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Estrogen receptor gene (*ESR*) and semen characteristics of boars

Abstract

Estrogen receptor gene (*ESR*) which is localized in the swine chromosome 1, has been recognized as a "candidate" gene of reproductive traits. The aim of this study was to determine the mutations in the *ESR/Ava*I (*ESR1*) and *ESR/Pvu*II (*ESR2*) gene in boars kept at the AI Station and its effect on selected quantitative and qualitative characters of the semen. The study included 217 boars maintained at the AI Station. The *ESR* genotypes were determined with PCR-RFLP. Two alleles *ESR1* were identified: A (109 and 76 bp), B (76, 62 and 47 bp) and also two alleles *ESR2*: *C* (120 bp), *D* (65 and 55 bp), with the frequencies: 0.79 (*A*), 0.21 (*B*), 0.83 (*C*) and 0.17 (*D*) respectively. In the studied population of boars, the genotype *AA* was detected with the frequency 0.69, *AB*-0.21, *BB*-0.10 of *ESR1* and *CC*-0.72, *CD*-0.23, *DD*-0.05 of *ESR2*.

The analysis showed statistically significant differences (P≤0,01) between boars carrying different *ESR1* and *ESR2* genotypes and all semen traits.

Key Words: estrogen receptor gene, *ESR*, boars, reproduction traits

Zusammenfassung

Titel der Arbeit: **Östrogenrezeptorgen (*ESR*) und Spermamerkmale von Ebern**

Das Östrogenrezeptorgen (*ESR*) ist auf dem Chromosom 1 des Schweines lokalisiert und es werden Effekte seiner Allele auf Fruchtbarkeitsmerkmale gezeigt. Das Ziel der Untersuchungen war die Bestimmung der Frequenz der Mutation im *ESR/Ava*I (*ESR1*) und *ESR/Pvu*II (*ESR2*) Gen sowie die Prüfung ihres Einflusses auf ausgewählte quantitative und qualitative Spermamerkmale bei 217 Ebern aus einer Besamungsstation. An Hand der Methode PCR-RFLP wurde die Mutationsfrequenz im *ESR*-Gen bestimmt. Identifiziert wurden die zwei Allele des *ESR1* Gen: *A* (109 und 76 Basenpaare) und *B* (76, 62 und 47 Basenpaare) und zwei Allele des *ESR2* Gen: *C* (120 Basenpaare) und *D* (65 und 55 Basenpaare), mit einer Frequenz von entsprechend 0.79 (*A*), 0.21 (*B*), 0.83 (*C*) und 0.17 (*D*). In der untersuchten Ebernpopulation werden die Genotypen: *AA*-0.69, *AB*-0.21, *BB*-0.10 von *ESR1* und *CC*-0.72, *CD*-0.23, *DD*-0.05 von *ESR2*.

Die ermittelten Ergebnisse wiesen eine signifikante (P≤0,01) Überlegenhei der Eber mit den Genotypen *ESR1* und *ESR2* hinsichtlich alles untersuchten Spermamerkmale auf.

Schlüsselwörter: Östrogenrezeptorgen, *ESR*, Eber, Spermamerkmale

Introduction

In younger time studies on porcine genome contributed to identification of polymorphic loci of the genes that may influence the level reproduction-relates traits in sows (KMIEĆ, et al., 2001; LINVILLE, et al., 2001; DRÖGEMÜLLER et al., 1999) and boars (MAĆKOWSKI, et al., 2004; KMIEĆ et al., 2003; SCHLINGMANN et al., 2002; KMIEĆ et al., 2001).

Progress in genetics and molecular techniques has enabled application of DNA polymorphism by selection of animals known as marker-assisted selection (MAS). Estrogen receptor (*ESR*) gene which is localized on the first swine chromosome (p25-p24) is the "candidate gene" for reproductive traits. It belongs to intacellular group of receptors. In pigs and human the length of ESR protein is the same (595 aminoacids) and consists the same domains A, B, C, D, E and F (BÖKENKAMP et al., 1994). ESR

plays an important role in many processes associated with reproduction. In females it influences on the maturation of milk gland during embryogenesis as well as Graafian follicle during menstruation cycle (TKACZYK and KALITA, 2001). In males ESR has impact on beginning and maintaining of spermatogenesis on different levels of hormone regulation: 1.hypothalmus-pituitary-testis axis, 2. Leydig, Sertoli and gametogenesis cells, 3. discharging tubule and epidydidymys (PAZIEWSKA and BILIŃSKA, 2003). The mutation within *ESR* gene results in changes leading to disturbance of reproduction (KMIEĆ et al., 2002). ROTHSCHILD et al. (1996) found associations between polymorphism in *ESR/Pvu*II locus and litter size in pigs by using PCR-RFLP method. An *Ava*I and *Msp*A1I polymorphisms were discovered in swine *ESR* gene by DRÖGEMÜLLER et al. (1997). Since that time many reports revealed status *ESR* gene as a "candidate" gene for performance traits in sows. Because estrogens have also significant impact on spermatogenesis, may presumed that mutations in its receptors cause changes in some semen parameters .

The aim of the present study was to estimate the frequencies of *ESR/Ava*I and *ESR/Pvu*II gene mutations and to find possible associations between different genotypes of these genes and characters of boars semen.

Materials and Methods

The experimental population included 217 boars kept at the same stations. Rearing and feeding conditions were equalized for all animals. Genomic DNA was isolated from blood leucocytes using Master Pure kit of Epicentre Technologies. ESR genotype was determined using the PCR procedure of SHORT et al. (1997) for *ESR1* and DRÖGEMÜLLER et al. (1997) for *ESR2*. Each sample prepared for the polymerase chain reaction included: 1.5 µL 10x PCR buffer, 1.3 µL 1.5 mM $MgCl_2$, 1.2 µL 10 m*M* dNTPs, 0.5 µL 10 µ*M* forward primer, 0.5 µL 10 µ*M* reverse primer, 1.5 µL genomic DNA, 1 µL *Taq* DNA polymerase (5 U), and 7.5 µL sterile deionised water. All reactions were performed on Biometra Cycler using the following temperature program: initial denaturation at 94ºC for 4 min, followed by denaturation at 94ºC for 40, primer annealing at 60ºC (for *ESR1*) or 55ºC (for *ESR2*), and primer extension at 72ºC, each for 30 sec, repeated for 30 cycles. A final extension step of 5 min at 72ºC completed the PCR amplification. Each sample was then digested with 5 I.U. of appropriate restriction endonuclease *Ava*I (*ESR1*) and *Pvu*II (*ESR2*) (MBI Fermentas) at 37ºC overnight. The restriction fragment of DNA were separated by electrophoresis in 3% agarose gel, stained with ethidium bromide at 0.5 µg/ml, and visualized by u.v. transillumination and recorded with the use of the Vilber Lourmat system.

The analysis of relationship between the *ESR1* and *ESR2* genotypes and ejaculate volume, sperm concentration, sperm alive percentage, number of live sperm in ejaculate were carried out by means of the GLM (General Linear Models) variation analysis procedure of the SAS® calculation package.

The following model was used:

$$Y_{ijkl} = \mu + g_i + ys_j + s_k + b_l + e_{ijkl}$$

where:

Y_{ijklm} - value of the character for the i–th *ESR* genotypes, j-th year season, k-th sire, l-boar breed

μ - population mean;

g_i – effect of i-th genotype (i = 1,2,3);

ys_j – constant effect of j-th year season (j = 1,2,…, 12) – (6 years*2 seasons);

s_k – constant effect of l-th sire;

b_l – constant effect of m-th boar breed (m = 1,2...,7);

e_{ijkl} – error.

The values of the studies traits were expressed as the means and their standard deviations.

Results

Two *ESR1* alleles were identified in boar herd under study: *A* and *B*. Three genotypes, namely *AA*, *AB* and *BB* were observed. The following lengths of restriction fragments were detected: 109 and 76 base pair (bp) for allele *A*, and 76, 62 and 47 bp for allele *B*. In the analyzed AI boars the alleles *A* and *B* occurred with a frequency of 0.79 and 0.21 respectively. The *AA* genotype occurred with a frequency of 0.69, *AB* with 0.21 and *BB* with 0.10 – Table 1.

Table 1
The number and frequency of *ESR1*/*Ava*I genotype and alleles of boars under study (Frequenz der Genotypen und Allele von *ESR1*/*Ava*I in der untersuchten Eberpopulation)

Breed	N	*ESR* genotype			*ESR* allele	
		AA	*AB*	*BB*	*A*	*B*
Polish Landrace	54	0.74	0.11	0.15	0.80	0.20
Duroc x Pietrain	50	0.76	0.16	0.08	0.84	0.16
Polish Large White	36	0.47	0.33	0.20	0.64	0.36
Hampshire x Pietrain	32	0.81	0.16	0.03	0.89	0.11
PIC	19	0.47	0.42	0.11	0.68	0.32
Pietrain	16	0.81	0.19	-	0.91	0.09
Polish synthetic line	10	0.60	0.40	-	0.80	0.20
Total	217	0.69	0.21	0.10	0.79	0.21

The polymorphism in the *ESR2* gene was also detected. Two different alleles of the *ESR2* gene were identified in the boar herd under study: alleles C and *D* that control the occurrence of three genotypes, namenly: *CC* , *CD* and *DD*. The lengths of restriction fragments detected during the experiment were as follows: 120 bp for allele *C* and 65 and 55 bp for allele *D*. The frequencies of the alleles and genotypes are presented in Table 2.

Table 2
The number and frequency of *ESR2*/*Pvu*II genotype and alleles of boars under study (Frequenz der Genotypen und Allele von *ESR1*/*Pvu*II in der untersuchten Eberpopulation)

Breed	N	*ESR* genotype			*ESR* allele	
		CC	*CD*	*DD*	*C*	*D*
Polish Landrace	54	0.78	0.18	0.04	087	0.13
Duroc x Pietrain	50	0.84	0.12	0.04	0.90	0.10
Polish Large White	36	0.39	0.44	0.17	0.61	0.39
Hampshire x Pietrain	32	0.91	0.09	-	0.95	0.05
PIC	19	0.42	0.53	0.05	0.68	0.32
Pietrain	16	0.94	0.06	-	0.97	0.03
Polish synthetic line	10	0.60	0.20	0.20	0.70	0.30
Total	217	0.72	0.23	0.05	0.83	0.17

Discussion

A higher frequency (0.87) of allele *A* was observed by DRÖGEMÜLLER et al. (1997) in Duroc, German Landrace, German Yorkshire and Synthetic Line pigs. A also higher frequency (0.91) was observed by DVORAK et al. (1998).

A similar frequency of allele C (0.83) was observed by SHORT et al. (1997) in Synthetic Line pigs. A higher frequency of allele C was revealed in Duroc (1.00), German Landrace (1.00) and Polish Landrace pigs (0.94) reported by DRÖGEMÜLLER et al. (2001), LINVILLE et al. (2001) and KMIEĆ et al. (2002) respectively. A lower frequency (< 0.60) was observed by LEGAULT et al. (1996), SHORT et al. (1997), and GIBSON et al. (2002) in Large White pigs.

Table 3 presents means and their standard errors for the boars semen traits across *ESR1* genotypes.

Table 3
Values of studied semen characters in reference to *ESR1*/*Ava*I genotype (Beziehungen untersuchter Spermamerkmale zu den *ESR1*/*Ava*I Genotypen)

Character		*ESR* genotype			Total
		AA	*AB*	*BB*	
Number of boars		149	46	22	217
Ejaculate volume [cm^3]	Mean	220.5^{A}	217.9^{B}	$207,9^{AB}$	218.7
	SD	77.3	81.0	70.5	77.6
Sperm concentration [mln/cm^3]	Mean	595.2^{A}	603.0^{B}	620.4^{AB}	599.5
	SD	121.8	127.2	126.0	123.7
Sperm alive percentage	Mean	72.8^{A}	72.4^{B}	71.0^{AB}	72.4
	SD	4.7	5.2	3.0	4.7
Number of alive sperms in ejaculate [mld]	Mean	91.6^{A}	91.9^{B}	88.7^{AB}	91.4
	SD	31.8	33.3	29.2	31.9

Means in rows designated with the same letter differ significantly at $P \leq 0.01$.

Table 4
Values of studied semen traits in reference to *ESR2*/*Pvu*II genotype (Beziehungen untersuchter Spermamerkmale zu den *ESR1*/*Pvu*II Genotypen)

Character		*ESR* genotype			Total
		CC	*CD*	*DD*	
Number of boars		156	48	13	217
Ejaculate volume [cm^3]	Mean	221.6^{AB}	211.1^{B}	$213,0^{A}$	218.7
	SD	77.3	78.4	78.5	77.6
Sperm concentration [mln/cm^3]	Mean	596.1^{AB}	607.3^{B}	610.1^{A}	599.5
	SD	120.7	134.6	119.4	123.7
Sperm alive percentage	Mean	72.5^{A}	72.2	71.9^{A}	72.4
	SD	4.8	4.4	4.7	4.7
Number of alive sperms in ejaculate [mld]	Mean	92.3^{AB}	88.9^{AC}	90.8^{BC}	91.4
	SD	31.7	32.1	34.0	31.9

Means in rows designated with the same letter differ significantly at $P \leq 0.01$

Mean ejaculate volume for all the boars was 218.7 cm^3 falling within the range of 50-245 cm^3 reported by DUBIEL (1985). The highest mean ejaculate volume was found in *AA* (220.5 cm^3) , while the lowest in *BB* genotype – 207.9 cm^3 (*ESR1*) boars and the recorded differences were statistically significant at $P \leq 0.01$ (Table 3).

For *ESR2* the highest mean ejaculate volume characterized boars with *CC* (221.6 cm^3) compared with the mean in *DD* genotype (213.0 cm^3) boars. The differences were confirmed statistically $P \leq 0.01$ (Table 4).
Mean spermatozoa concentration was 599.5 x $10^6/cm^3$. The highest concentration for *ESR1* showed the semen of *BB* (620.4 x $10^6/cm^3$), while the lowest of *AA* genotype (595.2 x $10^6/cm^3$) boars ($P \leq 0.01$) – Table 3.
For *ESR2* the greatest sperm concentration in ejaculates appeared in boars with *DD* genotype (610.1 x $10^6/cm^3$) and the lowest in boars with *CC* genotypes (595.2 x $10^6/cm^3$). The differences were confirmed statistically $P \leq 0.01$ (Table 4).
Average sperm alive percentage for all the analyzed boars was 72.4%. The highest sperm alive percentage (*ESR1*) was found for the boars with the *AA* genotype (72.8%), while the lowest for *BB* genotype (71.0%) and the recorded differences were statistically significant at $P \leq 0.01$ (Table 3).
The highest sperm alive percentage (*ESR2*) characterized boars with CC (72.5%), while the lowest boars with DD genotype (79.1%) and this differences were significant ($P \leq 0.01$) – Table 4.
The last, but also important analyzed semen traits was number of live sperm in ejaculate. Mean number of live sperm in ejaculate was 91.4 x 10^9. The greatest number appeared in boars with *AA* (91.6 x 10^9) and *CC* (92.3 x 10^9) genotype, while the smallest boars with *BB* (88.7 x 10^9) and *CD* (88.9 x 10^9) genotype for *ESR1* and *ESR2* respectively. The differences were confirmed statistically $P \leq 0.01$ (Table 3, 4).

Conclusion

The analysis of relation between the genotype of estrogen receptor and the studied reproductive traits showed statistically significant differences ($P \leq 0.01$) between all semen traits of boars carrying different *ESR1* and *ESR2* genotypes. The preliminary study showed that boars with *AA* (*ESR1*) and *CC* (*ESR2*) genotype produced ejaculates of large volume higher percentage and also higher number of sperm in ejaculate. The study suggest a possibility of using the existing polymorphisms in the estrogen receptor gene to improvement of some reproductive performance traits of boars. The results, however, should be verified by further research of *ESR/Ava*I and *ESR/Pvu*II polymorphisms on a larger number of animals.

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