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Relation Between ESR 2 Genotypes and Reproductive Performance of a Large White x Landrace Sow Population

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Abstract

The aim of the present study was to investigate any potential association of ESR2 genotypes with prolificacy in pigs. Polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) was performed for genotyping ESR2 gene in pig populations derived from a commercial farm in Greece. 400 sows were genotyped in total and the records of five litters concerning Total Number of Born (TNB), Number of Born Alive (NBA), Number of Born Dead (NBD), Number of Born Mummified (NBM) and Number of Weaned (NW) piglets were used in the association analysis. The population found to be in Hardy-Weinberg equilibrium, while the total number of born piglets, the number of piglets born alive, the number of piglets that born dead and the number of the piglets born mummified did not differ between the observed ESR2 genotypes. However, the AB genotype gave statistically significant ($P < 0.05$) higher values of the number of weaned piglets and lower values concerning the aborted pigs. According to our results, the AB genotype of ESR2 gene are in favor of producing larger litter size, suggesting that the certain gene may be used in Marker-assisted selection (MAS) programmes for a rapid improvement of the reproductive characteristics in pigs.

1. Introduction

The genetically improvement of swine litter size is of expanding interest for pig producers mainly, because ameliorations due to feeding regime and housing conditions are limited. It has been observed that during the past century improvements were implemented primarily on traits concerning growth and carcass characteristics, leading to stagnancy and a decrease of litter size in swine production [1,2].

As litter size belongs to the traits of economical importance in pig production, it can be inferred that any improvement of fecundity in pigs is highly desirable. Consequently, an improvement of swine reproduction traits has become of expanding interest, where moderate increases in litter size can equal large gain in profit [3]. The implementations of selection schemes based only on phenotypical traits were proved to be high laborious, expensive and time consuming due to the fact of the low heritability and intense selection of the sex-limited trait of litter size in pigs.

The advent of novel DNA technology assisted the association of genes with traits of economic interest in animal production and consequently impelled the design of novel selection schemes incorporating DNA-based information (i.e. marker assisted selection,

gene assisted selection). Several studies have been conducted in reference to genes, and their association with reproductive traits in pigs [3,4,5,6,7,8]. Among these, the estrogen receptor 2 (ESR2) gene has been referred that is associated with ovarian follicular growth and development at preimplantation [9], making the ESR2 gene into a strong candidate of genetic variation at prolificacy traits. The ESR2 gene has been characterized in the rat [10], mouse [11], human [12] and recently in pig (Kowalski *et al.*, 2002) [9] showing highly conserved regions. In pigs one missense mutation (c.949G>A; p.317Val>Met) in exon 5 has been shown to be associated with litter size [13], but with inconsistencies among different populations [13, 14,15,16].

Pig farming in Greece is considered as one of the most dynamic sectors of the rural economy [17], facing however many difficulties in prolificacy. As the latter is one of the most important parameters affecting reproductive efficiency of sows (weaned pigs /sow/year), the aim of the present study was to investigate whether exists an association of ESR2 genotype with prolificacy in a commercial pig farm in Greece, so as to be able such information to be used in future in breeding selection schemes.

2. Materials and Methods

2.1. Animals and Data Collection

The pig population was derived from a Greek commercial farm (North Western of Greece). 400 sows (crossbreed between Large White x Landrace) were randomly selected from the whole population of farm among those who gave their first birth at the age of 12 months and had at least 5 continuous litters and were genotyped. Sows were artificially inseminated with a constant amount of fresh semen derived from Duroc x Pietrain boars. All sows were kept under the same feeding and housing conditions and their reproductive performance by means of: i) the total number of born piglets (TNB), ii) the number of piglets born alive (NBA), iii) the number of piglets born dead (NBD), iv) the number of mummified piglets (NBM), v) the number of aborted piglets (ABRT) and vi) the number of weaned piglets (NW) was permanently recorded by the staff of the farm.

2.2. DNA Isolation and Genotyping

Polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) for the ESR2 gene was performed. For estimating the genotype distribution and allele frequencies, DNA was extracted from hair roots of the sows. The primer sequences were 5'-AAAATACTGATACCCACCCACAT-3' for the forward 5'-CGCCACATCAGCCCCACCAT-3' for the reverse primers [14].

Extraction of DNA was performed using the Nucleospin tissue kit (Macherey-Nagel, Germany). An electrophoresis was performed to ensure the integrity of the DNA samples. The PCR amplification was performed as follows:

Approximately 150 ng of genomic DNA was used as template and amplified in a final volume of 25 µl containing 200nM from each primer, 1mM dNTPs and 1 unit MyTaq™ DNA Polymerase (Bioline). PCR amplification was performed using the following conditions: Initial denaturation at 95 °C for 5 min, 30 amplification cycles including denaturation at 95 °C for 30s, annealing at 56 °C for 30s and extension at 72 °C for 45s and a final extension step at 72 °C for 10 min. Finally, 15 µl of PCR product (218 bp) was digested in a total volume of 20 µl, containing 10 U of enzyme Hsp92II (Takara), 2 µl of restriction buffer and 2 µl of BSA for 3 hours. Restriction fragments were examined by electrophoresis on 2.5 % agarose gel with 1x TBE buffer. The gels were stained with ethidium bromide and photographed under UV illumination.

2.3. Statistical Analysis

Genotype frequencies, allele frequencies and Hardy-Weinberg equilibrium estimations were calculated using PopGene Software v. 1.32 [18]. The statistical procedures were performed using the SPSS program (version 16.0). A mixed model was used for the analysis of associations between the ESR2 genotypes and the total number of born piglets (TNB), the number of piglets born alive (NBA), the number of piglets born dead (NBD), the number of piglets born mummified (BMUM), the number of aborted piglets (ABRT) and the number of weaned piglets (NW). Due to the uneven distribution of first litters, their parameters were ignored. The models were as follows:

$$Y_{ijk} = \mu + G_i + L_j + (G_i * L_j) + T_k + e_{ijk}, \text{ where}$$

Y_{ijk} = trait value,

μ = general mean,

G_i = the fixed effect of genotype ($i=1,2,3$),

L_j = the fixed effect of litter parity ($j=2,3,4,5$),

$G_i * L_j$ = the effect of interaction between the i genotype and j litter parity,

T_k = the random effect of the sow ($k=1,2, \dots, 400$)

e_{ijk} = random error.

The values were expressed as the mean and their standard error.

3. Results and Discussion

3.1. Genotype and Allele Distribution of ESR 2 Gene

The observed allelic and genotypic frequencies in the examined population are presented in Table 1. The allele A had a higher frequency ($p=0.53$) than the allele B ($q=0.47$) in the population while an excess of heterozygotes was noted ($P=0.48$). The analyzed population was found in Hardy-Weinberg equilibrium. Similar allelic frequencies have been reported for Large White populations [19,20,21,22] and in Black Slavonian sows [23]. Higher allelic values for the A allele have been previously reported in Duroc [24], Polish Landrace [25] and in Large White [26] sows populations. It

has been referred that B allele originates from Chinese pig [27]. Thus, the presence of B allele in high fertility hybrid

sows may reflect an interbreeding of Chinese and English pigs [28].

Table 1. Genotype and allele frequencies of ESR2 gene.

GENOTYPE DISTRIBUTION (OBSERVED)			GENOTYPE DISTRIBUTION (EXPECTED)			GENOTYPE FREQUENCY			ALLELE FREQUENCY	
AA	AB	BB	AA	AB	BB	AA	AB	BB	A (p)	B (q)
118	191	91	114	199	87	0.29	0.48	0.23	0.53	0.47
$\chi^2=0.66, df=1$										

3.2. Association of Sows' Reproductive Traits and ESR2 Genotypes

The results of mean TNB, NBA, NBD, BMUM, ABRT and NW values in association with the observed genotypes are presented in Table 2. None statistical significant difference was observed for the analyzed characteristics of TNB, NBA, NBD and BMUM. However, in the examined population statistical significant differences ($P<0.05$) were observed for the NW and ABRT values.

Specifically, for the AB genotype a higher value of NW (12.15 ± 0.08) was observed in respect to the AA (11.84 ± 0.11) and the BB (11.91 ± 0.12) genotypes, reflecting to an additive effect of the AB genotype in the final number of weaned pigs. In line to our result, [29] reported highest values for NW trait in a Prestice Black-Pied sow population, but in that case a lack of the BB genotype identification was observed. On the other hand, other authors [14] reported no significant association between litter size and ESR2 genotypes, but these may be affected by the small number of the examined animals. Other authors reported a positive effect of allele B on the litter size of European breeds and synthetics lines of sows [4,5,8], while Van Rens et al. [6] reported a favorable effect of allele A on the same reproductive trait. It seems that

in our case both alleles have a synergistic effect reflecting to an additive effect of AB genotype on the number of weaned pigs.

As far as it concerns ABRT trait, the respective values for the AA, AB and BB genotypes were 0.06 ± 0.01 , 0.03 ± 0.01 and 0.06 ± 0.01 piglets/birth, respectively. It is worth noting that the AB genotype revealed a lower value of aborted pigs than the other genotypes leading indirectly to an increase of the litter size. To the best of our knowledge this is the first time that a certain genotype is associated with the aborted number of piglet/birth.

In conclusion, we reported that the genotype AB of ESR2 gene had a greater "beneficial" effect as far as it concerns NW and ABRT reproductive traits in the analyzed sows' population. It is not easily to establish that a certain genotype will always express an improved reproductive trait [30]. The selection of a certain genotype remains not a simple procedure, as allele effects differ between lines or populations due to the different genetic background [24]. Thus, detailed analysis of a possible candidate genotype that may affect a desire trait in a specific population should be performed prior to its inclusion in a selection breeding scheme.

Table 2. Effect of ESR2 genotypes on reproductive traits of sows in the analyzed population.

GENOTYPE	TNB	NBA	BDEAD	BMUM	ABRT	WEANED
AA	13.49 ± 0.10^{NS}	12.61 ± 0.10^{NS}	0.60 ± 0.03^{NS}	0.28 ± 0.03^{NS}	0.06 ± 0.10^a	11.84 ± 0.11^a
AB	13.71 ± 0.08^{NS}	12.80 ± 0.08^{NS}	0.62 ± 0.03^{NS}	0.28 ± 0.02^{NS}	0.03 ± 0.01^b	12.15 ± 0.08^b
BB	13.44 ± 0.11^{NS}	12.67 ± 0.12^{NS}	0.68 ± 0.05^{NS}	0.22 ± 0.03^{NS}	0.06 ± 0.01^a	11.91 ± 0.12^a

Mean values with different superscripts are significant different ($P<0.05$).

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References

- [1] Kisner, V., Mollers, B., Brand, H., Glodek, P., 1995. Die Analyse von Sauenaufzuchtleistungen in der Versuchsstation Relliehausen zur Entwicklung von Kriterien der Wurfqualität. 1. Mitt.: Der Einfluss der fixen Effekte der Rassenkombination, der Wurfnummer und die Verteilung der Geburtsgewichte. Arch. Tierz., Dummerstorf 38, 73-86.
- [2] Merks, J.W.M., 2000. One century of genetic changes in pigs and the future needs. Proceedings of the BSAS Occasional Meeting: "The challenge of genetic change in animal production". Edinburgh, 8-20.
- [3] Vincent, A.L., Evans, G., Short, T.H., Southwood, O.I., Plastow, G.S., Tuggle, C.K., Rothschild, M.F., 1998. The prolactin receptor gene is associated with increased litter size in pigs. In: Proc., 6th World Congr. Genet. Livest. Prod., 27, 15-18, Jan, 11-16, Armidale, Australia.
- [4] Rothschild M.F., Jacobson C., Vaske D., Tuggle C., Wang L., Short T., Eckardt G., Sasaki S., Vincent A., McLaren D., Southwood O., van der Steen H., Mileham A., Plastow G., 1996. The estrogen receptor locus is associated with a major gene influencing litter size in pigs, Proc. Natl. Acad. Sci. 93, 201-205.
- [5] Chen, K. F., Huang, L. S., Li, N., Zhang, Q., Luo, M., Wu, C. X., 2000. The genetic effect of estrogen receptor (ESR) on litter size traits in pigs. Acta Genetica Sinica 27, 853-857.

- [6] Van Rens, B.T., de Groot, P.N., van der Lende, T., 2002. The effect of estrogen genotype on litter size and placental traits at term in F2 crossbred gilts. *Theriogenol.* 57, 1635-1649.
- [7] Goliasova, E., Wolf, J., 2004. Impact of the ESR gene on litter size and production traits in Czech Large White pigs. *Anim. Genet.* 35, 293-297.
- [8] Horogh, G., Zsolnai, A., Komlosi, I., Nyiri, A., Anton, I., Fesus, L., 2005. Oestrogen receptor genotypes and litter size in Hungarian Large White pigs. *J. Anim. Breed. Genet.* 122, 56-61.
- [9] Kowalski A.A., Graddy L.G., Vale-Cruz D.S., Choi I., Katzenellenbogen B.S., Simmen F.A., Simmen R.C., 2002. Molecular cloning of porcine estrogen receptor-beta complementary DNAs and developmental expression in preimplantation embryos, *Biol. Reprod.* 66, 760-769.
- [10] Kuiper G.G.J.M., Carlsson B., Grandien K., Enmark E., Haggblad J., Nilsson S., Gustafson J.A., 1997. Comparison of the ligand binding specificity and transcript tis-sue distribution of estrogen receptors alpha and beta, *Endocrinology* 138, 863- 870.
- [11] Tremblay, G.B., Tremblay, A., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Labrie, F., Giguere, V., 1997. Cloning, chromosomal localization, and funcional analysis of the murine estrogen receptor- β . *Mol. Endocrinol.* 11, 353-365
- [12] Mosselman, S., Polman, J., Dijkema, R., 1996. ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Letter.* 392, 49-53.
- [13] Buske, B., Sternstein, I., Reißmann, M., Brockmann, G., 2006. Detection of novel single-nucleotide polymorphisms (SNPs) in the CYP21 gene and association analysis of two SNPs for CYP21 and ESR2 with litter size in a commercial sow population. *J. Anim. Breed. Genet.* 123, 343-348.
- [14] Muñoz, G., Óvilo, C., Amills, M., Rodríguez, C., 2004. Mapping of the porcine oestrogen receptor 2 gene and association study with litter size in Iberian pigs. *Anim. Genet.* 35, 242-244.
- [15] Muñoz, G., Ovilo, C., Estellé, J., Silió, L., Fernández, A., Rodríguez, C., 2007. Association with litter size of new polymorphisms on ESR1 and ESR2 genes in a Chinese-European pig line. *Genet. Sel. Evol.* 39, 195-206.
- [16] Buske, B., Sternstein, I., Brockmann, G., 2006. QTL and candidate genes for fecundity in sows. *Anim. Reprod. Sci.* 95,167-183.
- [17] Aggelopoulos, S., Zioganas, M., Karipidis, 2006. Productivity analysis of pig farms in Greece in conjunction with their size. *NewMedit* 3, 53-58
- [18] Yeh, F.C., Yang, R.C., Boyle, I., 1999. POPGENE Version 1.32. Microsoft Windows-based Freeware for Population Genetic Analysis.
- [19] Legault, C., Gruand, J., Lebost, J. 1996. Frequency and effect on prolificacy of the ESR gene in two French Large White lines. *J. Recherche Porcine France* 28 (1996), 9-14.
- [20] Short, T.H., Rothshild, M.F., Southwood, O.I., McLaren, D.G., de Vries, A., Van der Steen, H., Eckardt, G.R., Tuggle, 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 pigs lines. *J. Anim. Sci.* 75, 3138-3142.
- [21] Gibson, J.P., Jiang, Z.H., Robinson, J.A.B., Archibald, A.L., Haley, C.S., 2002 No detectable association of the ESR PvuII mutation with sow productivity in a Meishan x Large White F2 population. *Anim. Genet.* 33, 448-450.
- [22] Dall'Olivo, S., Fontanesi, L., Tognazzi, L., Russo, V., 2010. Genetic structure of candidate genes for litter size in Italian Large White pigs. *Vet. Res. Commun.* 34(1); 203-206.
- [23] Kabalin E.A., Starčević K., Menčík S., Maurić M., Sušić V., Štoković I., 2013. Analysis of ESR and RBP polymorphisms in black Slavonian sows: preliminary results. *Acta argiculturae Slovenica Supplement* 4, 45-48,
- [24] Drogemuller, C., Hamann H., Distl O., 2001. Candidate gene markers for litter size in different German pig lines. *J. Anim. Sci.* 79, 2545-2570.
- [25] Kmiec M, Dvorak J, Vrtkova I 2002: Study on a relation between estrogen receptor (ESR) gene polymorphism and some pig reproduction performance characters in Polish Landrace breed. *Czech J Anim Sci* ,47: 189-193
- [26] Matoušek V., Kernerová N., Kolaříková O., Křížová H., Urban T., Vrtková I., 2003. Effect of RYR1 and ESR genotypes on the fertility of sows of Large White breed in elite herds. *Czech J. Anim. Sci.*, 48, 129-133
- [27] 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: 2334-2339
- [28] 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
- [29] Horák, P., Urban, T., Dvořák, J., 2005. The FUT1 and ESR genes – their variability and associations with reproduction in Přeštice Black-Pied sows. *J. Anim. Breed. Genet.* 122, 210-213.
- [30] 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