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# **Influence of** *ESR1* **and** *FSHB* **genes on litter size in Czech Large White sows** (short communication)

## Abstract

The effects of the candidate genes for reproduction, the estrogen receptor gene (*ESR1*) and the folliclestimulating hormone beta subunit gene (*FSHB*), on the performance of Large White sows in the three different herds have been examined. A total number of 271 sows (in case of the *ESR1* gene) and 171 sows (in case of the *FSHB* gene) were genotyped with PCR-RFLP method and analysed to determine whether polymorphisms of the genes influence the efficiency of Large White sows. The influence of these genes in the first, the first to the fourth and the second to the fourth litters was investigated. To discover the genes 'associations with reproduction traits, mixed linear models were used. The influence of *ESR1* and *FSHB* genes was not very conclusive in the studied populations. The influence of these genes was different depending on the herd, in which the sows were bred or on the set of analyzed litters.

Key Words: pig, gene marker, Large White, litters size, ESR1, FSHB

#### Zusammenfassung

Titel der Arbeit: Einfluss des *ESR1* und *FSHB* Gens auf die Wurfgröße bei Sauen des Tschechischen Edelschweines (Kurzmitteilung)

Es wurden in drei Edelschweinherden die Effekte der Kandidatengene Östrogenrezeptorgen (*ESR1*) und des follikelstimulierenden Hormongens (*FSHB*) auf die Wurfleistungen untersucht. Insgesamt 271 bzw. 171 Sauen wurden in Bezug auf *ESR1* bzw. *FSHB* mittels PCR-RFLP genotypisiert um festzustellen, ob und welchen Einfluss diese Genpolymorphien auf die Wurfleistungen im 1., dem 1. bis 4. bzw. 2. bis 4. Wurf nehmen. Die Varianzanalyse erfolgte mittels gemischter linearer Modelle. Es konnte kein überzeugender Einfluss der *ESR1* und *FSHB* Gene nachgewiesen werden, jedoch waren die Additiv- und Dominanzeffekte, abhängig von den einzelnen Herden und Wurfzahlen, unterschiedlich ausgeprägt.

Schlüsselwörter: Schwein, genetischer Marker, Edelschwein, Wurfleistung, ESR1 Gen, FSHB Gen

#### Introduction

One of the factors influencing economic efficiency of the pork production is a number of weaned piglets per sow. To increase the level of that trait, the knowledge of the gene markers and candidate genes is advisable to exploit in pig breeding. The estrogen receptor gene (*ESR1*) and the follicle-stimulating hormone gene (*FSHB*) are candidate genes for reproduction. The objective of our research was to determine the influence of these genes on the litter size in Czech Large White herds.

In the *ESR1* gene, the *Pvu*II polymorphism was found by ROTHSCHILD et al. (1991). The relationship between the genotypes in the *ESR1* gene and reproduction traits of crossbred sows was observed by DEPUYDT et al. (1999). Conversely, DROEGE-MUELLER et al. (1999) mapped genome region surrounding the *ESR* gene and they did not confirm an existence of QTL affecting litter size in the considered genome region.

Follicle-stimulating hormone consists of two subunits  $\alpha$  and  $\beta$  coded by different genes. The subunit  $\beta$  is specific for every animal genus and it is coded by the *FSHB* 

gene (MELLINK et al., 1995). The *FSHB* gene is considered as a candidate gene because of its function in maturation of small and medium follicles into ovulating large follicles (WANG and GREENWALD, 1993).

## Material and methods

The investigated population comprised 285 sows from three different herds of Czech Large White breed. The PCR-RFLP methods described by SHORT et al. (1997) and ROHRER et al. (1994) were employed for detection of genotypes in the *ESR1* and *FSHB* genes, respectively. In each of analysed polymorphisms of *ESR1* and *FSHB* genes, two alleles were detected: D (55 and 65 bp) and C (120 bp); A (332 bp) and B (173 and 159 bp), respectively. Genetic equilibriums were evaluated by  $\chi^2$  - test. The additive effect is estimated as half of the phenotypic difference between both of homozygous genotypes. The dominance effect is estimated as phenotypic values both of homozygous genotypes.

The associations of studied polymorphisms with a total number of born piglets (TNB); number of piglets born alive (NBA) and number of weaned piglets (NW) were estimated using a mixed linear model (REML) in SAS for Windows 8.2. The influence of the genes in the first (1<sup>st</sup>); the first to the fourth (1<sup>st</sup>-4<sup>th</sup>) and the second to the fourth  $(2^{nd}-4^{th})$  litters were monitored. Separate analyses were calculated for herd I and II. These analyses are called data set I (ds<sup>I</sup>) and data set II (ds<sup>II</sup>). In the analysis of data set III (ds<sup>III</sup>), sows from all herds were included. The low numbers of sows were genotyped in the herd III. Therefore, the separated analysis was not calculated for this herd (Table I). The genotypes in the *ESR1* gene and the *FSHB* gene, year of sow's birth (YB), month of litter (ML) and effect of litter order (OL) were included in models as a fixed effect. In models for ds<sup>III</sup>, the effect of herd of sows was included as a fixed effect. The effect of the boar (*BOAR*) was used as a random effect. Furthermore, linear regressions on backfat thickness (cm) (BT), and the age of the first conception (AFC) were included.

# Model 1 used in the 1<sup>st</sup> litters.

 $y_{ijklmnoprs} = \mu + ESR1_i + FSHB_j + YB_k + ML_l + BT_m + AFC_p + BOAR_r + e_{ijklmnoprs}$ Model 2 used in the 1<sup>st</sup> to the 4<sup>th</sup> litters and the 2<sup>nd</sup> to the 4<sup>th</sup> litters.

 $y_{ijklmnoprstuv} = \mu + ESR1_i + FSHB_j + YB_k + ML_l + OL_o + BT_p + AFC_t + BOAR_u + e_{ijklmnoprstuv}$ Table 1

Absolute (n) and relative frequencies of genotypes of the Estrogen Receptor Gene (*ESR1*) and the Follicle Stimulating Hormone Gene (*FSHB*) (Absolute (n) und relative Frequenzen der Genotypen des Östrogenrezeptorgens - *ESR1* und des follikelstimulierenden Hormongens - *FSHB*)

		Absolute (n) and relative frequencies of genotypes						
		herd I		herd II		herd III		
Locus	Genotypes	n	relative frequency	n	relative frequency	n	relative frequency	
	CC	45	0.26	31	0.43	2	0.09	
ESR1	CD	88	0.50	32	0.44	8	0.35	
	DD	43	0.24	9	0.13	13	0.57	
	H-W $(\chi^2)$	Α		Α			Α	
	AA	22	0.26	1	0.02	1	0.05	
FSHB	AB	28	0.32	7	0.11	9	0.41	
	BB	36	0.42	55	0.87	12	0.55	
	H-W $(\chi^2)$		no		Α		Α	

Note: <sup>A</sup> Population is in gene equilibrium by H-W for P = 0.01; <sup>no</sup> population is not in gene equilibrium by H-W

## Results

In herds I and II, the most frequent genotypes of the *ESR1* gene were the heterozygous genotypes *CD* (Table 1). On the other hand, genotypes *DD* achieved the highest frequency in the herd III. The *BB* genotypes in the *FSHB* gene was the most frequent in all examined herds. The populations were in Hardy-Weinberg equilibrium in all analysed herds and in herd II and III in the *ESR1* gene and in the *FSHB* gene, respectively.

#### Table 2

Sow's efficiency with different genotypes in the Estrogen Receptor Gene (*ESR1*) (Least square means and standard errors, LSM  $\pm$  SE) and additive (A) and dominance (D) effects for allele *D* (Reproduktionsleistungsmerk-male von Sauen mit unterschiedlichen Genotypen des Östrogenrezeptorgens - *ESR1* (Least-Square-Mittelwerte und Standardfehler, LSM  $\pm$  SE) und Additiv- (A) und Dominanzeffekte (D) für Allel *D*)

Genotypes		CC	CD	DD	А	D		
			the 1 <sup>st</sup> litters					
ds <sup>I</sup>	TNB	$12.46\pm0.57$	$12.57\pm0.52$	$11.95\pm0.55$	-0.26	0.37		
	NBA	$12.43\pm0.56$	$12.43\pm0.51$	$11.84\pm0.55$	-0.30	0.30		
	NW	$9.83 \pm 0.61$	$10.09\pm0.55$	$9.30\pm0.59$	-0.27	0.52		
	TNB	$12.52\pm0.30$	$12.27\pm0.28$	$12.73\pm0.41$	0.11	-0.36		
ds <sup>II</sup>	NBA	$11.88\pm0.44$	$11.36\pm0.41$	$12.29\pm0.60$	0.20	-0.73		
	NW	$11.88\pm0.53$	$10.74\pm0.49~^{a}$	$12.06\pm0.72~^{\mathrm{b}}$	0.09	-1.23		
	TNB	$12.61\pm0.41~^{\rm A}$	$12.67\pm0.36\ ^{\mathrm{A}}$	$11.64\pm0.40~^{\text{B}}$	-0.48	0.55		
ds <sup>III</sup>	NBA	$11.98\pm0.43$	$11.94\pm0.37$	$11.23\pm0.41$	-0.38	0.33		
	NW	$10.59\pm0.41$	$10.42\pm0.36$	$10.09\pm0.39$	-0.25	0.08		
the $2^{nd} - 4^{th}$ litters								
ds <sup>I</sup>	TNB	$13.81\pm0.56$	$13.48\pm0.51$	$13.47\pm0.59$	-0.17	-0.16		
	NBA	$13.48\pm0.52$	$13.17\pm0.48$	$13.48\pm0.55$	0.00	-0.31		
	NW	$11.15\pm0.46$	$10.53\pm0.42$	$11.09\pm0.48$	-0.03	-0.59		
	TNB	$12.25\pm0.18$	$12.13\pm0.17$	$12.38\pm0.27$	0.07	-0.19		
ds <sup>II</sup>	NBA	$11.32\pm0.22$	$11.29\pm0.21$	$11.25\pm0.33$	-0.04	0.00		
	NW	$11.02\pm0.30$	$10.54\pm0.28$	$11.02\pm0.46$	0.00	-0.48		
ds <sup>III</sup>	TNB	$12.98\pm0.31$	$12.74\pm0.27$	$12.54\pm0.31$	-0.22	-0.02		
	NBA	$12.25\pm0.30$	$12.04\pm0.26$	$11.91\pm0.30$	-0.17	-0.04		
	NW	$11.21\pm0.27~^{\rm A}$	$10.64\pm0.24~^{\text{B}}$	$10.86\pm0.27$	-0.18	-0.40		
the $1^{st} - 4^{th}$ litters								
ds <sup>I</sup>	TNB	$13.39\pm0.36$	$13.06\pm0.33$	$12.90\pm0.39$	-0.25	-0.08		
	NBA	$13.13\pm0.35$	$12.80\pm0.32$	$12.81\pm0.37$	-0.16	-0.17		
	NW	$10.52\pm0.33$	$10.20\pm0.31$	$10.31\pm0.36$	-0.11	-0.22		
ds <sup>II</sup>	TNB	$12.34\pm0.14$	$12.18\pm0.13$	$12.42\pm0.21$	0.04	-0.20		
	NBA	$11.50\pm0.20$	$11.34\pm0.18$	$11.58\pm0.29$	0.04	-0.20		
	NW	$11.17 \pm 0.25$ <sup>A</sup>	$10.59\pm0.23^{\text{ B a}}$	$11.19\pm0.37~^{\text{b}}$	0.01	-0.59		
ds <sup>III</sup>	TNB	$12.94\pm0.24~^{\rm A}$	$12.71\pm0.21~^{a}$	$12.23\pm0.24^{~\textbf{B}~\textbf{b}}$	-0.36	0.13		
	NBA	$12.20\pm0.24~^{\rm A}$	$11.97\pm0.21$	$11.62\pm0.23~^{\textbf{B}}$	-0.29	0.06		
	NW	$10.87\pm0.22$ ^ A a	$10.44 \pm 0.19$ <sup>B</sup>	$10.41 \pm 0.22$ <sup>b</sup>	-0.23	-0.20		

Note: Values with the different superscripts show significance level within rows:  $p \le 0.05$  (A, B); Approaching significance level  $p \le 0.10$  (a, b); TNB - total number of born piglets; NBA - number of piglets born alive; NW - number of weaned piglets

The *CC* genotypes in the *ESR1* gene showed significant tendency to higher litter size in the  $1^{st}-4^{th}$  litters in ds<sup>III</sup>. In the  $1^{st}$  and in the  $2^{nd}-4^{th}$  litters, this tendency was found

too (Table 2). The differences between homozygous genotypes in the *ESR1* gene were low in ds<sup>II</sup>. The number of piglets weaned in ds<sup>II</sup> of homozygous and heterozygous sows in the *ESR1* gene was different, especially in the  $1^{st}$ -4<sup>th</sup> litters. Furthermore, the significantly highest number of piglets in the  $1^{st}$  litters had sows with *BB* genotypes in the *FSHB* gene in ds<sup>I</sup> and ds<sup>III</sup> (Table 3). This tendency can be observed in ds<sup>III</sup> for  $2^{nd}$ -4<sup>th</sup> litters; in ds<sup>II</sup> for the  $2^{nd}$ -4<sup>th</sup> and the  $1^{st}$ -4<sup>th</sup> litters as well.

#### Table 3

Sow's efficiency with different genotypes in the Follicle Stimulating Hormone Gene (*FSHB*) (Least square means and standard errors, LSM  $\pm$  SE), and additive (A) and dominance (D) effects for allele *B*. (Reproduktionsleistungsmerkmale von Sauen mit unterschiedlichen Genotypen des follikelstimulierenden Hormongens - *FSHB* (Least-Square-Mittelwerte und Standardfehler, LSM  $\pm$  SE), Additiv- (A) und Dominanzeffekte (D) für Allel *B*)

		AA	AB	BB	А	D
			the 1 <sup>st</sup> litters			
ds <sup>I</sup>	TNB	$11.49 \pm 0.55$ <sup>A</sup>	$11.89 \pm 0.51$ <sup>A</sup>	$13.60 \pm 0.68$ <sup>B</sup>	1.06	-0.65
	NBA	$11.32 \pm 0.54$ <sup>A</sup>	$11.78\pm0.51~^{\rm A}$	$13.59 \pm 0.68$ <sup>B</sup>	1.14	-0.68
	NW	$8.91\pm0.58~^{\rm A}$	$9.44 \pm 0.55$ <sup>a</sup>	$10.87\pm0.73\ ^{\textbf{A}\textbf{b}}$	0.98	-0.45
	TNB	/	$12.49\pm0.40$	$12.51\pm0.22$	/	/
ds <sup>II</sup>	NBA	/	$12.15\pm0.59$	$11.54 \pm 0.33$	/	/
	NW	/	$11.92\pm0.70$	$11.20\pm0.39$	/	/
	TNB	$11.59 \pm 0.52$ <sup>A</sup>	$12.38\pm0.40$	$12.95 \pm 0.34$ <sup>B</sup>	0.68	0.11
ds <sup>III</sup>	NBA	$10.85\pm0.55$ $^{\rm A}{}^{\rm a}$	$11.99 \pm 0.42$ <sup>b</sup>	$12.31\pm0.35^{\text{ B}}$	0.73	0.41
	NW	$9.66 \pm 0.53^{a}$	$10.59\pm0.41$	$10.85 \pm 0.34$ <sup>b</sup>	0.60	0.34
			the $2^{nd} - 4^{th}$ litters			
ds <sup>I</sup>	TNB	$14.04\pm0.59$	$13.06\pm0.58$	$13.67\pm0.60$	-0.19	-0.80
	NBA	$13.64\pm0.55$	$13.13\pm0.54$	$13.36\pm0.56$	-0.14	-0.37
	NW	$11.59\pm0.48$	$10.61\pm0.47$	$10.57\pm0.49$	-0.51	-0.47
	TNB	/	$11.93\pm0.27~^{\rm A}$	$12.58\pm0.12\ ^{\text{B}}$	/	/
ds <sup>II</sup>	NBA	/	$11.06\pm0.33$	$11.52\pm0.15$	/	/
	NW	/	$10.76\pm0.45$	$10.96\pm0.22$	/	/
	TNB	$13.06\pm0.44$	$12.55\pm0.32$	$12.65\pm0.25$	-0.21	-0.31
ds <sup>III</sup>	NBA	$12.11 \pm 0.42$	$12.08\pm0.30$	$12.00\pm0.24$	-0.05	0.03
	NW	$11.40 \pm 0.37$ <sup>B</sup>	$10.70\pm0.27~^{\rm A}$	$10.61 \pm 0.22$ <sup>A</sup>	-0.40	-0.31
			the $1^{st} - 4^{th}$ litters			
ds <sup>I</sup>	TNB	$13.25\pm0.39$	$12.74\pm0.36$	$13.37\pm0.39$	0.06	-0.57
	NBA	$12.90\pm0.37$	$12.68\pm0.35$	$13.16\pm0.37$	0.13	-0.35
	NW	$10.56\pm0.36$	$10.06\pm0.34$	$10.41\pm0.36$	-0.08	-0.42
	TNB	/	$12.10 \pm 0.21$ <sup>A</sup>	$12.53 \pm 0.10^{\text{ B}}$	/	/
ds <sup>II</sup>	NBA	/	$11.39\pm0.28$	$11.56\pm0.14$	/	/
	NW	/	$10.99\pm0.36$	$10.98\pm0.19$	/	/
ds <sup>III</sup>	TNB	$12.67 \pm 0.34$	$12.55 \pm 0.24$	$12.66 \pm 0.18$	0.00	-0.11
	NBA	$11.75\pm0.33$	$12.02\pm0.23$	$12.03\pm0.18$	0.23	0.22
	NW	$10.69 \pm 0.31$	$10.51\pm0.22$	$10.52\pm0.17$	-0.09	-0.10

Note: Values with the different superscripts show significance level within rows:  $p \le 0.05$  (A, B); Approaching significance level  $p \le 0.10$  (a, b); TNB - total number of born piglets; NBA - number of piglets born alive; NW - number of weaned piglets

# Discussion

All investigated sows were collected from three nucleus herds of Large White pigs, where an intensive selection for reproduction traits has been carried out. It can be

assumed, that this selection was performed in favour of sows with the alleles positively influencing reproduction traits. The most frequent genotype in herd I and II was the heterozygous genotype CD in the ESR1 gene (Table 1). A similar result was published by ISLER et al. (2002) who found highest frequency of heterozygous genotypes in Yorkshire pigs, Large White pigs and their crossbred sows. In the herd III, the most frequent homozygous genotypes DD were found. In the FSHB gene, homozygous genotypes BB showed the highest frequency in all of analysed herds (Table 1). LINVILLE et al. (2001) described similar frequencies in four synthetic lines.

In the present study, the significantly higher efficiency had sows with CC genotype in the *ESR1* gene. This tendency was observed especially in  $ds^{III}$  in  $1^{st}-4^{th}$  litters where the sows with CC genotype in the ESR1 gene had higher TNB, NBA and NW (Table 2). In contrast, SHORT et al. (1997) noted that sows with DD and CD genotypes exceeded homozygous CC sows. Higher efficiency of heterozygous sows was described by SOUTHWOOD et al. (1995) in Meishan sows. MATOUŠEK et al. (2003) described dissimilar influence of the ESR1 gene on litter size in two herds of Large White. In their analysis, higher efficiency had sows with DD genotype in one herd and sows with CC genotype in another herd. In present study, the sows with homozygous genotypes BB in the FSHB gene had significantly highest TNB, NBA and NW in 1<sup>st</sup> litters in ds<sup>I</sup> and ds<sup>III</sup>. The highest TNB of sows with *BB* genotype was found in 1<sup>st</sup>-4<sup>th</sup> litters in ds<sup>II</sup>. These results corresponded to the findings published by LI et al. (1998). They observed that sows with the BB genotypes achieved the highest total number of piglets born and born alive. In the present study, additive and dominance effects were unequal depending on the herd, in which the sows were bred, and on the analyzed litters (Table 2 and 3). GOLIÁŠOVÁ and WOLF (2004) found a negative additive effect of the B allele of the ESR1 gene in a population of Large White sows on litters size traits across parities as well as in the first and in the second and subsequent parities.

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