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Polymorphism of *ESR, FSHß, RBP4, PRL, OPN* genes and their influence on morphometric traits of gilt reproductive tract before sexual maturity

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Abstract

The aim of the study was to examine the effect of gene polymorphism on the development of the reproductive system in 100 Polish Large White and 100 Polish Landrace gilts. Gilts were slaughtered when they reached 100 kg, and their uterus weight and length, as well as the ovary weight and volume were evaluated. Differences between gilts of both breeds were observed in some traits of the reproductive system, such as the uterine weight without the broad ligament ($P \leq 0.05$) and the uterine capacity ($P \leq 0.01$). Polish Landrace gilts were characterized with higher uterine weight and higher uterine capacity; they were also younger at the time of slaughter ($P \leq 0.05$). No *BB* homozygote relative to the oestrogen receptor gene was found in the examined populations of Polish Landrace gilts. Also, a very low number of gilts with the *AA* genotype relative to follicle stimulating hormone gene was observed in both populations. Polymorphism of the examined genes had an effect on numerous traits which define the size of particular uterine sections, and thus it influenced the development of potential fertility of gilts before they entered the reproductive cycle. Based on our results we can conclude that genetic determination of reproductive potential occurs already in prepubertal gilts. The results may be used for the prognosis of fertility potential in gilts.

Pigs, prepubertal gilts, reproductive system, genetic markers, gene effects

Reports on the possibility of genetic selection of animals with a better potential for reproduction are becoming more frequent (Spötter and Distl 2006; Rempel et al. 2010). Studies have been conducted on the polymorphisms of many candidate genes which could be used as markers of phenotypic traits for sow breeding performance (Korwin-Kossakowska 2007). A selection intended to increase the frequency of favourable alleles in nucleus herd sows ensures increased litter sizes (Bjerre et al. 2010). This project studies the polymorphism of five genes potentially affecting breeding traits, and examines traits defining the morphometric development of the reproductive system in gilts before they reach sexual maturity.

The aim of our study was to evaluate the polymorphism of five genes potentially affecting breeding traits, and examine the traits defining the morphometric development of the reproductive system in gilts before they reach sexual maturity. The second aim of this study was to determine the relationship between gilt genotypes relative to the oestrogen receptor gene (*ESR*), follicle stimulating hormone gene (*FSHß*), retinol binding protein gene (*RBP4*), prolactin receptor gene and osteopontin gene (*OPN*), and the morphometric characteristics of their reproductive system for the early recognition of potential breeding effectiveness.

Materials and Methods

The research was conducted on 100 Polish Large White gilts (PLW) and 100 Polish Landrace gilts (PL) at the Swine Slaughter Performance Control Station. The pigs were slaughtered when they reached approximately 100 kg (101.6 ± 2.96 kg) of live weight, and their blood was taken in order to mark the genotypes of examined genes. The experimental procedures were approved by the Local Ethics Committee (No 21/2008).

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Immediately after the slaughter, the following indicators of morphometric evaluation of the reproductive tract were measured: the weight of the uterus with and without the broad ligament, the length of the vagina and cervix, the length of the uterine horns, the proportion of the uterus weight to the length of its horns and the length of the oviducts and their diameter. Uterine capacity was measured using the volumetric method (Kapelański et al. 2013); similarly the weight of the ovaries and their volume were determined volumetrically. Apart from that, the number of teats on the gilt's carcass was also recorded.

The genomic DNA was isolated with the use of the Wizard Genomic DNA Purification Kit (PROMEGA, Madison, WI, USA). Based on the PCR-RFLP technique, we examined the polymorphisms of the oestrogen receptor gene (*ESR*) with the use of restriction enzyme *Pvu*II (Short et al. 1997), follicle stimulating hormone gene *(FSHß)* with *Hae*III restriction enzyme (Rohrer et al. 1994), retinol binding protein gene *(RBP4*) with *Msp*I restriction enzyme (Rothschid et al. 2000) and prolactin receptor gene *(PRLP)* with *Alu*I restriction enzyme (Kmieć and Terman 2004). Based on the PCR technique, we also determined the polymorphism of the osteopontin gene (*OPN*) (Knoll et al. 1999). The frequency of alleles and gilt genotypes relative to particular genes were determined.

Both the arithmetic mean (x) and standard deviations (s) were calculated. A two-way analysis of variance ANOVA/MANOVA was carried out, taking into account the breeds and genotypes of gilts in relation to particular genes. The significance of differences was estimated using Duncan test. Calculations were made with the use of the STATISTICA 8 PL software (2008).

Results

Table 1 presents the comparison of PLW gilts and PL gilts in terms of some of the most important reproductive system traits. The age of gilts at which organs were examined was also taken into consideration. The PL gilts compared to PLW gilts were significantly younger at the time of slaughter ($P \leq 0.05$). Nevertheless, the weight of the uterus without the broad ligament was significantly higher in PL gilts ($P \leq 0.05$). Other uterus characteristics, such as the length of the vagina, cervix and uterine horns were similar in both breeds and did not display any significant diversity related to the breed. However, considerable differences between gilts of both breeds were found in relation to the volumetrically determined uterine capacity ($P \leq 0.01$). PL gilts were characterized by larger uterine capacity than PLW gilts at the time of slaughter. No significant difference between gilts of both breeds in terms of oviduct and ovary size was found. The weight and volume of the ovaries as well as teat numbers were also similar in PLW and PL gilts.

Table 2 shows the frequency of gilt genotypes, reflecting how often particular variants of the examined gene occur. What deserves special attention is the complete lack of PL gilts with the *BB* genotype in the *ESR* gene locus and negligible percentage in both breeds of animals with the *AA* genotype in relation to follicle stimulating hormone gene (*FSHß*).

Table 1. The characteristics of the reproductive systems in Polish Large White and Polish Landrace gilts.

Indicator	PLW	PL	Significance of differences
Age at slaughter (days)	171.17 ±18.04	166.07 ± 18.09	$P \leq 0.05$
Uterine weight without ligament (g)	129.19 ± 52.77	146.87 ± 66.14	$P \leq 0.05$
Vaginal length (cm)	10.53 ± 2.41	9.55 ± 2.58	NS
Cervical length (cm)	12.60 ± 2.24	12.35 ± 2.35	NS
Length of both uterine horns (R +L) (cm)	97.72 ± 17.28	99.38 ± 19.10	NS
Uterine capacity (cm^3)	132.55 ± 63.79	155.99 ± 67.28	$P \leq 0.01$
Length of both oviducts, (R + L) (cm)	39.15 ± 5.65	41.04 ± 7.61	NS
Average oviduct diameter, (mm)	2.57 ± 0.72	2.61 ± 0.68	NS
Weight of both ovaries (R + L) (g)	7.24 ± 1.90	7.15 ± 1.98	NS
Volume of both ovaries (R +L) (cm^3)	5.26 ± 2.12	5.24 ± 2.11	NS
Teat number (n)	14.38 ± 1.73	14.53 ± 1.02	NS

PLW - Polish Large White, PL - Polish Landrace, NS – not significant, R – right, L - left

Table 2. The frequency of genotypes and number of gilts within each genotype in relation to examined genetic markers population of Polish Large White and Polish Landrace gilts.

Genes	Genotype	PLW	PL	Total
ESR	*AA*	0.384 (n = 38)	0.848 (n = 84)	0.616 (n = 122)
	AB	0.485 (n = 48)	0.152 (n = 15)	0.318 (n = 63)
	BB	0.131(n = 13)	-	0.066 (n = 13)
Total		99	99	198
FSHβ	*AA*	0.052 (n = 5)	0.011 (n = 1)	0.032 (n = 6)
	AB	0.206 (n = 20)	0.168 (n = 15)	0.188 (n = 35)
	BB	0.742 (n = 72)	0.820 (n = 73)	0.779 (n = 145)
Total		97	89	186
RBP4	*AA*	0.309 (n = 30)	0.444 (n = 44)	0.377 (n = 74)
	AB	0.289 (n = 28)	0.414 (n = 41)	0.352 (n = 69)
	BB	0.402 (n = 39)	0.141 (n = 14)	0.270 (n = 53)
Total		97	99	196
PRLR	*AA*	0.112 (n = 21)	0.402 (n = 39)	0.306 (n = 60)
	AB	0.404 (n = 40)	0.423 (n = 41)	0.413 (n = 81)
	BB	0.384 (n = 38)	0.175 (n = 17)	0.281 (n = 55)
Total		99	97	196
OPN	*AA*	0.102 (n = 10)	0.320 (n = 31)	0.210 (n =4 1)
	AB	0.071 (n = 7)	0.175 (n = 17)	0.123 (n = 24)
	BB	0.826 (n = 81)	0.505 (n = 49)	0.667 (n = 130)
Total		98	97	195

PLW - Polish Large White, PL - Polish Landrace, ESR - estrogen receptor gene, FSH - follicle stimulating hormone gene, RBP4 - retinol binding protein gene, PRLR - prolactin receptor gene, OPN - osteopontin gene

Table 3 presents complete results indicating to what extent particular genes affected the examined reproductive system traits.

Significant effect of the genotype on the *ESR* locus was observed only in PLW gilts, and it was related to the weight of the uterus including the broad ligament and the length of uterine horns ($P \leq 0.05$). As expected, the influence of the genotype in the *FSHβ* locus was related to the weight of ovaries and their volume ($P \leq 0.05$), and it occurred only in PLW gilts. The effect of the genotype in the *RBP4* locus was related to the length of uterine horns in PLW gilts ($P \leq 0.01$), and the proportion of the uterus weight to the length of uterine horns in PL gilts ($P \leq 0.05$), as well as the volume of ovaries ($P \leq 0.05$). The effect of genotype in the prolactin locus was related to the capacity of the uterus in PL gilts, the length of oviducts of PLW gilts and the number of teats in PL gilts ($P \leq 0.05$). As regards PL gilts, the last of the examined *OPN* genotype markers had an influence on the weight of the uterus with and without the broad ligament ($P \leq 0.05$).

Discussion

The studied Polish Large White and Polish Landrace breeds showed that both breeds are similar to each other in most performance traits (Tyra and Żak 2010; Różycki and Tyra 2012). There were no big differences in the morphometric characteristics of the reproductive tract, either. They were found in the weight of the uterus without the broad ligament and in volumetrically determined uterine capacity. PL gilts were characterized

Table 3. The effect of gilt genotype relative to particular genes on the reproductive system characteristics.

Indicator	Breed	Genotype				
		ESR	*FSHβ*	*RBP4*	*PRLR*	*OPN*
Uterine weight incl. ligament	PLW	a	-	-	-	-
	PL	-	-	-	-	a
Uterine weight excl. ligament	PLW	-	-	-	-	-
	PL	-	-	-	-	a
Vaginal length	PLW	-	-	-	-	-
	PL	-	-	-	-	-
Cervical length	PLW	-	-	-	-	-
	PL	-	a	-	-	-
Length of horns (R + L)	PLW	a	-	b	-	-
	PL	-	-	-	-	-
Weight/length of horns	PLW	-	-	-	-	-
	PL	-	-	a	-	-
Uterine capacity	PLW	-	-	-	-	-
	PL	-	-	-	a	-
Length of both oviducts (R + L)	PLW	-	-	-	a	-
	PL	-	-	-	-	-
Weight of ovaries (R + L)	PLW	-	a	-	-	-
	PL	-	-	-	-	-
Volume of both ovaries (R + L)	PLW	-	a	-	-	-
	PL	-	-	a	-	-
Number of teats	PLW	-	-	-	-	-
	PL	-	-	-	a	-

PLW - Polish Large White, PL - Polish Landrace, ESR - estrogen receptor gene, FSH - follicle stimulating hormone gene, RBP4 - retinol binding protein gene, PRLR - prolactin receptor gene, OPN - osteopontin gene, – no significant differences, a – differences between genotypes significant with $P \leq 0.05$, b – differences between genotypes significant with $P \leq 0.01$

by better development of the intrauterine space, which guarantees better developmental conditions and enough space for a larger number of foetuses (Foxcroft et al. 2009; Vallet 2000; Wu and Dziuk 1989). The size of uterine capacity may probably be indicative of the high breeding value of gilts.

Gilt fertility estimated according to litter size can be increased by selection based on genetic markers. A number of important relations between genotypes of some markers and the litter size have been reported (Rotschild et al. 1996, 2000; Linville et al. 2001; Van Rens et al. 2002). In the opinion of numerous researchers, selection aimed at the increase of litter size based on several markers is more effective (Spötter and Distl 2006).

Lack of complete heterogeneity in respect to a particular genetic marker may be an obstacle in the breeding selection of suitable gilt genotypes. Analysing the frequency of both alleles in each gene, we established that the largest disproportion in alleles of the estrogen receptor gene *(ESR)* and follicle stimulating hormone gene *(FSHβ)* occurs in Polish Landrace gilts. As a result, there were no animals with the *BB* genotype in the *ESR* gene locus within the examined group, and only one gilt with the *AA* genotype in the *FSHβ* locus. Lack of polymorphism in certain genes and the unstable frequency of the occurrence of both alleles of a given gene have been observed in some breeds, lines and populations of pigs bred in the USA, Europe and Asia (Drögemüller et al. 2001; Kmieć et al. 2002; Terman et al. 2006; Wang et al 2006; Wu et al. 2006).

The breeding potential of gilts is determined by many factors and fully reveals itself after reaching sexual maturity and giving birth to the first and further litters. Our study focused only on the morphometric characteristics of the gilts' reproductive system before they reach sexual maturity. However, we were able to determine a number of significant effects of the genotype of the examined markers on the indicators of the reproductive system in gilts of both breeds. They concerned the weight of the uterus, the length of uterine horns and the uterine capacity as well as the weight and volume of the ovaries. The results may be used for fertility prognosis in gilts.

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