Associations between the microsatellite DNA sequence in the IGF1 gene, polymorphism in the ESR gene and selected reproduction traits in F1 (Zlotnicka Spotted × Polish Large White) sows

Agnieszka **Korwin-Kossakowska**, Grażyna **Sender**, Jolanta **Kurył**

Polish Academy of Sciences Institute of Genetics and Animal Breeding,
Jastrzębiec, 05-552 Wólka Kosowska, Poland

In 53 F_1 sows (Zlotnicka Spotted boars × Polish Large White sows) the insulin-like growth factor 1 (IGF1) and estrogen receptor (ESR) genes were assessed as candidate genes for total number of piglets born (TNB), number of piglets born alive (NBA), litter weight at birth (LWB), litter weight at weaning (LWW), number of piglets weaned (NPW), average piglet weight at weaning (AWW) and gestation length (GL). The *IGF1 locus* genotype (microsatellite sequence) was found to affect (P≤0.01) the TNB, NBA and GL when based on analysis of sows with all litters born. No association between reproductive traits and ESR genotype was found in any restrictive site. However, interesting appeared the results of the analysis including an interaction between ESR genotypes in three polymorphic sites where almost all traits investigated appeared associated (P≤0.05) with ESR genotype. However, due to the limited number of animals, conclusions on the relationship between gene polymor-phisms and reproduction traits must be considered as preliminary, and thus treated with care.

KEY WORDS: estrogen receptor / insulin-like growth factor 1 / reproduction / sows

One of the main objectives of molecular genetics is to find the markers or major genes with large effects on trait of interest. In farm animals, especially pigs, repro-duction traits play the most important role in the effectiveness of production. Most important and easily measured reproductive trait is the litter size, a typical quantitative trait, controlled by the genetic background and the environment. Increasing the litter size by selection gives only a partial effect due to the low heritability of the trait (0.1)

– Haley *et al*. [1988]. Furthermore, litter size is expressed only in females and cannot be measured before reproduction. Marker-assisted selection (MAS), however, makes it possible to select directly for genes that control litter size.

The candidate gene approach was proposed by Rothschild and Soller [1997] as a procedure to identify genes with significant phenotypic performance effects for possible use in genetic improvement programmes. A gene is selected to be a candidate gene because of an important physiological role it regulates in a given process or pathway. In this study investigated were two genes associated with pig reproduction: insulin-like growth factor 1 gene (*IGF1*) and estrogen receptor gene (*ESR*).

IGF-system plays a major role in the reproduction of mammalian species. IGFs may have an important role in the control of ovarian function [Schams *et al*. 1999]. Locally produced peptide/protein growth factors, together with endocrine signals are responsible for different folliculogenic processes such as recruitment, selection, and dominance. IGF1 has been reported to stimulate the proliferation and differentiation of the granulose cells isolated from antral follicles in various species, among which pigs and rats were the first ones [Zhao *et al*. 2001]. IGFs and the binding proteins controlling their activity are also crucial for foetal growth and development. The knock-out of either gene reduced litter size to 60% of normal litters in mice [Gibson *et al*. 2001].

The *ESR locus* was chosen as a candidate gene for litter size due to its integral role in several reproductive pathways as described by Rothschild *et al*. [1996] and Short *et al*. [1997]. Using data from nearly 10 000 litters from several lines they reported that one of the two alleles (allele B) identified at the *ESR locus* seems to increase the number of piglets born alive. Estrogen is involved in maternal recognition of pregnancy [Geisert *et al*. 1990]. It is produced by the growing conceptus and is recognized by receptors in the uterus of the sow [Linville *et al*. 2001].

The objective of the research reported here was to test the polymorphism of the IGF1 and ESR genes (*IGF1* and *ESR*) and their effects on reproductive traits in sows.

Material and methods

Animals

Total number of piglets born (**TNB**), number of piglets born alive (**NBA**), litter weight at birth (**LWB**), litter weight at weaning (**LWW**), number of piglets weaned (**NPW**), average piglet weight at weaning (**AWW**), and gestation length (**GL**) were recorded in 53 F_1 sows (Zlotnicka Spotted boars × Polish Large White sows) from the National Institute of Animal Production, Kołbacz Farm. Forty-six out of 53 sows were recorded for the second parity.

Molecular test

DNA was isolated from the leukocytes of blood samples. The PCR-RFLP procedure described by Kirkpatrick [1992] was used to establish the genotypes of *IGF1* and those by Short *et al*. [1997] and Drögemüller *et al*. [1997] for *ESR* genotyping. The

IGF1 microsatellite was amplified using PCR with primers from INTERACTIVA. The PERKIN-ELMER ABI PRISM 310™ Genetic Analyser was used to analyse PCR products obtained by amplification with fluorescence-marked starters. The interpretation of alleles was based on graphs by the automatic analyser. Allele size was determined in base pairs (bp) comparing the length of the PCR product subjected to capillary electrophoresis separation (polymer POP4) with a standard length – TAMRA 500. ESR-specific DNA was amplified using PCR with primers from BIONOVO. Three different PCR products were obtained in *ESR locus*. Two of them appearing in exon 8 [Drögemüller *et al*. 1997] were digested with restriction enzymes *Ava*I and *Msp*A1I, depending on the mutation site. The third PCR product was obtained in intron and was digested with restriction enzyme *Pvu*II. Electrophoresis was conducted in 4% agarose gel (SIGMA).

Statistical

The relationship between *IGF1* and *ESR* genotypes and reproductive traits was evaluated with a least squares method. All litter data were analysed using GLM procedure of SAS (8.2 software, copyright 1999-2001, SAS Institute, Inc., Cary, NC. USA).

The model for TNB, NBA, LWB, LWW, NPW, AWW and GL included the fixed effects of *IGF1* or *ESR* genotypes of a sow, parity, year and season of mating, number (mark) of a boar and covariate of additive and dominance effects of *RYR1* genotype.

In the model for TNB, NBA and LWB the GL was included as covariate, while in that for GL – the TNB.

Additionally the data were analysed using model including the interaction between *ESR* genotypes, the remaining factors in the model being unchanged.

Linear contrasts between model-adjusted least squares means were used to test for differences between genotypes.

Table 1. Number of sows of each *IGF1* genotype

IGF1 genotype	First litter (n=53)	First and later litters (n=99)
124/128	4	7
124/132	6	15
128/128	10	20
128/132	22	44
132/0	1	2
126/128	2	4
126/132	1	3
130/132	3	6

Results and discussion

At *IGF1 locus* six microsatellite variants were identified (Tab. 1). The $(AC)_{21}$ repeat was identified by Wintero *et al.* [1994] in the first intron at position 921 to 1002. The DNA fragments 124, 126, 128, 130 and 132 bp, and a null allele were observed with the frequencies of 0.11, 0.03, 0.47, 0.03, 0.35 and 0.01, respectively.

Because in some genotype groups the numbers of first parity sows were really low the data of first parity and second parities were pooled, thus providing a greater number of data for statistical evaluation and increasing the credibility of results and conclusions (Tab. 1). Moreover, there were only a few sows with 132/0, 126/128, 126/132 genotypes (Tab. 1) that were excluded from the statistical evaluation.

At *IGF1 locus* the sows with the 130/132 genotype produced the greater TNB than 124/132 (P≤0.01), and 128/128 and 128/132 (P≤0.05) sows. Also the NBA of 130/132 sows was found higher than of 128/132 (P≤0.01) and 124/132 and 128/128 sows (P≤0.05). The former showed also higher LBW than 128/128 sows (P≤0.05). The GL was significantly higher in 128/128 compared to 124/132 (P≤0.01) and 124/128 (P≤0.05) sows (Fig. 1, 2, 3, 4). There are no earlier results published on the relationship between *IGF1* genotypes and reproduction traits in pigs, but the factor was found involved in certain reproductive processes, and the ovary was demonstrated to be a site of IGFs production and gene expression, reception and action [Zhao *et al.* 2001].

At *ESR locus* three point mutations were detected. Two in exon 8 where a T→C mutation at position 1665 creates an *Ava*I restriction site, and A→G mutation at posi-
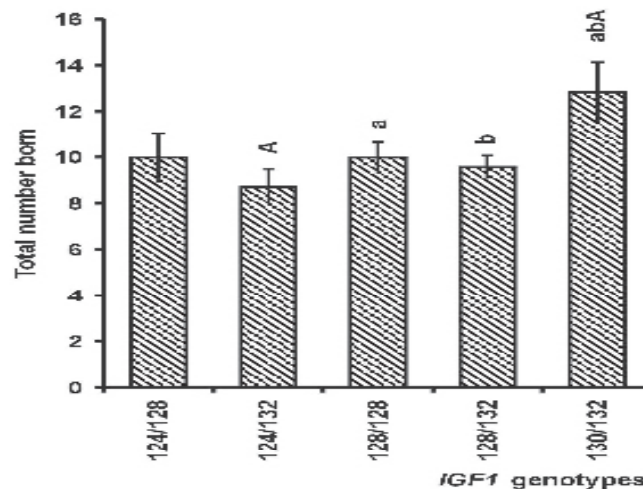

Fig. 1. Effect of *IGF1* genotypes on total number of piglets born (TNB).
[a][A]...Means represented by bars bearing the same letters differ significantly at: small letters – P≤0.05; capitals – P≤0.01.
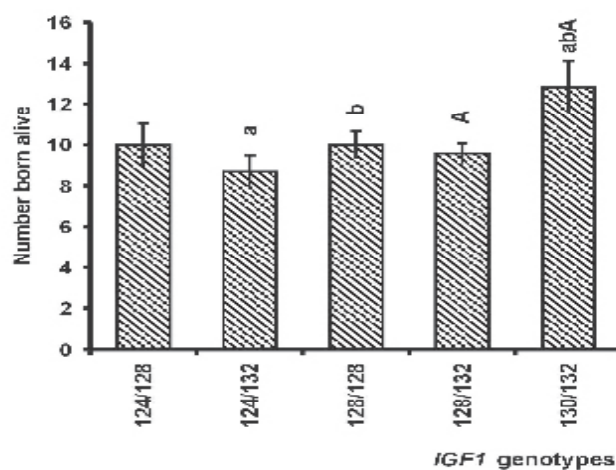
Fig. 2. Effect of *IGF1* genotypes on number of piglets born alive (NBA).
aA...Means represented by bars bearing the same letters differ significantly at: small letters – P≤0.05; capitals – P≤0.01.
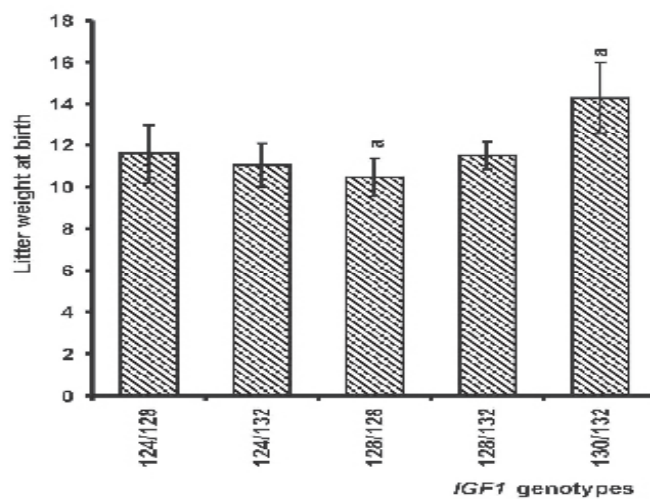

Fig. 3. Effect of *IGF1* genotypes on litter weight (kg) at birth (LWB).
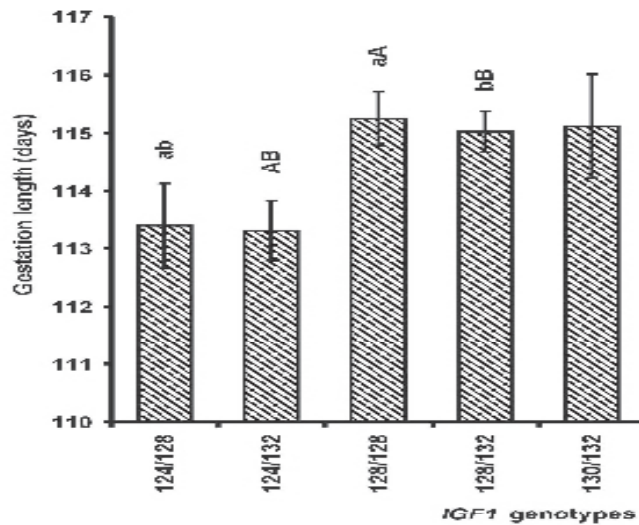a...Means represented by bars bearing the same letters differ significantly at: small letters – P≤0.05.

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Fig. 4. Effect of *IGF1* genotypes on gestation length (GL).
aA...Means represented by bars bearing the same letters differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

tion 1756 creating *Msp*A1I restriction site. At *ESR locus* only two alleles appeared, as expected for type I markers. For *Ava*I, allele "3" (109 and 76 bp) and "4" (76 , 62 and 47 bp) with the frequencies 0.6 and 0.4 were distinguished, while for *Msp*A1I restriction site allele "5" (98, 55, and 18 bp) and "6" (98 and 73 bp) with the frequencies 0.6 and 0.4. The third mutation, detected by *Pvu*II restriction enzyme [Gibson *et al*. 2002], was located in an intron with two alleles – "A" (120 bp) and "B" (65 and 55 bp) with the frequencies of 0.7 and 0.3.

Least squares means and standard errors for the *ESR* genotype effects on sows' reproductive traits are shown in Tables 2, 3 and 4. No association appeared between reproduction traits and *ESR* genotype in any of the observed restriction sites. For the *ESR*/*MspA*1I polymorphism for TNB, NBA, LWB, LWW and NPW, animals of the genotype 5/6 showed not significant trends for a lower reproductive efficiency than those of 5/5 or 6/6 genotype (Tab. 3). Considering the *ESR*/*PvuII* polymorphism (Tab. 4) sows of the B/B genotype were excluded from the analysis due to the low number of animals in the group (n=2).

These results are contradictory with those of Korwin-Kossakowska *et al*. [1999] who found significant differences between *ESR* genotypes in litter weight and gestation length analysing a group of similar animals (from the same crossing and herd, but different in number). In both cases *AA/Pvu*II genotype was favourable, but in the quoted report the *RYR1* genotype was not considered. Van Rens [2001] reported that crossbred (Large White × Meishan) *AB* sows produced more piglets born and born alive

Table 2. Least squares means (LSM) and their standard errors (SE) for the *ESR/AvaI* genotype effects on reproductive traits in sows

Trait	ESR/AvaI genotype					
	3/3 (n=32)		3/4 (n=60)		4/4 (n=13)	
	LSM	SE	LSM	SE	LSM	SE
TNB	9.14	0.64	9.71	0.46	9.37	0.96
NBA	8.76	0.62	9.33	0.45	9.27	0.94
LWB	11.09	0.83	11.22	0.59	12.27	1.24
LWW	47.43	4.77	49.45	3.42	46.87	7.10
NPW	6.76	0.61	7.02	0.43	6.60	0.90
AWW	7.02	0.23	7.03	0.17	7.07	0.34
GL	114.67	0.47	114.57	0.33	114.99	0.69

TNB – total number of piglets born.
NBA – number of piglets born alive.
LWB – litter weight at birth.
LWW – litter weight at weaning.
NPW – number of piglets weaned.
AWW – average weight at weaning.
GL – gestation length.

Table 3. Least squares means (LSM) and their standard errors (SE) for the *ESR/MspA1I* genotype effects on reproductive traits in sows

Trait	ESR/MspA1I genotype					
	5/5 (n=45)		5/6 (n=39)		6/6 (n=15)	
	LSM	SE	LSM	SE	LSM	SE
TNB	10.06	0.56	9.07	0.51	10.08	0.77
NBA	9.62	0.54	8.76	0.50	9.54	0.76
LWB	11.26	0.71	10.81	0.66	11.82	0.99
LWW	52.18	4.14	45.92	3.83	50.99	5.65
NPW	7.47	0.52	6.52	0.48	7.10	0.71
AWW	6.92	0.20	7.12	0.18	7.06	0.27
GL	114.93	0.28	114.73	0.25	115.48	0.37

Traits symbols are explained at the bottom of Table 2.

in a litter than did sows *BB*. The significant increase of estrogen synthesis by the rapidly elongating conceptus before establishment of pregnancy in pigs indicates the major influence of estrogen receptors on embryonic mortality [Pope 1994]. Drögemüller *et al*. [2001] reported no significant differences in number of piglets born alive between the *AA* and *AB* sows from the synthetic line Duroc × Large White. No further analyses were possible due to missing *BB* animals.

Interesting results appeared from the analysis that included effect of interactions

Table 4. Least squares means (LSM) and their standard errors (SE) for the *ESR/PvuII* genotype affects on reproduction traits in sows

Trait	*A/A* (n=44)		*A/B* (n=53)		*B/B* (n=2)	
	LSM	SE	LSM	SE	LSM	SE
TNB	9.95	0.56	9.49	0.47	6.72	1.76
NBA	9.63	0.55	9.07	0.46	6.77	1.72
LWB	11.48	2.02	11.22	2.11	9.25	1.35
LWW	50.88	4.28	49.25	3.60	29.80	13.26
NPW	7.08	0.54	7.07	0.45	4.21	1.66
AWW	7.15	0.20	6.98	0.17	7.26	0.63
GL	114.76	0.42	114.62	0.34	113.30	1.26

Traits symbols are explained at the bottom of Table 2.

between *ESR* genotypes in three polymorphic sites. Almost all traits analysed were found associated (P≤0.05) with genotypes, but because of a small number of sows in some of them the relations shown could have been accidental and must be treated with care. All results are shown in Tables 5, 6 and Figure 5. More favourable interactions

Table 5. Least squares means (LSM) and their standard errors (SE) for the *ESR PvuII/MspI/AluI/AvaI* genotype effects on reproductive traits TNB, NBA and NPW in sows

Genotype	N	Trait					
		TNB		NBA		NPW	
		LSM	SE	LSM	SE	LSM	SE
AA5533	4	9.09ᶠ	1.30	8.91	1.28	6.64	1.24
AB5533	4	11.08ᵃᵉᶠ	1.35	11.00ᵃᵇᶜ	1.33	8.82ᵃᵈ	1.27
AA5534	6	9.37ᵉ	1.09	9.01	1.07	6.46	1.04
AB5534	18	9.55ᵇ	0.71	9.13	0.70	7.60ᵇᶜ	0.67
AA5544	6	9.27	1.25	8.96	1.24	7.05	1.19
AB5544	4	10.97ᵈ	1.57	10.37	1.65	6.34	1.54
AA5633	6	8.14	1.34	7.95	1.35	6.57	1.21
AB5633	8	7.73ᵃᵇᵃ	1.05	7.11ᵃᵈᶠ	1.04	6.16ᶠ	1.00
AA5634	12	10.20ᵇʳ	0.76	9.77ᵈ	0.75	6.83	0.72
AB5634	12	9.93ᵂⁿˢ	0.88	9.30	0.86	7.39ᶠ	0.84
BB5634	2	6.50ᵐᵏᵒ	1.74	6.66ᵇ	1.72	3.79ᵇᶻ	1.65
AB5644	2	3.68ᵈᶠᵗʰⁿᵘ	2.3	5.88	2.33	4.34	2.22
AA6633	6	10.76ʳᵉˢˢ	1.14	10.46ᵉ	1.13	8.71ᶜᵗʰ	1.08
AB6633	4	7.90	1.35	6.90ᶠᵉ	1.33	4.44ᵈᵗʰ	1.23
AA6634	6	10.89ᵏⁿᵒᵘ	1.26	10.29ʳ	1.26	7.66	1.21

Traits symbols are explained at the bottom of Table 2.
* Means in columns bearing the same superscripts differ significantly at P<0.05.

Table 6. Least squares means (LSM) and their standard errors (SE) for the *ESR* *Pvu*II/*My*aI/*Ava*I genotype effects on reproductive traits LWB, LWW and AWW in sows

Genotype	N	Trait						
		LWB		LWW		AWW	
		LSM	SE	LSM	SE	LSM	SE
*AA*55/33	4	9.92[a]	1.65	46.69	9.97	7.11	0.49
*AB*55/33	4	12.57	1.71	57.49	10.26	6.41[a]	0.51
*AA*55/34	6	10.93	1.38	43.85	8.37	6.56[b]	0.41
*AB*55/34	18	10.75[b]	0.90	54.57[a]	5.40	7.08	0.26
*AA*55/44	6	10.71	1.59	53.47	9.61	7.40	0.48
*AB*55/44	4	15.21[abcdef]	2.12	40.06	12.37	6.33[c]	0.61
*AA*56/33	6	10.85	1.70	52.26	9.79	7.93[abcd]	0.48
*AB*56/33	8	9.37[ac]	1.34	42.11[b]	8.06	6.80[d]	0.40
*AA*56/34	12	10.51[d]	0.96	47.06	5.79	6.95	0.29
*AB*56/34	12	12.33[h]	1.12	53.38	6.76	7.35	0.33
*BB*56/34	2	9.35[e]	2.22	25.19[c]	13.26	7.08	0.66
*AB*56/44	2	9.28	3.00	31.79	17.87	7.45	0.86
*AA*66/33	6	13.06[g]	1.45	62.74[bcd]	8.75	7.09	0.43
*AB*66/33	4	8.31[fh]	1.71	33.65[d]	9.90	7.33	0.49
*AA*66/34	6	12.91	1.62	56.35	9.77	7.11	0.48

Traits symbols are explained at the bottom of Table 2.
[a] Means in columns bearing the same superscripts differ significantly at P<0.05.

(genotypes) – AB/55/33, AB/55/44, AA/66/33 and AA/66/34 – gave higher (P≤0.05) TNB than AB/56/33 or BB/56/34. AB/55/33 sows had the NBA significantly (P≤0.05) higher than *AB*/56/33, *BB*/56/34 and *AB*/66/33 sows. *AA*/66/33 and *AB*/55/33 sows showed higher NPW compared with *AB*/66/33 (Tab. 5). The highest LWB was shown in *AB*/55/44, while the highest LWW in *AA*/66/33 sows. Animals of genotype *AA*/56/33 showed the highest AWW (Tab. 6).

Sows of the genotype *AA*/56/33 had the shortest GL and differed (P≤0.01) from those bearing *AB*/55/44, *AA*/56/34, *AB*/56/34, *AB*/66/33 and *AA*/66/34 genotypes, and *AB*/56/44 compared to *AB*/55/44 (P≤0.01) – Figure 5. Van Rens [2001] found no association between *Pvu*II mutation and other mutations in the porcine *ESR* gene. Also linkage analysis performed by Drögemüller *et al*. [1999] with the use of mutation in exon 8 and microsatellite covering 40 cM of the *ESR* surrounding genome did not show any evidence for the existence of a QTL affecting the number of piglets born by German Landrace sows.

Concluding, it should be stated that because of a small number of animals included, the results presented here must be treated with care. However, at *IGF1 locus,* sows of 130/132 genotype had larger litter size (TNB, NBA) and higher litter weight (LWB), while those of genotype 128/128 and 128/132 showed the longest gestation (GL) and significantly smallest litter size (TNB, NBA) and lowest litter weight (LWB). On the
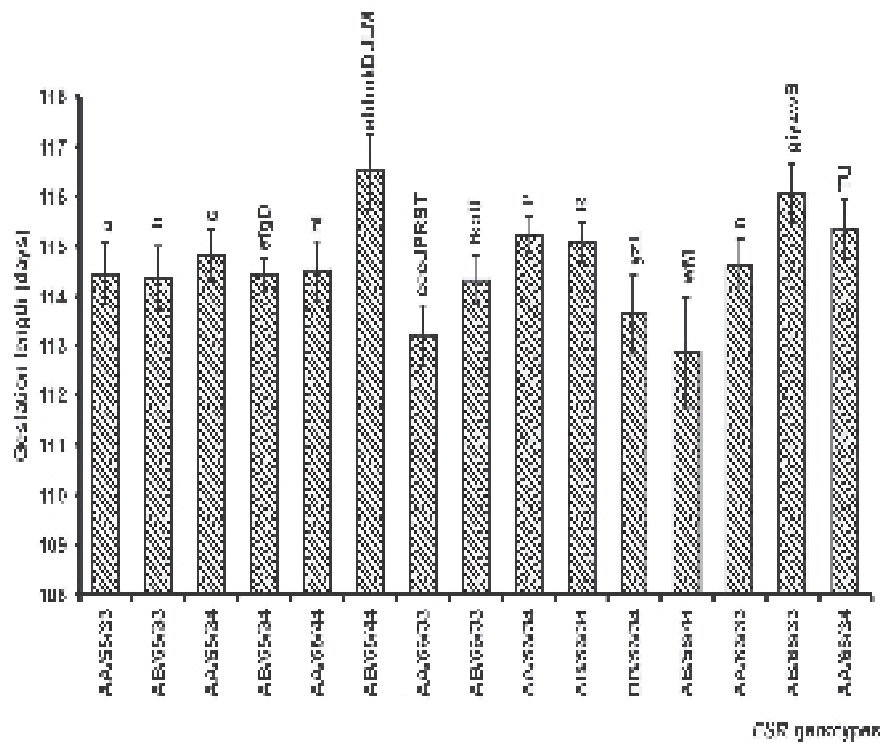
Fig. 5. Effect of *ESR* genotypes on gestation length (GL).
aA...Means represented by bars bearing the same letters differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

basis of the results reported here it can hardly be concluded whether the *IGF1* gene is, or is not, a marker for reproductive traits in pigs. It should be further examined on a larger number of animals. Future studies shall also involve a new polymorphism in the coding region within the *IGF1* gene.

No association was found between reproduction traits and *ESR* genotype in any of the observed restriction sites. This may have been affected by small sample size or a lack of segregation at the *ESR locus*. The *Pvu*II mutation identified in an intron and caused by the polymorphism at the *ESR locus* makes a difference in *ESR* expression or structure very unlike to appear, and thus increases the probability of *ESR* to be a marker for litter size. Still the existence of a difference in the *ESR* structure produced by the different *ESR* alleles cannot entirely be excluded. Alternative splicing, involving retention of introns has been described for other genes [Freeman *et al*. 2000] and might have occurred in porcine *ESR* gene, as well. According to van Rens [2001] this mutation can also be a marker for a mutation in another closely linked gene, or for

another mutation within the same gene.

To the authors' knowledge no analysis similar to the one reported here on the interactions between *ESR* genotypes in three polymorphic sites has so far been published. However, the research presented here does not determine which of combined genotypes is most favourable, but may suggest a possibility of selecting such genotype by testing the more numerous animal material.

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Agnieszka Korwin-Kossakowska, Grażyna Sender, Jolanta Kurył

Zależność między sekwencją mikrosatelity położonego w obrębie genu IGF1 i polimorfizmem genu ESR a wybranymi wskaźnikami rozpłodu loch pokolenia F_1 (złp × wbp).

S t r e s z c z e n i e

Na 53 lochach pokolenia F_1 (knury złp × lochy wbp) badano polimorfizm genu insulino-podobnego czynnika wzrostu 1 (*IGF1*) i genu receptora estrogenu (*ESR*), określając jego wpływ na liczbę prosiąt urodzonych ogółem (TNB), liczbę prosiąt urodzonych żywo (NBA), masę miotu przy urodzeniu (LWB), masę miotu w dniu odsadzenia (LWW), liczbę odsadzonych prosiąt (NPW), średnią masę prosięcia przy odsadzeniu (AWW) i długość ciąży (GL).

Stwierdzono istotną (P≤0.01) zależność między genotypem *IGF1* (sekwencja mikrosatelitarna) a TNB, NBA i GL. Zależności takiej nie stwierdzono natomiast między genotypem ESR a badanymi cechami reprodukcyjnymi, w żadnym z analizowanych miejsc restrykcyjnych. Z drugiej strony, interesujące rezultaty uzyskano w wyniku statystycznej analizy dokonanej z uwzględnieniem interakcji między genotypami ESR w trzech miejscach polimorficznych, gdzie prawie wszystkie badane wskaźniki rozrodu okazały się istotnie zależne od genotypu. Ze względu jednak na niewielką liczbę zwierząt, wnioskowanie o zależności między polimorfizmami badanych genów a cechami reprodukcyjnymi jest jedynie wstępne i należy je traktować