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ANALYSIS OF ESTROGEN RECEPTOR POLYMORPHISM AND LITTER SIZE TRAITS IN PRIMIPAROUS SOWS: PRELIMINARY RESULTS

ANALIZA POLIMORFIZMA ESTROGENOG RECEPTORA TE VELIČINE LEGLA U PRVOPRASKINJA: PRELIMINARNI REZULTATI

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SUMMARY

Identification of individual genes controlling litter size and their use in selection programs could contribute to an increased reproductive rate in pig population. Estrogen receptor gene (ESR) is a candidate gene marker for reproductive traits and growth in pigs. The aim of this preliminary study was to investigate ESR gene polymorphism as well as the frequencies of different ESR-*Pvu*II genotypes and their litter size in primiparous sows. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method with the *Pvu*II restriction enzyme was used on genotyping 30 primiparous Topigs 20 hybrid sows. The following reproductive traits of litter size were analyzed: Total Number of Born piglets (TNB), Number of piglets Born Alive (NBA), Number of Stillborn piglets (NSB) and Number of Mummified piglets (NMUM). Allele and genotype frequencies were calculated, and Hardy-Weinberg equilibrium was tested using χ^2-test. The ANOVA test was used to analyze litter size differences between sows with different genotypes. Three genotypes were detected with absolute frequencies (AA (13), AB (14) and BB (3)), while the gene relative frequency was 0.67 for A allele and 0.33 for B allele. Study animals were in Hardy-Weinberg equilibrium. The primiparous sows with the BB genotype showed highest TNB (14.33) and NSB (2.33), while NBA and NMUM were highest in primiparous sows with AA genotype (12.66 and 0.38, respectively). Although some differences were found in litter size according to genotypes they were not significant ($P>0.05$). Further investigations in a larger sample size will contribute to more conclusive evaluation of the possible correlation between ESR-*Pvu*II polymorphism and litter size in sows.

Key words: estrogen receptor gene, Topigs 20, litter size

INTRODUCTION

Effectiveness and sustainability of pig production depend mostly on animal health, longevity, and welfare. Reproductive traits, litter size and preweaning viability in particular, are important components for reducing the costs of producing pork and survivability of producers. Selection for improving reproduction traits is in the focus of interest within breeding programs all over the world (Spötter and Distl, 2006). The success depends on the value of heritability traits, which is low, with a mean value of 0.08-0.11 for litter size traits, as one of the most easily measured reproductive traits (Johnson et al.,

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1999; Holm et al. 2005; Canario et al., 2006). For this reason, the selection process took 20 years resulting in 2.6 piglets *per* litter increase. Genetic markers associated with reproductive traits are often used to increase the rates of genetic response and bring more economic profit to pig industry (Buske et al., 2006). The estrogen receptor gene (ESR) is one of the first and most utilized candidate genes to analyze litter size in many studies that include pig population under experimental control. A candidate gene approach provides a more direct understanding of the genetic basis and physiological interactions involved in the expression of quantitative differences between individuals (Noguera et al., 2003; Distl, 2007). Estrogens are steroid hormones and with their receptors they play an important role in some reproductive traits and hormonal regulation of the reproductive process (Matoušek et al., 2003; Li et al., 2006; Humpolicek et al., 2009). ESR gene polymorphism is related to prolificacy and productivity. Prolificacy is also a multigene trait of low heritability, and an increase in this trait is determined by various factors. In this case, ESR has been identified as an intragenic molecular marker for selection of more prolific sows (Goncalves et al., 2008).

ESR genes are located on the intron region pig chromosome 1 p.2.5 - p2.4 (Munoz et al., 2007). The *Pvu*II polymorphism at porcine ESR gene was designated in 1994 as a major gene for litter size. One ESR allele with a 3.7-kb fragment, called B allele, has been reported to be significantly associated with a higher total number born (TNB) and number born alive (NBA) in a 50% Meishan synthetic line (Rothschild et al., 1996). The difference between homozygote genotype in synthetic line of Meishan (MS) pigs were 2.3 piglets more *per* litter for TNB and NBA. Also, the influence of ESR was associated with other important reproductive traits like embryo survival, maternal recognition of pregnancy (Spencer and Bazer, 2004), fetal survival (Linville et al., 2001) and successful embryo implantation (Van Rens et al., 2000). One of the first selections to increase litter size in high fertility sows was based on French Large White hyperprolific scheme and Landrace population. The selection scheme was used in a study conducted by Legault et al. (1996). Short et al. (1997) report on a difference between two homozygote genotypes in primiparous sows of PIC lines of 0.83 more piglets *per* litter for TNB and NBA. Many studies have been published on the role of ESR gene in reproductive traits. Numerous studies have been performed on purebred, crossbred and hybrid sows and there are many reports of mutation and its role in reproductive and some performance traits (Ye et al., 2009). We report on preliminary study results and analysis of ESR polymorphism related to litter size traits in primiparous Topigs 20 sows.

MATERIAL AND METHODS

Thirty primiparous Topigs 20 sows from the Krmiva pig breeding farm (Croatia) were used in this preliminary study. The animals were kept in a modern, properly equipped service unit with uniform microclimatic, rearing and feeding conditions. Blood samples were collected by puncture of cranial vena cava using a sterile tube containing EDTA anticoagulant, in accordance with the approved farm management practice. DNA was isolated using DNeasy Blood® & Tissue Kit (Qiagen, GmbH, Hilden, Germany). Estrogen receptor genotype was identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based on the method described by Short et al., (1997). The conditions were 30-50 ng/μL genomic DNA, 10 x PCR Buffer (Promega, USA), 25 mM $MgCl_2$, 5μM of each primer (designed according to Short et al., 1997), 2mM of dNTP mix and 5U/μL of Taq DNA polymerase (Promega, USA), in a final volume of 25 μL PCR reaction. The reaction was performed in a Mastercycler® personal 5332 thermocycler (Eppendorf, Germany). The amplification conditions were as follows: one denaturation cycle at 94 °C for 4 min followed by 34 cycles at 94 °C for 1 min, 57 °C for 1 min and 72 °C for 1 minute, followed by final extension of 72 °C for 8 min and finally held at 4 °C. Amplified products were visualized with 1.5% agarose gel containing ethidium bromide. The amplicons of ESR were digested using the *Proteus vulgaris* II (*Pvu*II) restriction enzyme, a mixture of 7.3 μL of ultrapure water, 2 μL Buffer (Promega, USA), 0.2 μL of BSA (10mg/mL), 0.5 μL (12U/μL) of *Pvu*II and 10 μL of the amplified product. The PCR products were digested for 3 h at 37 °C in water bath. Digested products were submitted to electrophoresis on 3.25% agarose gel containing ethidium bromide at 85V for 45 min. The bands were visualized under ultraviolet (UV) light and size of the fragments was determined with 25bp DNA Step Ladder (Promega, USA). The results were recorded using a Mini BIS Pro®, DNR

Bio-Imaging Systems (Jerusalem, Israel). The genotyped animals were classified as genotype AA (120 bp), AB (120, 65, 55 bp) and BB (65, 55 bp), as outlined previously (Short et al., 1997). Data on litter size including Total Number of Born piglets (TNB), Number of Born Alive piglets (NBA), Number of Stillborn piglets (NSB) and Number of Mummified piglets (NMUM) were collected from the breeding farm. Descriptive statistics analysis of analyzed traits was performed using the Statistica v.10 reference program. Data are presented as mean±SD. Allele and genotype frequencies were calculated, and Hardy-Weinberg equilibrium was tested using χ^2-test. Kruskal-Wallis ANOVA was used to analyze significance of differences in litter size traits according to genotypes and 2 sample T-test in order to calculate the difference between them.

RESULTS AND DISCUSSION

Three genotypes were detected in 30 primiparous sows: AA (13), AB (14) and BB (3) (Table 1). The relative frequencies of genotypes were 0.4333, 0.4667 and 0.1, respectively. The relative frequency of AB genotype recorded in primiparous Topigs 20 sows was in agreement with the studies conducted by Rothschild et al. (1994; 1996), Omelka et al. (2005), Santana et al. (2006), Wang et al. (2006), Gonçalves et al. (2008) and Chvojková and Hraška (2008), in which the relative values ranged from 0.448 to 0.493.

The BB genotype was detected in the smallest number of primiparous sows and its relative frequencies were consistent with those reported by Omelka et al. (2005), Santana et al. (2006) and Chvojková and Hraška (2008), with the values ranging from 0.09 to 0.119. For the ESR-*Pvu*II polymorphism, the A allele and allele B frequency was 0.67 and 0.33, respectively. Rothschild et al. (1996) demonstrated the favorable effect of B allele in Meishan (MS) and LW synthetic lines/hybrid combinations of Western breeds, manifested as 1.15 more piglets *per* litter for TNB and NBA in first parity sows. The same results have been reported by Short et al. (1997) in four LW-based commercial lines with the additive effect of 0.4 more piglets in first parity sows. The frequency of B allele was similar in three Large White lines (range 0.64 to 0.74), but was considerably lower in the 3/4 Duroc line (0.17) (Alfonso, 2005). In previous studies including LW breed, the A allele occurred at the frequency from 0.63 to 0.6828 (Matoušek et al., 2003; Omelka et al., 2005; Santana et al., 2006; Chvojková and Hraška, 2008). The population of 30 primiparous sows were found to be in Hardy-Weinberg equilibrium according to the method described by Rodriguez et al. (2009). The data on primiparous sows with BB genotype showed highest values for TNB (14.33) and NSB (2.33) piglets (Table 2). According to Herpin et al. (2001), piglets from very large litters have a higher probability of stillbirth, which is associated with prolonged farrowing, or with longer birth intervals because piglets are more prone to be asphyxiated or suffer a greater risk of hypoxia. Goliášová and Wolf (2004) analyzed an LW population for ESR polymorphism and found the AA genotype primiparous sows to have better performance in litter size for NBA and NSB piglets. Sows with AA genotype produced approximately 0.5 more live born piglets *per* litter than BB sows. The results on TNB were similar to NBA. The NBA and NMUM were highest in primiparous sows with AA genotype (12.66 and 0.38, respectively). This is in agreement with the crossbred gilt population LW x MS F_2 for NMUM, reported by van Rens et al. (2002). In the present study, there was no statistically significant

Table 1. Frequency of ESR-*Pvu*II genotypes and alleles

Tablica 1. Frekvencija genotipova i alela za ESR-*Pvu*II

Number of primiparous sows - Broj prvopraskinja	ESR-*Pvu*II genotype - ESR-*Pvu*II genotip	Frequency - Učestalost genotipa	ESR-*Pvu*II allele - Alel ESR-*Pvu*II	Frequency - Učestalost alela
13	AA	0.4333	A	0.67
14	AB	0.4667		
3	BB	0.10	B	0.33

Table 2. Litter size traits according to ESR-*Pvu*II genotype in 30 primiparous sows Topigs 20

Tablica 2. Vrijednosti veličine legla 30 prvopraskinja Topigs 20 s obzirom na ESR-*Pvu*II genotip

Genotype - Genotip	TNB-UOO Mean±SD - arit.sred.±SD	NBA–ŽO Mean±SD - arit.sred.±SD	NMUM-MM Mean±SD - arit.sred.±SD	NSB-MO Mean±SD - arit.sred.±SD
AA	13.84±2.96	12.76±2.48	0.38±0.86	0.69±1.18
AB	12.71±2.46	12.0±2.18	0	0.71±1.63
BB	14.33±3.21	12.0 ±1	0	2.33±2.3

TNB = Total Number of Born piglets, UOO = Ukupan broj oprasenih odojaka
NBA = Number of piglets Born Alive; ŽO = broj živooprasenih odojaka
NMUM = Number of Mummified piglets; MM = broj mumificiranih odojaka
NSB = Number of Stillborn piglets; MO = broj mrtvooprasenih odojaka
Mean±SD = Mean ± Standard deviation; arit.sred.±SD = aritmetička sredina ± standardna devijacija

Table 3. Differences in litter size traits according to ESR-*Pvu*II genotypes

Tablica 3. Razlike u veličini legla između tri ESR-*Pvu*II genotipa

Genotype - Genotip	TNB-UOO Mean±SD - arit.sred.±SD	NBA–ŽO Mean±SD - arit.sred.±SD	NMUM-MM Mean±SD - arit.sred.±SD	NSB-MO Mean±SD -arit. sred.±SD
AB- AA	-1.13±1.05	-0.76±0.9	-0.38±0.23	0.02±0.54
BB - AB	1.62±1.97	0±0.82	0	1.62±1.39
BB - AA	0.49±2.02	-0.76±0.89	-0.38±0.23	1.64±1.36

Note: differences in litter size traits according to ESR-*Pvu*II were not significant (P>0.05)
Napomena: nisu utvrđene značajne razlike u veličini legla između tri ESR-*Pvu*II genotipa (P>0,05)

TNB = Total Number of Born piglets, UOO = ukupan broj oprasenih odojaka
NBA = Number of piglets Born Alive; ŽO = broj živooprasenih odojaka
NMUM = Number of Mummified piglets; MM = broj mumificiranih odojaka
NSB = Number of Stillborn piglets; MO = broj mrtvooprasenih odojaka
Mean±SD = Mean ± Standard deviation; arit.sred.±SD = Aritmetička sredina ± standardna devijacija

difference in any litter size according to genotypes (P>0.05). Variability in the frequency of A and B alleles in the study populations of pigs may be partially caused by different history of each population. It has been postulated that B allele has a Chinese pig origin. Additional copies of ESR B allele decrease litter weight, which may be the result of an increased number of fetal losses in the animal with more copies of B allele (Isler et al., 2002).

The presence of B allele in high fertility and hybrid sows may be the result of interbreeding of Chinese and English pigs (Alfonso, 2005). The number of sows analyzed in this preliminary study was limited. It is necessary to examine a large sample size, which will contribute to more conclusive evaluation of the possible correlation between ESR-*Pvu*II polymorphism effects on litter size in the study population.

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SAŽETAK

Identifikacijom pojedinih gena odgovornih za veličinu legla te njihovom primjenom u selekciji jedinki, možemo doprinijeti povećanju reprodukcijske učinkovitosti svinja**.** Estrogeni receptor (ESR) jedan je od kandidatnih gena povezanih s rastom i reproduktivnim svojstvima. Cilj ovog preliminarnog istraživanja bio je analizirati polimorfizam odsječka gena ESR-*Pvu*II, utvrditi frekvencije različitih genotipova kao i veličinu legla za svakog od njih. Primjenom lančane reakcije polimerazom genotipizirano je 30 prvopraskinja hibrida Topigs 20. Polimorfizam estrogenog receptora utvrđen je primjenom restrikcijske endonukleaze *Pvu*II. Analizirani su sljedeći reproduktivni pokazatelji: ukupan broj oprasenih odojaka (UOO), broj živooprasenih odojaka (ŽO), broj mrtvooprasenih odojaka (MO) i broj mumificiranih odojaka (MM). Izračunate su frekvencije alela i genotipova, te je χ^2-testom analizirano odstupanje od Hardy-Weinbergove ravnoteže. Značajnost razlike u promatranim svojstvima između pojedinih genotipova testirana je analizom varijance. Utvrđena su tri genotipa sa sljedećom zastupljenošću: AA (13), AB (14) i BB (3). Frekvencija alela A iznosila je 0,67, a alela B 0,33, pri čemu nije utvrđeno odstupanje od Hardy-Weinbergove ravnoteže. U prvopraskinja genotipa BB utvrđen je najveći broj UOO (14,33) i MO (2,33) za razliku od genotipa AA s najvećim brojem ŽO (12,66) i MM (0,38) odojaka. Iako su pronađene pojedine razlike u veličini legla kod jedinki različitih genotipova, one nisu bile statistički značajne ($p>0,05$). Vjerujemo da će daljnje analize provedene na većem broju jedinki doprinijeti konkretnijim zaključcima o mogućoj povezanosti ESR-*Pvu*II genotipa i veličine legla u krmača Topigs 20.

Ključne riječi: odsječak gena estrogenog receptora, Topigs 20, veličina legla