The Effects of Estrogen Receptor Locus on Reproductive Tracts Components and Performance Traits in Large White×Meishan F2 Offspring*

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ABSTRACT : Previously candidate gene approach revealed estrogen receptor (ESR) locus was associated with increased litter size. In this study, *Pvu*II polymorphisms of ESR gene was detected by PCR-RFLP, and ESR locus was evaluated for its association with reproductive tracts components in the Large White×Meishan (LW×M) F2 offspring. Ninety seven gilts with reproductive tracts components records and 136 offspring with performance traits records were genotyped and the results were used to estimate allele substitution effects. The results showed that two alleles (A and B) were identified, and 121 bp fragments were observed for the AA genotype and 65 bp and 56 bp fragments for the BB genotype; the length of uterine body (LUB) of BB gilts were significantly shorter than AA gilts', the additive effect was -1.762 cm; the uterine weight (UW) of AB gilts were significantly lighter than AA gilts' with the additive effect -18.058 g; no significant associations of ESR alleles with ovulation rate (OR), length of uterine horn (LUH), length of uterine cervix (LUC), weight of two ovaries (OW), volume of uterine lumen (VUL), length of oviduct (LO) were observed. BB genotypes gilts need significantly less days to 100 kg ($D_{100\ kg}$) than AA genotypes ($p<0.01$), the additive effect was per copy of B allele. Allele B is also favorable for average daily gain (ADG), with additive effect 0.015 kg/d ($p<0.05$). There was no difference between genotypes for backfat thickness at the 13th rib (SF13), loin meat height (ELMH), and loin meat percentage was estimated (ELMP), individual birth weight (IBW) and teat number (TN). ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 9 : 1223-1226)***

Key Words : Pigs, Estrogen Receptor Gene, *Pvu*II Locus, Female Reproductive Tract Components, Performance Traits

INTRODUCTION

Estrogen receptor (ESR) is a ligand-activated transcription factor which binds to specific cis-acting hormone responsive to DNA element which behaves as an enhancer (Green et al., 1986: Ying et al., 2003). ESR and its hormone ligand play critical roles in the development of feminine secondary sexual characteristics as well as in the female reproductive cycle, infertility, and maintenance of pregnancy (Lubahn et al., 1993). In the recent years, there are many reports about ESR locus and its relationship between reproductive traits and some performance traits (Rothschild et al., 1994; Southwood et al., 1995; Short et al., 1997; Southwood et al., 1998; Zhu et al., 2004). This major gene was discovered with a Restriction Fragment Length Polymorphism (RFLP) for the ESR gene. At present, it is also not clear whether the RFLP polymorphism at the ESR locus is causally related to variation in litter size or whether this polymorphism merely provides a marker for a closely linked polymorphism (in or outside the ESR gene) for litter size. The objective of this study is to determine the physiological mechanism behind the difference in prolificacy as discovered by Rothschild et al. (1994) and confirmed later by Short et al. (1997).

* Supported by the National "863" project (2002AA211041) and "973" project (G2000016105) of P. R. China and the Key Project of Chinese Ministry of Education (104132).
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Received August 25, 2003; Accepted May 17, 2004

MATERIALS AND METHODS

Pig populations

In this study a three-generation resource family was investigated. The ancestors were three unrelated Large White grand boars and seven unrelated Meishan grand sows. Five F1 sires and 21 F1 dams, and 289 F2 offspring were produced, including 97 gilts with reproductive tracts traits records and 136 F2 offspring with performance traits records used in present study. All the pigs were bred and raised at the genetic nucleus station owned by Huazhong Agricultural University.

Traits

Reproductive tracts components : Reproductive tract characteristics, including length of uterine horn (LUH), length of uterine cervix (LUC), length of uterine body (LUB), uterine weight (UW), weight of two ovaries (OW), volume of uterine lumen (VUL), length of oviduct (LO) and ovulation rate (OR) were recorded. Ovulation rates have mostly been estimated by counting the corpora lutea on the surface of the ovaries. LUH, LUC and LUB were measured according to the method of Lin (1992); ULV was the maximum volume of filled water (Li et al., 2002).

Performance traits : Individual birth weight (IBW), teat number (TN), weight at 60 d (WT_{60}), weight and days at slaughter were recorded. The average daily gain (ADG) was

estimated according to days of the testing and total gain.

Backfat thickness at the 13th rib (SF13) and loin meat height (ELMH) were determined by ultrasonic measurement (Pig log105), and loin meat percentage was estimated (ELMP).

And the SF13 and days to 100 kg ($D_{100\ kg}$) were corrected according to the days and weight to slaughter, and the correction formula was described as National Protocol for Swine Genetic Evaluation in China. The correction formula was as follows.

Corrected $D_{100\ kg}$=testing age-[(testing weight-100)/CF];

CF=(testing weight/testing age)×1.826040 (boars);

CF= (testing weight/testing age)×1.714615 (gilts).

Corrected SF13=testing SF13×CF;

CF=A/{A+[B× (testing weight-100)]}

A=12.402, B=0.106530 (boars); A=13.706, B=0.119624 (gilts).

DNA preparation

Blood was collected in 50 mM EDTA at pH 8.0 to prevent coagulation and genomic DNA was extracted from blood white cells. DNA extraction procedure was described as Xiong (1999). ESR gene amplified using a PCR protocol that was developed by Iowa State University and Pig Improvement Company (PIC) (Short et al., 1997). Primers were as follows: 5′-CCT GTT TTT ACA GTG ACT TTT ACA GAG-3′; 5′-CAC TTC GAG GGT CAG TCC AAT TAC-3′(Short et al., 1997). Amplified products were digested with *Pvu*II restriction endonuclease, separated on a 4% agarose gel, and visualized under UV light following ethidium bromide staining. Two alleles (A and B) were identified, and 121 bp fragments were observed for the AA genotype and 65 bp and 56 bp fragments for the BB genotype.

Statistical analysis

All the data obtained were analyzed using General Linear Model (GLM) Procedure of SAS package (windows V8). Pairwise t test were used to test difference among ESR genotypes. Allele substitution effects were estimated by substituting or ESR genotype a covariate that included the number of B alleles present (0, 1 or 2) (Liu, 1998). Dominance effects were estimated as the deviation of heterozygotes from the mean of the homozygous genotypes.

RESULTS

Table 1 summarized the association of porcine ESR locus with the female reproductive tracts component. The

Table 1. Effects of ESR *Pvu*II locus on the female reproductive tracts component in L×M F2 offspring

Traits	Least square means±standardized error			Additive effect, a	Dominant effect, d	Dominance D
	AA	AB	BB			
N	17	48	32			
VUL (cm^3)	573.524±83.091	427.016±50.856	519.411±61.540	-45.477	-61.367	1.350
LUH (cm)	60.682±5.199	61.582±3.173	57.972±3.729	-0.611	0.714	-1.170
LUC (cm)	7.567±0.411	7.073±0.256	7.425±0.294	-0.038	-0.161	4.263
LUB (cm)	18.058±1.077[a]	15.747±0.657	15.098±0.772[b]	-1.762**	-0.516	0.293
UW (g)	386.890±30.765[a]	304.505±18.780[b]	327.170±22.066	-18.058	-16.133	0.893
OW (g)	13.271±2.398	12.206±1.4646	13.664±1.720	0.864	-0.396	-0.435
OR	13.750±1.094	12.625±0.774	12.667±1.031	-0.542	0.292	-0.539
LO (cm)	20.200±2.446	20.500±2.101	19.500±2.887	-0.700	1.300	-1.857

N: Total number of pigs observed. Means in the same line with different superscripts significantly differ at $p<0.05$; additive effects with superscripts.
** Significantly differ from zero at $p<0.01$; dominance degree (D)=d/a.

Table2. Effects of ESR *Pvu*II locus on performance traits in LW×M F2 offspring

Traits	Least square means±standardized error			Additive effect, a	Dominant effect, d	Dominance D
	BB	AB	AA			
N	91	40	5			
SF13 (mm)	15.968±0.850	16.436±0.570	16.696±0.908	-0.364	0.052	0.142
ELMH (mm)	24.961±0.907	24.894±0.669	26.192±1.699	0.127	0.113	0.890
ELMP (%)	52.309±0.721	52.613±0.533	53.471±1.355	0.274	-0.001	-0.004
IBW (kg)	1.447±0.043	1.457±0.037	1.351±0.059	0.047	0.042	0.913
ADG (kg/d)	0.493±0.007	0.490±0.006	0.474±0.010	0.015*	0.005	0.333
$D_{100\ kg}$ (d)	195.601±3.800[b]	202.342±3.267	214.012±5.442[a]	-11.642**	-2.079	0.178
WT_{60} (kg)	21.321±1.810	22.720±1.461	21.354±1.666	-0.039	0.391	-10.000
TN	14.758±0.067	14.880±0.083	14.334±0.151	0.212	0.334	1.632

Means in the same line with different superscripts significantly differ at $p<0.01$; additive effects with superscripts.
* and ** significantly differ from zero at $p<0.05$ and $p<0.01$, respectively; dominance degree (D)=d/a.

results showed that the LUB of BB gilts were significantly shorter than AA gilts', the additive effect was -1.762 cm ($p<0.01$); the UW of AB gilts were significantly lighter than AA gilts' ($p<0.05$), with the additive effect -18.058 g. From Table 2, we can see that BB genotype gilts need significantly less days to 100 kg than AA genotype ($p<0.01$), the additive effect was per copy of B allele. Allele B is also favorable for ADG, with additive effect 0.015 kg/d ($p<0.05$). There is no difference between genotypes for SF13, ELMH, ELMP, IBW and TN.

DISCUSSION

Our research results showed that the UW of AB gilts were significantly lighter than that of AA gilts' ($p<0.05$), with the additive effect of -18.058 g; while Isler et al. (1999) showed that the ESR genotypes were not significantly associated with UW. The difference might result from the different physiological conditions the pigs used by Isler et al. (1999) were at approximately 75 days of gestation, while the pigs used in this study were non-pregnant gilts, additionally the genetic background were different. Interestedly, though AA sows had relatively more ovulation rate, while they had smaller litter size, so we suggest that ESR gene probably influences the embryo development and embryo survival and then affects the litter size.

Rens et al. (2000) studied periovulatory hormone profiles and components of litter size in gilts with different ESR genotypes. Their results showed that no differences in periovulatory plasma luteinizing hormone (LH), estradiol (E2) or progesterone (P4) profiles between genotypes AA and BB gilts were detected; although the B allele was associated with a larger litter size, no differences existed in the number of corpora lutea, or number and percentage of vital day 35/36 embryos, this indicated that the difference in the litter size is likely due to the embryo survival. Furthermore, they found embryos of BB gilts had a larger placental size than embryos of AA gilts, and so they had a higher chance for placental insufficiency in AA gilts leading to the expected higher fetal mortality compared with the BB gilts. Although Wilson et al. (1999) suggested that smaller placentas were relatively more efficient and were linked to higher prolificacy. The results of Rens et al. (2000) also supported the hypothesis of our study.

In this study, we found AA pigs grew more slowly than BB pigs, however there was no different between genotypes for ADG or food/gain (F/G) ($p>0.1$) in Short et al. (1997).

TN and average daily food consumed (ADF) were the only traits to display negative effects of ESR in Short et al. (1997). Pigs with AB and BB genotypes had 0.1 fewer teats than AA animals. While, Rothschild et al. (1994) suggested the B allele was associated with increased TN in Meishan synthetic pigs but the results were not confirmed for lines of Large White background. In our study, there was no significantly different between genotypes for TN.

Favorable pleiotropic effects were detected for backfat thickness (BF) ($p<0.05$) with the additive effect of -0.11 mm per copy of the B allele (Short et al., 1997), while earlier research of Rothshcild et al. (1996) had suggested the effect of the favorable B ESR allele might be antagonistic relative to BF. Presently, no effect of ESR locus was found on TN, which was the same to the results of Mei et al. (1997).

The ESR *Pvu*II locus is located in the first intron, which probably had some important regulatory sequences such as enhances, promoters and so on, so the variation in this locus maybe affects the transcriptory ability (Ushiyama et al., 1998; Hill et al., 1989). If such a major gene would be located at the ESR locus; it should be confirmed in other total genome scans. It's strange that no other research group could confirm this QTL (Linville et al., 1999; Wilkie et al., 1999; Cassady et al., 2001). So the ESR gene was not the major gene of litter size, but a marker gene associated with the major gene. Why ESR has so a large effect on litter size and how does it works need to be studied further.

IMPLICATION

Litter size is one of the most economically important traits in pig production, and because of its low heritability and sex-limited nature, the improvement is very slow. Rothschild et al. (1996) chose ESR gene as a candidate gene of the major genes influencing litter size, studied the linkage of allele B with litter size, and found allele B could control 0.5 pigs/litter. In this study, the beneficial B alleles for a litter size have no antagonistic relationship with other performance traits, so it is an encouraging information for the marker assisted selection (MAS). However, at present there is insufficient information to encourage MAS for any QTL influencing prolificacy (Linville et al., 1999). A selection strategy should be designed for each line separately and should always consider possible pleiotropic effects. So next step we will research on the pleiotropic effects of ESR gene.

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