

ANALYSIS OF ESR AND RBP POLYMORPHISMS IN BLACK SLAVONIAN SOWS: PRELIMINARY RESULTS

Anamaria EKERT KABALIN ¹, Kristina STARČEVIĆ ¹, Sven MENČIK ¹, Maja MAURIĆ ¹,
Velimir SUŠIĆ ¹, Igor ŠTOKOVIĆ ¹

ABSTRACT

Black Slavonian pig belongs to the breeds of medium fertility with relatively high variation in litter size, denoting that it could be included in selection using molecular genetics. Estrogen receptor gene (*ESR*) and retinol binding protein 4 (*RBP4*) are candidate gene markers for sows' reproductive traits. The aim of this preliminary study was to investigate frequencies of different *ESR-PvuII* and *RBP4-MspI* genotypes in fourteen Black Slavonian sows, as well as their reproductive traits. Sows were genotyped with PCR-RFLP method. Allele and genotype frequencies were calculated and Hardy-Weinberg equilibrium was tested using χ^2 -test. Litter size differences between sows of different genotypes were analyzed with analysis of variance. Three *ESR-PvuII* genotypes were detected with relative frequencies AA (0.54), AB (0.38) and BB (0.08), while the gene relative frequency was 0.73 for allele A and 0.27 for allele B. In the case of *RBP4-MspI* those values were: AA (0.54), AB (0.23) and BB (0.23), while allele frequencies were 0.65 for A and 0.35 for B allele. Analyzed genes were in accordance with Hardy-Weinberg equilibrium. There were no statistically significant differences detected between genotypes for any of the studied trait ($P > 0.05$). Further research on a larger sample size is needed for more conclusive evaluation of the possible correlation between *ESR-PvuII* and *RBP4-MspI* polymorphisms and reproductive parameters in Black Slavonian sows.

Key words: Black Slavonian sow / estrogen receptor gene / retinol binding protein / reproductive parameters

1 INTRODUCTION

Black Slavonian pig is a breed of medium fertility. According to Croatian Agricultural Agency (2013), the average litter size in the year 2012 was 6.12 total piglets born (TNB). As Spötter and Distl (2006) pointed out, no considerable progress in litter size has been made using traditional quantitative genetic methods. Litter size, as a composite trait, has a low heritability which is estimated on average at 0.09 for the number of piglets born alive (NBA). Furthermore, this is one of sex-limited traits which can be observed only after the onset of sexual maturity (Spötter and Distl, 2006). Genetic markers associated with reproductive parameters allow identification of animals carrying beneficial alleles early in life (in both sexes), resulting in reduced generation interval and faster genetic improvement of the trait (Drogemüller *et*

al., 2001). *ESR* gene is located on the intron region of chromosome 1 (SSC1) (Muñoz *et al.*, 2007). The *PvuII* polymorphism at porcine *ESR* gene was recognized as a major gene for litter size. The retinol-binding protein 4 gene (*RBP4*) is located on chromosome 14 (SSC14), and it has been suggested as a candidate gene for litter size (Rothschild *et al.* 2000; Drogemüller *et al.*, 2001).

The aim of this preliminary study was to determine *ESR-PvuII* and *RBP4-MspI* polymorphisms in Black Slavonian sows, as possible candidate genes for litter size performances.

2 MATERIAL AND METHODS

Fourteen Black Slavonian sows with a total of 57 litters were used in this preliminary study. Animals were

¹ Department of Animal Husbandry, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, e-mail: akabalin@vef.hr

extensively reared in quite equable way. DNA was isolated from hair follicles using *One-tube Hair DNA Extraction Kit* (Bio Basic Inc., Markham Ontario, Canada). *ESR-PvuII* and *RBP4-MspI* genotypes were identified separately, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based on methods described by Short *et al.* (1997) and Rothschild *et al.* (2000), and later in papers of Lemus-Flores *et al.* (2009) and Terman *et al.* (2011). PCR amplification (mixture final volume of 13 μ l) was performed using 20-50ng DNA, 1 U of Hot start *Taq* polymerase, 0.2mM dNTPs, 0.4 μ M of each primer, 1x buffer (Promega, USA), 1.5mM $MgCl_2$ and 6.9 μ l H_2O , per reaction. Primers utilized for *ESR* gene amplification were designed according to Short *et al.* (1997), while for *RBP4* gene we used primers described by Rothschild *et al.* (2000) Reactions were performed in a Mastercycler[®] personal 5332 thermocycler (Eppendorf, Germany). Ten microliter of PCR products were digested with 2.5U of *PvuII* and *MspI* enzymes for 3 h at 37 °C. Digested products were submitted to electrophoresis on 2% agarose gel stained by 4% ethidium bromide. Bands were visualized in an ultraviolet transilluminator (Mini BIS Pro[®], DNR Bio-Imaging Systems (Jerusalem, Israel). Restriction fragments identified in *ESR-PvuII* genotypes were as follows: 120 bp for AA, 65, 55 bp for BB and 120, 65, 55 bp for AB. In *RBP4-MspI* genotypes, restriction fragments were: 190, 154, 136 bp in AA homozygote animals, 154, 136, 125, 70 bp in BB, and 190, 154, 136, 125, 70 bp in AB heterozygote sows.

Litter size, including total number of piglets born (TNB), number of piglets born alive (NBA), number of stillborn (NSB) and number of weaned piglets (NW), were collected from the sows' reproductive cards. Data were analysed with Statistica v.10 program. Results are presented as mean \pm standard deviation (SD) and coefficient of variability (CV, %). For NSB median with minimum and maximum are given. Allele and genotype frequencies were calculated, and Hardy-Weinberg equilibrium was tested using χ^2 -test. One-way analysis of variance (ANOVA) and Kruskal-Wallis ANOVA were used to analyze significance of differences between genotypes for studied reproductive traits.

3 RESULTS AND DISCUSSION

For each candidate gene three genotypes were detected in Black Slavonian sows (Table 1). Average reproductive parameters of litter size for *ESR-PvuII* and *RBP4-MspI* are presented in Table 1.

In the case of *ESR-PvuII* genotype, relative frequencies of AA homozygote animals were 0.54, AB heterozygote 0.38, while we detected only one sow with BB geno-

type. It has been postulated that B allele originates from Chinese pig (Isler *et al.*, 2002). So, the presence of B allele in high fertility hybrid sows may be the result of interbreeding of Chinese and English pigs (Alfonso, 2005). This result is in agreement with findings of Omelka *et al.* (2005), and Chvojková and Hraška (2008), whereas some other researchers found higher frequency of B allele in commercial pig breeds and crossbreeds (Alfonso, 2005). As for *RBP4-MspI* the sows with AA were the most frequent (0.54). The same frequency (0.23) was obtained for AB heterozygote and BB homozygote animals. Rothschild *et al.* (2000) mentioned that favourable allele A had higher frequency than the alternative allele in populations included in their survey. According to Drogemüller *et al.* (2001) frequency of allele A ranged from 0.62 to 0.85 in different commercial breeds and synthetic line. Animals included in this survey were found to be in Hardy-Weinberg equilibrium according to the method described by Rodriguez *et al.* (2009).

According to the research results described by Rothschild *et al.* (1996), allele B of *ESR-PvuII* was associated with a higher TNB and NBA in a 50% Meishan synthetic line. These results were confirmed by Short *et al.* (1997) in four synthetic lines of European breeds and Chen *et al.* (2001) in five different breeds. In contrast to their findings, some other researchers did not detect any significant association between the *ESR-PvuII* polymorphism and litter size (Linville *et al.*, 2001; Gibson *et al.*, 2002; Isler *et al.*, 2002; Noguera *et al.*, 2003), or found favourable effect of the allele A on NBA (Van Rens *et al.*, 2002; Goliášová and Wolf, 2004). Although not significant, we found the highest value of TNB, NBA and NW in BB homozygote sow (Table 1). As presented in Table 1, the highest litter size values are recorded in heterozygote sows for *RBP4* gene. Rothschild *et al.* (2000) found an additive effect of allele A on TNB and NBA in some commercial lines, but with no statistically significant effect of any allele on litter size in synthetic line. Similarly, no effect of individual allele was reported in the study of Drogemüller *et al.* (2001), Linville *et al.* (2001) and Korwin-Kossakowska *et al.* (2005).

As Kernerova *et al.* (2009) stated, it is not possible to establish without doubt that certain (so called "beneficial") genotype will always express improved reproductive traits in different populations. Furthermore, allele effects differ between lines or populations due to the genetic background (Drogemüller *et al.*, 2001). For that reason, detailed analysis of possible candidate genes in specific populations should be performed prior to their inclusion in the selection program.

Table 1: Frequency of genotypes/alleles and reproductive parameters of Black Slavonian sows according to candidate gene

Candidate gene	Genotype	Frequency of certain genotypes	Allele frequency	TNB Mean \pm SD (CV)	NBA Mean \pm SD (CV)	NSB Median (min-max)	NW Mean \pm SD (CV)
<i>ESR-PvuII</i>	AA	0.54	A (0.73)	7.15 \pm 1.07 (14.96%)	7.05 \pm 1.03 (14.62%)	0 (0–1)	6.39 \pm 1.32 (20.61%)
	AB	0.38		5.97 \pm 1.32 (22.21%)	5.84 \pm 1.46 (25.02%)	0 (0–1)	5.84 \pm 1.46 (25.02%)
	BB	0.08	B (0.27)	7.44 \pm 1.94 (26.11%)	7.44 \pm 1.94 (26.11%)	0	7.44 \pm 1.94 (26.11%)
<i>RBP4-MspI</i>	AA	0.54	A (0.655)	6.22 \pm 1.39 (22.27%)	6.08 \pm 1.42 (23.45%)	0 (0–1)	6.06 \pm 1.40 (23.18%)
	AB	0.23		7.65 \pm 0.77 (10.07%)	7.65 \pm 0.77 (10.07%)	0	6.15 \pm 1.87 7 (4–8)*
	BB	0.23	B (0.345)	6.83 \pm 1.01 (14.84%)	6.72 \pm 0.95 (14.10%)	0 (0–1)	6.72 \pm 0.95 (14.10%)

TNB – total number of piglets born; NBA – number of piglets born alive; NSB – number of stillborn; NW – number of weaned piglets

*for NW in AB genotype of *RBP4-MspI* median value (min-max) are also included because of high variability

4 CONCLUSIONS

These preliminary results indicate a possible prediction of sows' reproductive capacity on the basis of their genotypes. Methods of molecular genetics can be successfully included in traditional swine improvement programmes based mostly on animal selection according to their observable phenotype. But it is important to note that detailed analysis of possible candidate genes in specific populations should be performed prior to their inclusion in selection program.

5 ACKNOWLEDGEMENT

This study is part of projects supported by Ministry of science, education and sports of the Republic of Croatia, number 053-0532265-2238, 053-1780469-2118 and 053-0531854-1849.

6 REFERENCES

Alfonso, L. 2005. Use of meta-analysis to combine candidate gene association studies: application to study the relationship between the ESR PvuII polymorphism and sow litter size. *Genetics Selection Evolution*, 37: 417–435

Chen, K., Li N., Huang L., Zhang Q., Zhang J., Sun S., Luo M., Wu C. 2001. The combined genotype effect of ESR and FSH β genes on litter size traits in five different pig breeds. *Chinese Sci Bul*, 46: 140–143. Cited in: Alfonso, L. 2005. *Genetics Selection Evolution*, 37: 417–435

Chvojková Z., Hraška Š. 2008. Changes in reproductive traits of Large White pigs after estrogen receptor gene-based se-

lection in Slovakia: preliminary results. *Asian-Australasian Journal of Animal Science*, 21, 3: 320–324

Croatian Agricultural Agency, 2013. Annual report 2012 – Pig breeding. Zagreb, Croatia.

Drogemüller C., Hamann H., Distl O. 2001. Candidate gene markers for litter size in different German pig lines. *Journal of Animal Science*, 79: 2565–2570

Gibson J. P., Jiang Z.H., Robinson J.A.B., Archibald A.L., Haley C.S. 2002. No detectable association of the ESR Pvu II mutation with sow productivity in a Meishan \times Large White F2 population. *Animal Genetics*, 33: 448–450

Goliášová E., Wolf J. 2004. Impact of the ESR gene on litter size and production traits in Czech Large White pigs. *Animal Genetics*, 35, 4: 293–297

Isler B. J., Irvin K. M., Neal S.M., Moeller S.J., Davis M.E. 2002. Examination of relationship between estrogen receptor gene and reproductive traits in pig. *Journal of Animal Science*, 80, 9: 2334–2339

Kernerova, N., Matoušek V., Čermakova A., Forbelska M. 2009. Role of genetic markers in the prediction of classification of Czech Large White gilts to a hyperprolific line. *Archiv für Tierzucht*, 52, 1: 40–50

Lemus-Flores C., Mejia-Martinez K., Rodriguez-Carpa J.G., Barreras-Serrano A., Herrera-Haro J.G., Alonso-Morales R. 2009. Genetic diversity and Variation of ESR, RBP4 and FUT1 genes in Mexican Creole and Yorkshire pig populations. *Journal of Biological Sciences*, 9, 8: 878–883

Linville R.C., Pomp D., Johnson R.K., Rotschild M.F. 2001. Candidate gene analysis for loci affecting litter size and ovulation rate in swine. *Journal of Animal Science*, 79, 1: 60–67

Muñoz G., Ovilo C., Estellé J., Fernández L.A., Rodríguez C. 2007. Association with litter size of new polymorphisms on ESR1 and ESR2 genes in a Chinese European pig line. *Genetics Selection Evolution*, 39, 2: 195–206

Noguera J. L., Varona L., Gomez-Raya L., Sanchez A., Babot D., Estany J., Messer L.A., Rothschild M.F., Perez-Enciso M.

2003. Estrogen receptor polymorphism in Landrace pigs and its association with litter size performance. *Livestock Production Science*, 82, 1: 53–59
- Omelka R., Bauerová M., Mlynek J., Bauchová B., Peškovičová D., Bulla J. 2005. Effect of oestrogen receptor (ESR) gene on reproductive traits of Large White, White Meaty and Landrace pigs. *Czech Journal of Animal Science*, 50, 6: 249–253
- Rodriguez S., Gaun T.R., Day I.N.M. 2009. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *American Journal of Epidemiology*, 169: 505–514. doi 10.1093/aje/kwn359
- Rothschild M. F., Messer L. A., Day A., Wahs R., Short T., Southwood O., Plastow G. 2000. Investigation of the retinol binding protein (RBP4) gene as a candidate gene for litter size in the pig. *Mammalian Genome*, 11: 75–77
- Rothschild M.F., Vaske D.A., Tuggle C.K., Masser L.A., McLaren D.G., Short T.H. 1996. Estrogen receptor locus is a major gene for litter size in the pig. In: *Proceedings of the 46th Annual Meeting of European Association of Animal Production*, Prague, Czech Republic: Vol. 1: 53
- Short T.H., Rothschild M.F., Southwood O.I., McLaren D.G., De Vries A., van der Steen H., Eckardt G. R., Tugge C.K., Helm J., Vaske D.A., Mileham A.J., Plastow G.S. 1997. Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. *Journal of Animal Science*, 75, 12: 3138–3142
- Spötter A., Distl O. 2006. Genetic approaches to the improvement of fertility traits in the pig. *Veterinary Journal*, 172, 2: 234–247
- Terman A., Kmiec M., Polasik D., Rybarczyk A. 2011. Association between RBP4 gene polymorphism and reproductive traits in Polish sows. *Journal of Animal and Veterinary Advances*, 10, 20: 2639–2641
- Van Rens B.T.T.M., Hazeleger W., van der Lende T. 2000. Periodovulatory hormone profiles and components of litter size in gilts with different estrogen receptor (ESR) genotypes. *Theriogenology*, 53, 6: 1375–1387