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Association Between *RBP4* Gene Polymorphism and Reproductive Traits in Polish Sows

[1]Arkadiusz Terman, [1]Marek Kmiec, [1]Daniel Polasik and [2]Artur Rybarczyk
[1]Department of Genetics and Animal Breeding, West Pomeranian University of Technology, ul. Doktora Judyma 6, 71-466 Szczecin, Poland
[2]Department of Livestock Product Exaluation, West Pomeranian University of Technology, ul. Doktora Judyma 24,71-466 Szczecin, Poland

Abstract: The aim of the experiment was to detect polymorphism in the *RBP4* gene to determine associations between the genotype and litter size in Polish large white x Landrace sows. Reproductive traits investigated were: Total Number of piglets Born (TNB), Number of piglets Born Alive (NBA) and number of piglets weaned (TW). The polymorphism in *RBP4* gene was detected using the PCR-RFLP method with specific primers and the restriction enzyme (MspI). Two different alleles were identified: alleles A (0.34) and B (0.66). The relationship between the RBP4 genotypes and TBN, NBA and NW were analyzed. The analysis of *RBP4* gene showed that sows with BB genotype had the largest litter size compared to AB and BB sows and the difference was statistically significant ($p \leq 0.01$). Analysis of the interaction PARITY×RBP4 showed small and statistically not significant differences.

Key words: RBP4, polymorphism, reproductive traits, sows, polish, genotype

INTRODUCTION

The reproduction, particularly in the species having large litters (like pigs), plays an important role in the successful production of farm animals. The advancement in research on swine genome enabled identification of polymorphic loci of individual genes that control the level of reproductive traits which are know to have influence on reproductive performance in sows (Wang *et al*., 2006; Terman, 2005; Linville *et al*., 2001; Droegemueller *et al*., 1999; Rothschild *et al*., 1996). Currently, genetic markers for sow production are identified by two main approaches, Quantitative Trait Loci (QTL) and candidate gene (Rohrer *et al*., 1999).

One of these genes can be: Retinol Binding Protein 4 (*RBP4*) gene. The *RBP4* gene was localized in chromosome 14 in pigs (Harney *et al*., 1993) and have shown that there is an increasing *RBP4* gene expression in gravid porcine endometrium from day 10-12. Their results support an important role for this vitamin A transport protein in uterine and conceptus physiology during the establishment of pregnancy. Therefore, RBP4 was investigated as a candidate gene for litter size owing to its role at the time of high embryonic mortality rate. Messer *et al.* (1996) were detected the polymorphism in pig genomic DNA by hybridization of Southern blots with a pig Retinol Binding Protein 4 (RBP4). This polymorphism was significantly associated with litter size in pigs (Drogemuller *et al*., 2001; Rothschild *et al*., 2000). The goal of the experiment was to estimate the frequencies of *RBP4* gene mutations and to find possible associations between different genotypes of these genes and litter size traits in polish hybrid sows.

MATERIALS AND METHODS

The experimental populations included in total 444 Polish Large White x Landrace crossbreed sows. The animals were bred and raised at a farm in Western Pomerania (Poland). Rearing and feeding conditions were equalized for all animals. Genomic DNA was extracted from blood sample using Master pure kit of Epicentre technologies. Genotypes of the *RBP4* gene were determined by the PCR-RFLP method reported by Rothschild *et al.* (2000). Information on primer sequences, restriction enzyme and allele sizes are shown in Table 1. Each sample prepared for the PCR included: 1.5 μL 10×PCR buffer, 1.3 μL 1.5 mM $MgCl_2$, 1.2 μL 10 mM dNTPs, 0.5 μL 10 μM forward primer, 0.5 μL 10 μM reverse primer, 1.5 μL genomic DNA, 1 μL Taq DNA

Corresponding Author: Arkadiusz Terman, Department of Genetics and Animal Breeding, Agriculture University of Szczecin, ul. Doktora Judyma 6, 71-466 Szczecin, Poland

polymerase (MBI fermentas) and 7.5 μL sterile deionised water. All reactions were performed on Biometra Cycler using the following temperature program: 93°C for 3 min followed by 40 cycles of 93°C from 30 sec, 56°C for 45 sec, 72°C for 45 sec and ending with a final step of 72°C for 5 min.

Digestion of PCR product (550 bp) was performed with 3 IU of appropriate restriction endonuclease MspI at 37°C overnight. The PCR product was then examined by electrophoresis on a 2% agarose gel stained with ethidium bromide. After that the gels were analyzed in UV rays transillumination and recorded with the use of the Vilber Lourmat system.

Performance traits data were collected from farm records. All data were analyzed with SAS/STAT. The procedure mixed was used for analyzing the following linear model:

$$Y_{ijklm} = \mu+g_i+ys_j+p_k+d_l+s_m+e_{ijklm}$$

Where:

Y_{ijklm} = Total Number Born (TNB), Number Born Alive (NBA), Number Weaned (NW)
μ = Population mean
g_i = Effect of ith genotype (i = 1, 2, 3)
ys_j = Effect of jth year season (j = 1, 2,..., 24) (6 years *4 seasons)
p_k = Effect of kth the parity (k = 1 ≥2)
d_l = Random polygenic effect of the lth dam (l = 1, 2, ..., 301)
s_m = Random polygenic effect of the mth sire (k = 1, 2, ..., 46)
e_{ijklm} = Random residual term

The variance components for random effect were estimated by MIXED procedure using the expectation-maximisation REML method.

Table 1: Endonuclease and allele sizes of *RBP4* gene

Gene	PCR product size (bp)	Endonuclease	Allele size (bp)	Source
RBP4	550	MspI	A-190, 154, 136, 70 B-190, 136, 125, 70, 29	Rothschild *et al.* (2000)

RESULTS AND DISCUSSION

Two RBP4 alleles were identified in sow herd under study: A and B. Three genotypes, namely AA, AB and BB were observed. The length of restriction fragments are shown in Table 1. The allele and genotype frequency were distributions in Hardy-Wainberg equilibrium. In the analyzed sows herd, the allele A occurred with the frequency 0.61 whereas the allele B with the frequency 0.39. The AA genotype occurred with the frequency 0.37, AB with frequency 0.48 and BB with 0.15 (Table 2). Researchers were analyzed effects of genotypes of the *RBP4* gene on litter size and the results are shown in Table 3.

The analysis of the Total Number Born (TNB), Number Born piglets Alive (NBA) showed in first and second parity sows statistically significant ($p \leq 0.05$) differences between sows carrying BB genotypes compared to AA and AB genotypes.

The sows with BB genotypes had also larger number of piglets weaned (NW) than sows carrying AA and AB genotypes and this differences were statistically

Table 2: The frequency of RBP4 genotypes and alleles of Polish Large White x Landrace crossbreed sows

		Genotype			Allele	
Breed	Parameters	AA	AB	BB	A	B
Large White x Landrace	Number	165.00	212.00	66.00	0.61	0.39
	Frequency	0.37	0.48	0.15	-	-

Table 3: Effects of *RBP4* genotypes on reproductive traits of Large White x Landrace crossbred sows (least square means±standard error of the mean)

RBP4 genotype	Parity	n	TNB[1]	NBA[1]	NW[1]
AA	I	143	9.07±0.23^{a}	8.69±0.21^{a}	8.12±0.22^{A}
AB		198	9.05±0.17^{a}	8.85±0.19^{a}	8.52±0.21^{A}
BB		61	9.31±0.32^{b}	9.18±0.31^{b}	9.00±0.30^{B}
AA	II	138	8.83±0.20^{a}	8.73±0.22^{a}	8.69±0.22
AB		185	9.07±0.19^{a}	8.85±0.22^{a}	8.67±0.21
BB		59	9.22±0.29^{b}	9.11±0.31^{b}	8.88±0.28
AA	≥III	285	9.85±0.18	9.67±0.20	9.54±0.20
AB		485	9.98±0.16	9.71±0.19	9.63±0.20
BB		98	10.08±0.21	9.81±0.25	9.69±0.23

Small letters (a, b) significance difference ($p \leq 0.05$); capital letters (A, B) significance difference ($p \leq 0.01$), [1]TNB: Total Number Born; NBA: Number of piglets Born Alive; NW: Number of piglets Weaned, n: number of sows within parities

significant ($p \leq 0.01$) in 1st parity (Table 3). In later (≥III) parities sows with the BB genotype still had the largest litter size compared to AA and AB sows but the difference was statistically not significant. Analysis of the interaction PARITY×RBP4 showed small and non-significant differences. A similar frequency of allele A (0.62) was observed in German Landrace x Duroc crossbreed sows (Drogemuller *et al*., 2001; Rothschild *et al*., 2000). A higher frequency of allele A (0.67) was observed by Drogemuller *et al*. (2001) who studied the breed German Landrace and 0.65 by Terman *et al*. (2006) in Large White sows. A lower frequency of allele A compared to the present study was revealed in Landrace (0.59), Large White (0.55) and Landrace x Large White crossbreed (0.42) pigs (Rothschild *et al*., 2000; Wang *et al*., 2006; Linville *et al*., 2001). Rothschild *et al*. (2000) using data of nearly 2,800 L of 1,300 RBP4 genotyped sows of six commercial lines reported a significant additive effect associated with RBP4 of +0.15 NBA.

Rothschild *et al*. (2000) stated that it is difficult to determine whether it is linked to one or more genes having this effect. Wang *et al*. (2006) showed that sows with BB genotype of RBP4 locus had more piglets per litter than sows with AA and AB genotypes. Researchers showed that the sows with BB genotype of RBP4 had an advantage of 1.60 pigs L^{-1} over the AB although, the difference was not significant. These results are comparable to the results reported in this study. The analysis of the Total Number Born (TNB) and Number Born Alive (NBA) reported by Drogemuller *et al*. (2001) showed no significant effect RBP4 locus on litter size in pigs. The results showed that the sows with AA>AB genotype has the same trend inall analyzed reproductive traits.

CONCLUSION

The study of retinol binding protein 4 gene showed that the sows with the BB genotype has more piglet than sows with the AA and AB genotypes. This result was confirmed statistically ($p \leq 0.01$ and $p \leq 0.05$) in 1st and 2nd parity. The present study showed that the *RBP4* gene is strongly associated with reproductive traits in Polish sows. In later parities sows carrying the BB genotype had still the largest litter size compared to AB and AA sows but the difference was statistically not significant. Thus, the researchers hypothesize that the *RBP4* gene might be a marker or gene for litter size in White Large x Landrace crossbreed sows with the B allele as the favorable allele. However, this should be verified by a larger number of animals.

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