Abstract M71

Genetic Selection Tool for Number Born Alive and Stillbirth Piglets in Commercial Thai Populations

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SUMMARY

Genetic markers are widely used for selection and prediction in commercial pig populations. Number of piglets born alive (**NBA**) and stillborn piglets (**STB**) within litters have a major impact on overall economic returns. The aim of this study was to determine the association between estrogen receptor gene (***ESR***) and paternally-expressed gene 1 (***PEG1***) and **NBA** and **STB**. The dataset included information from two commercial pig populations, one in Northern Thailand (210 sows; 123 Landrace and 87 Yorkshire) and another one in Northeastern Thailand (130 sows; 70 Landrace and 60 Large White). Each population was analyzed separately using two models. Model 1 included farrowing year-season, parity, age at first farrowing and breed group as fixed effects, and animal and residual as random effects. Model 2 included all effects from model 1 plus genotypes for ***ESR*** (AA, AB, and BB) and ***PEG1*** (AA, GA, and GG) as fixed effects. Association between predicted **EBV** from models 1 and 2 were analyzed using Spearman rank correlations. Genotype frequencies were 0.67, 0.29 and 0.04 for ***ESR*** and 0.01, 0.40, and 0.59 for ***PEG1*** in the Northern population, and 0.42, 0.34, and 0.24 for ***ESR*** and 0.05, 0.25, and 0.70 for ***PEG1*** in the Northeastern population. Large White ***ESR*** BB sows had the highest average **EBV** for **NBA** (1.31 piglets; SD = 0.45 piglets), whereas Yorkshire ***ESR*** BB sows had the lowest average **EBV** for **STB** (-0.003 piglets; SD = 0.14 piglets). The ***PEG1*** genotype affected the **EBV** of Landrace, Yorkshire and Large White sows for **NBA** and **STB** ($P < 0.05$). Landrace ***PEG1*** GG sows had the highest average **EBV** for **NBA** (1.18 piglets; SD = 0.46 piglets), and Large White ***PEG1*** GG sows had the lowest average **EBV** for **STB** (-0.05 piglets; SD = 0.15 piglets). Rank correlations between **EBV** from models 1 and 2 varied across traits (**NBA** and **STB**), breeds (Landrace, Yorkshire, and Large White), and regions (Northern and Northeastern Thailand). ***These rank correlations indicated that selecting sows with higher NBA and lower STB by using EBV from models with and without ESR and PEG1 marker effects would yield similar or different outcomes depending on trait, breed, and region.***

INTRODUCTION

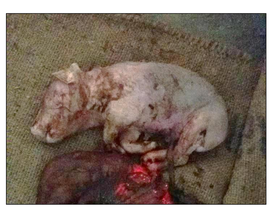
Genetic improvement of replacement gilts has been focused on high number of piglets born alive per litter in order to maximize annual pig production and increase economic returns. However, genetic improvement has been limited because of the low heritability for this trait. To help identify superior prospective parents and speed up genetic progress pigs can be genotyped for markers known to be positively associated with litter size and number of piglets born alive. The favorable genotype of estrogen receptor gene (***ESR***; Rothschild et al., 1996; Short et al., 1997) and paternally expressed gene 1 (***PEG1***; Isler, 2003) are recognized as genetic markers for increasing number of piglets within a litter in Meishan and European sows raised in temperate zones. Sows with the B allele of the ***ESR*** gene had larger litter size, whereas sows with the G allele of ***PEG1*** gene had a greater percentage of surviving ova and number of mummified piglets at farrowing. The genetic of the latter gene is directly transmitted from boar of the sow. Using these two genetic markers would help producers to improve number of piglets born alive per litter by selecting sow and boar lines. However, this modern technology is not widely applied for swine selection at Thai commercial farm levels due to the high cost of marker identification and the requirement of complete individual pedigree and phenotypic information from the herd. There are currently no reports on the use of genetic makers for prediction and selection in Thai commercial swine populations. Thus, the aim of this study was to determine the association between predicted **EBV** from models with and without ***ESR*** and ***PEG1*** genotypes as fixed effects. The hypothesis was that selection of sows using marker, phenotypes, and pedigree information would result in a different outcome from selection of sows based only on phenotypes and pedigree information.

MATERIALS AND METHODS

Data and Animals. Animals used in this study were from two commercial swine farms located in Northern Thailand (210 sows; 123 Landrace and 87 Yorkshire) and in Northeastern Thailand (130 sows; 70 Landrace and 60 Large White). The breeding stocks of these two farms were originally imported from Europe and have been improved for higher number of piglets born by using the performance records.

Sow Managements. Sows from both populations were managed under the open-house systems. Similarly, pregnant sows and weaned sows were managed in similar fashion in both populations. Pregnant sows were moved to individual farrowing pens approximately one week before the expected farrowing date. Individual records of number of piglets (number of piglets born alive, mummified piglets and stillborn piglets) were collected after parturition. Weaned sows were moved to mating pens. Estrus was routinely detected twice a day with the presence of a boar, once in the morning and once in the afternoon. Sows in standing heat were artificially inseminated twice; the second mating was done within 12 hours of the first mating.

Nutrition. Sows from the Northern Thailand population were fed 2.5 kg per day of gestation diet (3,200 to 3,500 kcal/kg and 16% of crude protein) at 7:00 a.m. and 13:00 p.m. during gestating period and 6 kg per day of a lactation diet (4,060 kcal/kg and 16 to 17% of crude protein) at 7:00 a.m., 10:00 a.m., 13:00 p.m. and 15:00 p.m. during the nursing period. Sows from the population in Northeastern Thailand were fed 1.8 kg per day of gestation diet (2,200 kcal/kg and 14.5% of crude protein) twice a day (7:30 a.m. and 13:30 p.m.) during the first 21 days of gestation, and then they were fed 2.5 to 3.0 kg of the gestation diet until parturition. After parturition, sows were fed 4.5 kg per day of the lactation diet (2,340 kcal/kg and 16.5% of crude protein) three times a day (7:30 a.m., 13:30 p.m. and 16:00 p.m.) during the first week of the lactation period, followed by a 1.5 kg increment each week until 7.5 kg. The feeding time during the second week of lactation until weaning was scheduled at 7:30 a.m., 11:00 a.m., 13:30 p.m. and 16:00 p.m.



DNA extraction. Genomic DNA was extracted from 200 µl of whole blood sample using the MasterPure™ DNA Purification kit for blood version II (Epicentre® Biotechnologies, USA) following the manufacturer's instructions. The 340 DNA samples were diluted to 2 ng/µl of final concentration.

Primer Design. The referenced polymorphism areas of ***ESR*** (Short *et al.*, 1997) and intron 11 of ***PEG1*** (***PEG1-3-105***; Isler, 2003) genes were used to design new sets of primers (**Table 1**) by using Pyrosequencing™ Assay Design Software version 1.0.6 (Biotage AB, Sweden). The forward and reverse primers were used to amplify the target regions of the studied genes by PCR. Then, the biotinylated PCR products and sequencing primers were used in the pyrosequencing process.

Polymerase Chain Reaction. The total volume per PCR reaction for amplification of the ***ESR*** and ***PEG1*** target regions was 25 µl as recommended in the protocol of PyroMark® PCR Kit (GIAGEN®, Germany). The reaction included 12.5 µl of PyroMark PCR Master Mix, 2.5 µl of CoralLoad Concentrate, 10 µM of each primer and 10 ng of genomic DNA. The reaction was initiated at 95°C for 15 min. Then, the reaction was followed by 45 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s. The final extension time was 10 min. The fragment size of biotinylated PCR product was 61 bp for the ***ESR*** target region and 101 bp for the ***PEG1*** target region.

Pyrosequencing. The amplified biotinylated products were genotyped by using PyroMark® Q96 ID Instrument (Biotage AB, Sweden). Firstly, the biotinylated products were immobilized on Streptavidincoated Sepharose HP beads (GE Healthcare, USA) with a total volume of 80 µl per well. The binding reaction included 3 µl of Sepharose HP beads, 40 µl of Binding buffer (GIAGEN®, Germany), 10 µl of biotinylated products, and 27 µl of high-purity water. Then, the sequencing primer was annealed to the immobilized biotinylated products at 80°C for 2 min. The final concentration of sequencing primers was 4 µM. The PSQ™ 96 reagent cartridges were prepared following the recommendations of the instrument's manufacturer. The dispensation order for sequence analysis were designed to be '**G**A**C**A**G**A**C**T**C**T' for detection of SNP from ***ESR***, and '**C**G**A**C**G**T**A**C**A**' for detection of SNP from ***PEG1***. Samples were analyzed using the PyroMark™ ID Software version 1.0. All individual ***ESR*** and ***PEG1*** genotypes were used for genetic prediction for **NBA** and **STB**.

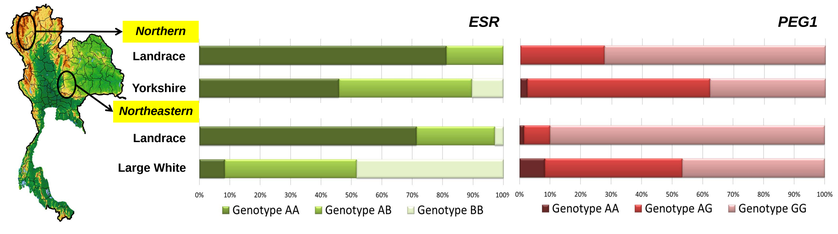
Statistical Analysis. Each population was analyzed separately using two single-trait mixed models. Model 1 included farrowing year-season, parity, age at first farrowing and breed group as fixed effects, and animal, permanent environment, and residual as random effects. Model 2 included all effects from model 1 plus additive ***ESR*** and ***PEG1*** effects as functions gene content (0, 1, 2 second alleles) as fixed effects. Seasons were winter (November to February), summer (March to June) and rainy (July to October). Animal **EBV** were computed as breed solution plus predicted animal deviation (model 1) plus ***ESR*** and ***PEG1*** solutions times gene content (model 2 only). Association between **EBV** from models 1 and 2 were analyzed using Spearman rank correlations.

Table 1 New primer sets for pyrosequencing

ESR	5'-AGATGCAGAATCAAGTTTTATGAG-3'	*forward primer*
	5'-AATAGGGTGGAATGGGGACT-3'	*reverse biotinylated primer*
	5'-GCAGAATCAAGTTTTATGAG-3'	*sequencing primer*
PEG1	5'-ATTGGCACAGGTGAAGGGCTTTTTC-3'	*forward primer*
	5'-AGGCTTCACTCGATTAGGTCTGG-3'	*reverse biotinylated primer*
	5'-ATTGGCACAGGTGAAGGGCTTTTTC-3'	*sequencing primer*

RESULTS AND DISCUSSION

Genotypic frequency. The genotypic frequencies of ***ESR*** gene were 0.67, 0.29 and 0.04 in the Northern population and 0.42, 0.34 and 0.24 in the Northeastern population. Different proportions of ***ESR*** genotypes were found among breeds. Landrace sows in both populations and Yorkshire sows had the highest frequencies of genotype AA (0.81, 0.71 and 0.46), while Large White sows had the highest frequency of genotype BB (0.48). However, the BB genotype was absent in Landrace sows from the Northern population. The genotypic frequencies for ***PEG1*** genotypes were 0.05, 0.41 and 0.59 in the Northern population and 0.05, 0.25 and 0.70 in the Northeastern population. In both populations, the AA genotype was present at a lower frequency than the other genotypes in all breed groups, especially in Landrace sows from the Northern population. Landrace and Large White sows had the highest frequencies of genotype GG, whereas Yorkshire sows had high frequency of genotype AG. For paternally imprinted genes such as ***PEG1***, the expressed allele could be detected in sows with homozygous genotypes (AA and GG). However, the expressed allele could not be determined heterozygous AG sows because their parents were not genotyped.



Estimated breeding values. Large White ***ESR*** BB sows had the highest average **EBV** for **NBA** (1.31 piglets; SD = 0.45 piglets). This result disagreed with the unfavorable effect of the ***ESR*** B allele on **NBA** found by Isler (2003) in Yorkshire, Large White and their reciprocally crossbred sows, but it was in agreement with studies by Rothschild et al. (1996) and Short et al. (1997) who found a favorable impact of the B allele of ***ESR*** on **NBA**. Moreover, Yorkshire ***ESR*** BB sows had the lowest average **EBV** for **STB** (-0.003 piglets; SD = 0.14 piglets). The estimated **EBV** for **NBA** and **STB** of Landrace, Yorkshire and Large White sows varied among ***PEG1*** genotypes. The highest **EBV** for **NBA** was found in Landrace ***PEG1*** GG (1.18 piglets; SD = 0.46 piglets). Conversely, Isler (2003) reported that Large White sows with genotype GG had higher **EBV** for **NBA** than sows with other genotypes. Positive effects of ***PEG1*** genotype on **STB** were found only in Large White GG sows (-0.05 piglets; SD = 0.15 piglets) from the Northeastern population.

Rank correlations. Rank correlations between **EBV** for **NBA** and **STB** from models with and without ***ESR*** and ***PEG1*** genotypic effects in the Northern and Northeastern populations were mostly medium positive (0.29 to 0.69; $P < 0.01$) and some near zero (**Table 2**). Rank correlations between **EBV** from models 1 and 2 were higher for **NBA** than for **STB**, except for the Large White population in Northeastern Thailand. The highest rank correlations between **EBV** from the two models the one for **NBA** in Landrace sows from Northeastern Thailand. Results showed that correlations between **EBV** rankings from models 1 and 2 varied across traits (**NBA** and **STB**), breeds (Landrace, Yorkshire, and Large White), and regions (Northern and Northeastern Thailand).

Table 2 Mean EBV (SD) and Spearman rank correlations between EBV predicted for NBA and STB using animal models with and without *ESR* and *PEG1* genotype effects by breed and region

Trait	Model with genotype effects	Model without genotype effects	r (P-value)	Model with genotype effects	Model without genotype effects	r (P-value)
	Landrace sows in Northern Thailand			**Yorkshire sows in Northern Thailand**		
NBA, piglets	0.18 (0.49)	0.41 (0.48)	**0.31 (0.0004)**	-0.17 (0.48)	0.11 (0.43)	**0.29 (0.0065)**
STB, piglets	1.20 (0.18)	-0.04 (0.18)	0.17 (0.0540)	1.25 (0.23)	0.02 (0.15)	0.03 (0.7756)
	Landrace sows in Northeastern Thailand			**Large White sows Northeastern Thailand**		
NBA, piglets	1.13 (0.48)	0.24 (0.44)	**0.69 (<0.0001)**	0.79 (0.67)	-0.23 (0.45)	-0.05 (0.7163)
STB, piglets	1.18 (0.19)	0.001 (0.16)	**0.33 (0.0047)**	0.03 (0.15)	-0.14 (0.12)	**0.33 (0.0101)**

FINAL REMARKS

- Genotypic frequencies of ***ESR*** and ***PEG1*** genes varied across regions and sows breeds
- Large White ***ESR*** BB sows had the highest average **EBV** for **NBA**
- The largest effect of ***PEG1*** on **EBV** for **NBA** occurred in GG Yorkshire and Large White sows
- **EBV** rank correlations from models with and without genotypes varied across traits, breeds, and regions

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