	0.13	11.99	0.12	12.52	0.29
4NBA	264	12.45	0.16	12.89	0.16	13.09	0.40
4N21d	264	11.77	0.16	12.20	0.15	12.58	0.38
1-4NBA	264	12.25	0.08	12.30	0.08	12.33	0.21
1-4N21d	264	11.81	0.08	11.82	0.08	11.98	0.20

(1, 2, 3, 4)NBA – number of piglets born alive in the first and subsequent parities; (1, 2, 3, 4)N21d – number of piglets at 21st day in the first and subsequent parities; 1-4NBA – average number of live-born piglets from parities 1–4; 1-4N21d – average number of piglets at 21st day from parities 1–4.

Discussion

The present study searched for mutations in the *WNT7A* gene and their relationships with reproductive traits in sows. Three new mutations in this gene were identified. The polymorphic change in exon 3 *WNT7A* g.6270G>A is a silent mutation that does not change the amino acid sense and has no immediate effect on protein quality, but may be linked to another causal mutation, which will be reflected in the phenotype. Both polymorphic changes in intron 4 (g.36251G>A and g.36220delinsAT) may have regulatory functions, because they can affect gene expression by binding appropriate transcription factors as well as influencing mRNA maturation (pre mRNA>mRNA).

In the present study, in most cases higher values were found for litter size traits in the sows of AA genotypes at locus g.6270G>A, ins/ins at locus g.36220delinsAT and AA at locus g.36251G>A. However, statistically significant differences ($P \leq 0.05$) were found only between genotypes AA and GG at locus g.6270G>A for the number of piglets born and reared to day 21 in parity 1 (1NBA and 1N21d, by 0.57 and 0.62 piglet, respectively) and between ins/ins and del/del genotypes at locus g.36220delinsAT for the number of piglets reared in parity 4 (4N21d by 1.02 piglets). It should be noted, however, that considerable differences were also found for some other traits in litter size from sows of different genotypes. For the number of piglets born in parity 4 (4NBA) and for the number of piglets born and reared in parities 1 to 4 in total (1-4NBA and 1-4N21d) there were differences of 0.37, 0.36 and 0.31 piglet between sows of AA and AG genotypes at locus g.6270G>A. Even greater differences were obtained for sows of ins/ins and ins/del genotypes at locus g.36220delinsAT, by 0.47 piglet for the number of piglets reared in parity 3 (3N21d) and for sows of ins/ins and del/del genotypes, by 0.88 piglet for the number of piglets born in parity 4 (4NBA). Also between sows of del/del and ins/del genotypes for the number of piglets born in parity 3 (3NBA) and the number of piglets born in parities 1 to 4 (1-4NBA) by 0.43 and 0.52 piglet, respectively. At locus g.36251G>A, also more than 0.5 piglet was found for the number of piglets born and reared in parity 3 (3NBA and 3N21d) between sows of AA and AG genotype, and for the number of piglets born and reared in parity 4 (4NBA and 4N21d) between AA and GG sows. However, sows with the greater number of piglets born and reared per litter belonged to the groups of animals with lowest frequency of genotypes at different loci (total breeds – from 0.09 to 0.13; PLW – from 0.11 to 0.17; PL – from 0.06 to 0.10), which could influence the statistical power of analysis. Furthermore, frequency analysis of genotypes in the above polymorphisms at the *WNT7A* gene – in accordance with the Hardy-Weinberg law – showed departure from Hardy-Weinberg equilibrium for PLW at locus g.36220delinsAT and for total breeds and PLW at locus g.36251G>A. Departure from Hardy-Weinberg equilibrium may indicate potential genotyping errors, but also the actual relationship with the analysed trait (Turner et al., 2011). It seems that in the test protocol we can eliminate the genotyping error. In turn, the lack of accordance with the Hardy-Weinberg law could be due to long-term selection for production traits not associated with reproduction, especially since the experimental sows originated from nucleus herds (active population), which are selected intensively based on breeding value estimation using BLUP. For many years, these herds were selected for fattening and slaughter traits. Currently the improvement in the number of piglets born and reared per litter is one of the main selection criteria in the breeding programmes, especially for maternal lines such as the PLW and PL breeds. However, litter size is a complex trait that depends mainly on environmental factors but is also determined by many genes. The analyses of candidate gene, QTL mapping or genome-wide association studies contribute to better understanding of the genetic determination of litter size.

Few studies addressed the effect of the *WNT7A* gene polymorphism on phenotypic characteristics. In humans, few mutations such as the recessive form of missense in exon 4 c.1179C>T and exon 3 c.630G>A, and three synonymous g.13896140A>G

and g.13896284C>T (Woods et al., 2006), in exon 4 c.664C>T (Kantapura et al., 2010), cause a range of limb malformations, including Al-Awadi/Raas-Rothschild/ Schinzel phocomelia syndrome (AARRS) and Fuhrmann syndrome in humans. AARRS may also be caused by two other mutations, c.713T>G (Sahin et al., 2013) and c.610G>A (Eyaid et al., 2011). In addition, sequencing of the *WNT7A* gene in humans with Santos syndrome revealed a new homozygous mutation c.934G>A linked to this disease (Alves et al., 2014). The polymorphic change in exon 1 of the *WNT7A* gene was also analysed for its effect on congenital clubfoot, but it was found not to be the immediate cause of the disease (Shyy et al., 2009; Sinnwell et al., 2006).

In turn, studies in farm animals were only performed with Chinese Qinchuan cattle, in which the effect of *WNT7A* on growth traits was analysed (Xue et al., 2013). Sequencing of this gene in cattle showed the presence of three mutations: g.T4926C and g.A21943G localized in introns and g.C63777T in exon 4. At the g.C63777T locus, animals with G_3G_3 genotype were characterized by significantly greater height, chest width and height at hip cross compared with genotype A_3A_3 ($P<0.01$ or $P<0.05$). The other polymorphisms, g.T4926C and g.A21943G had no significant effect on growth traits. In our study, the *WNT7A* gene was analysed for the effect on reproductive traits in pigs, but we cannot compare the results of our research with those of other authors.

The *WNT7A* gene plays a major role in postmenstrual regeneration and growth of endometrial epithelium (Fan et al., 2012) as well as during embryo implantation (Hayashi et al., 2009) and early pregnancy (Hayashi et al., 2007). Therefore, the *WNT7A* gene appeared to be a candidate gene for reproductive traits in pigs. Three new mutations were identified in this gene. For most traits under analysis, the largest litters were found in sows with AA genotype for the g.6270G>A and g.36251G>A mutations and in sows with ins/ins genotype for the g.3622delinsAT mutation compared to sows with the other genotypes. This suggests that *WNT7A* gene polymorphisms may be associated with litter size in pigs. However, this was statistically confirmed in only a few cases. In the analysed group of animals, sows with larger litters were rather infrequent, especially at locus g.3622delinsAT. This could contribute to showing the statistical correlation between *WNT7A* gene polymorphisms and reproductive traits. Unfortunately, our results cannot be compared with those of other authors. But we cannot rule out the impact of newly identified polymorphisms on reproductive traits in other breeds and lines of pigs.

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