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Prolactin receptor gene polymorphism in Polish Landrace boars

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Prolactin receptor gene (*PRLR*), which is localized in chromosome 16 of pigs, is a candidate gene marker for reproductive traits. The polymorphism in *PRLR* gene *locus* was detected using PCR-RFLP method, with specific primers and restriction enzyme *Alu*I. Two different alleles were identified – $PRLR^A$ and $PRLR^B$ – with the estimated frequencies of 0.4068 and 0.5932, respectively. In the studied population of boars, the genotype $PRLR^APRLR^A$ was detected with the frequency of 0.2373, $PRLR^APRLR^B$ with 0.3390, and $PRLR^BPRLR^B$ with 0.4237. Significant differences ($P \leq 0.01$) were shown in ejaculate volume and sperm concentration between boars of different *PRLR* genotypes.

KEY WORDS: boars / gene polymorphism / Landrace / prolactin receptor gene

The progress in the research on porcine genome enabled identification of polymorphic *loci* of individual genes determining the reproduction traits of animals. One of them is the prolactin receptor gene (*PRLR*) mapped in pigs to chromosome 16 with regional assignment to 16q1.4 or 16q2.2-2.3 [Vincent *et al*. 1997]. The gene is a strong candidate gene for reproductive traits in pigs evidently affecting the litter size. In the first litter, its effect was estimated from zero to 0.71 piglet per litter for the total number born and number born alive, respectively [Rothschild *et al*. 1998, Vincent *et al*. 1998, Southwood *et al*. 1999].

The presented study aimed at estimating the frequency of *PRLR* gene mutation and to find possible relations between *PRLR* genotype and reproductive traits in boars.

Material and methods

The study was carried out on 59 Polish pedigree Landrace boars kept at pedigree stations. The semen was obtained to inseminate sows kept at the same stations (Tab. 1).

Genotypes of the *PRLR* were determined by the PCR-RFLP. Fragment of PRLR DNA 457 base pair (bp) long was amplified with primers of sequences proposed by Vincent *et al* [1997]: forward 5'–CCC AAA ACA GCA GCA GGA GGA CG–3', reverse 5'–GGC AAG TGG TTG AAA ATG GA–3'. Conditions of PCR were as follows: 94°C for 5 min followed by 35 cycles of 40 s at 94°C, 40 s at 60°C, 40 s at 72°C and the last extension 5 min at 72°C. PCR product was than digested with 6 I.U. of restriction enzyme *Alu*I (MBI FERMENTAS) at 37°C overnight. The restriction fragments were separated by 5% agarose gel electrophoresis and stained with ethidium bromide. The results were visualized in UV rays.

Analysed was the relationship between the polymorphism in the *PRLR/Alu*I gene and ejaculate volume, spermatozoa concentration, per cent of live spermatozoa, number of insemination doses and age of the boars at the moment of ejaculate collection, despite the fact that the ejaculates were collected from the boars at age range 221-400 days only. The relations between *PRLR* genotype and studied reproductive traits were evaluated with analysis of variance and the significance of differences was verified using Duncan test with Likehood Computer Programme PC – 1, according to Harvey [1987]. The following model was used:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + d_l + e_{ijklm}$$

where:

Y_{ijkl} – value of the trait for the *i*-th *PRLR* genotype, *j*-th year, *k*-th month, and *l*-th father;

μ – population mean;

a_i – effect of *i*-th *PRLR* genotype (*i*=1,2,3);

b_j – constant effect of *j*-th year (*j*=1,2,…, 6);

c_k – constant effect of *k*-th month (*k*=1,2);

d_l – constant effect of *l*-th father;

e_{ijklm} – error.

Results and discussion

In the group of boars studied, two alleles of *PRLR* gene were identified with PCR-RFLP method: $PRLR^A$ and $PRLR^B$ responsible for three genotypes – $PRLR^APRLR^A$, $PRLR^APRLR^B$ and $PRLR^BPRLR^B$. The following lengths of restriction fragments were detected : 124, 90, 79, 77, 67 and 20 base pair (bp) for allele $PRLR^A$, and 124, 110, 79, 77, and 67 bp for $PRLR^B$. In the analysed AI boars the alleles $PRLR^A$ and $PRLR^B$ occurred

with the frequency of 0.4068 and 0.5932, respectively. The *PRLR*^{A}*PRLR*^{A} genotype occurred with the frequency of 0.2373, *PRLR*^{A}*PRLR*^{B} with 0.3390 and *PRLR*^{B}*PRLR*^{B} with 0.4237 – Table 1.

Table 1. Frequency of *PRLR/AluI* genotypes and alleles in 59 Landrace boars

Item	*PRLR/AluI* genotype			*PRLR* allele	
	PRLR^{A} *PRLR*^{A}	*PRLR*^{A} *PRLR*^{B}	*PRLR*^{B} *PRLR*^{B}	*PRLR*^{A}	*PRLR*^{B}
Number of animals	14	20	25	0.4068	0.5932
Frequency	0.2373	0.3390	0.4237		

et al. [2001] and Linville *et al*. [2001] in the pedigree Landrace sows, while higher in Duroc pigs by Drogemüller *et al*. [2000].

Table 2 presents means and their standard errors for the boar semen traits across *PRLR/Alu*I genotypes.

Mean ejaculate volume for all the boars was 233.9 cm^3 falling within the range

Table 2. Means and their standard deviations (SD) for semen traits across *PRLR/AluI* genotypes in Landrace boars

PRLR/AluI genotype	Number of ejaculate	Ejaculate volume (cm^3)		Sperm concentration (10^6/cm^3)		Live spermatozoa (%)		Number of live spermatozoa per ejaculate (billion)		Number of insemination doses	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
PRLR^{A} *PRLR*^{A}	422	225.6**	8.3	598.6**	11.9	73.4	0.2	93.8	3.5	25.2	1.0
PRLR^{A} *PRLR*^{B}	342	242.4**	7.0	563.0**	10.0	73.4	0.2	93.5	3.0	25.8	0.8
PRLR^{B} *PRLR*^{B}	429	233.7**	6.3	557.6**	9.1	73.6	0.1	91.1	2.7	24.5	0.7
Total	1193	233.9	5.2	573.1	7.5	73.5	0.1	92.8	2.2	25.2	0.6

**P≤ 0.01.

of 50-245 cm^3 reported by Dubiel [1985]. The highest mean ejaculate volume (242.4 cm^3) was found in the *PRLR*^{A}*PRLR*^{B}, while the lowest (225.6 cm^3) in *PRLR*^{A}*PRLR*^{A} genotype boars (P≤0.01) – Table 2.

Mean spermatozoa concentration was 573.1 × 10^6/cm^3. The highest concentration showed the semen of *PRLR*^{A}*PRLR*^{A} (598.6 × 10^6/cm^3), while the lowest of *PRLR*^{B}*PRLR*^{B} (557.6 × 10^6/cm^3) boars (P≤0.01) – Table 2.

The inter-genetype differences for per cent of live spermatozoa, number of live spermatozoa in ejaculate and number of insemination doses were small and not significant.

Summarizing, two *PRLR* alleles were identified in 59 Polish Landrace boars.

The analysis of relation between the *PRLR* genotype and the studied reproductive traits showed statistically Significant differences (P≤0.01) were shown in ejaculate volume and spermatozoa concentration of ejaculates of boars of different *PRLR* genotypes.

The preliminary study showed that $PRLR^{A}PRLR^{B}$ boars produced ejaculates of large volume and higher spermatozoa percentage as well as high spermaatozoa number per ejaculate and number of insemination does. A possibility is anticipated of using the existing polymorphism in the *PRLR/Alu*I gene to improve some reproductive performance traits in boars such as qualitative and quantitative characters of semen. The results, however, should be verified by further research of *PRLR/Alu*I polymorphism on a larger number of animals.

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Polimorfizm genu receptora prolaktyny u knurów rasy polskiej białej zwisłouchej

Streszczenie

Gen receptora prolaktyny (*PRLR*) położony jest w 16 chromosomie świni jako gen kandydat cech reprodukcyjnych.U 59 knurów pbz oznaczono metodą PCR-RFLP polimorfizm genu *PRLR*, posługując się specyficznymi primerami oraz enzymem restrykcyjnym *Alu*I. Zidentyfikowano dwa allele – $PRLR^{A}$ i $PRLR^{B}$ – o frekwencjach odpowiednio 0,4068 i 0,5932. Frekwencje genotypów $PRLR^{A}PRLR^{A}$, $PRLR^{A}PRLR^{B}$ i $PRLR^{B}PRLR^{B}$ wyniosły odpowiednio 0,2373, 0,3390 i 0,4237. Wykazano istotne różnice ($P \leq 0,01$) w objętości ejakulatu i koncentracji plemników między knurami o poszczególnych genotypach *PRLR*.