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(Original Research)

Testes Morphometry, Semen Characteristics, and Reproductive Performance of Landrace and Large White Boars Genotyped for Estrogen Receptor (*ESR1*) Gene

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Abstract

Background: The allelic and genotypic frequencies of *ESR1* in Landrace and Large White boars, and the effect of breed and genotype on reproductive traits of boars were investigated. **Methods:** Landrace (n=12) and Large White boars (n=15) were genotyped for *ESR1*, and the data was subjected to Fisher's exact test. Testes length and width, semen volume, and sperm concentration of boars, and litter records (n=988) of their mates were analyzed using different statistical models which include breed and genotype effects. **Results:** Majority (83%) of the Landrace boars were AA, while Large White boars were mostly AB (67%) genotypes. No BB genotype was detected. Distribution of genotypes were different between breeds ($p<0.05$). Testes length and width, semen volume and sperm concentration were neither different between breeds nor genotypes. Litter size and average birth weight of progenies sired by these boars were comparable between breeds and genotypes. **Conclusion:** The proportion of boars with AA and AB genotypes differ between breeds. Landrace and Large White are both dam breeds, which may explain their consistent similarities in the traits measured. No significant associations between boar traits and *ESR1* were detected under the conditions and limitations of the study. Further investigation using a population of boars with a more balanced number *ESR1* genotypes is warranted.

Keywords

Swine, *ESR1*, Estrogen receptor, Boar, Litter size

1. Introduction

Herd productivity is measured by the quality and quantity of pigs produced per year, and this is largely influenced by the performance of breeder boars and sows. Among the animals in the herd, boars are the most expensive individual. It serves a dual role: to pass on its superior growth and feed efficiency to its offspring, and to sire large litters. Although the effect of service sire to the variation in litter size appears minimal at ~2-3% [1], the boar's reproductive efficiency should be given importance.

Markers of boar fertility and prolificacy encompasses testes size and weight, semen characteristics, and litter size of mates [2]. Among these, testes size is the most observable trait indicative of sperm output. Boars selected for testes size had higher sperm concentration [3]. More recent findings showed that testes width and length were correlated with sperm concentration [4]. Furthermore, correlation between testes width and litter size [5] was seen, whereas, testes length tends to correlate with higher steroid concentrations, particularly estradiol, in growing crossbred Landrace x Large White boars [6]. Collectively, these relationships point to underlying genetic drivers in the form of breed differences and/or pleiotropic effects of candidate genes involved in steroid regulation or signaling.

Estrogen's biological function is mediated through its estrogen receptors (ESR1 and ESR2). Testicular estrogen receptors were localized in Leydig cells, Sertoli and germ cells [7, 8] and efferent ducts connecting the rete testis to the epididymis [9]. This indicates that estrogen is involved in spermatogenesis and possibly affects the volume and composition of seminal fluid. Estrogen was seen to be influential in young boars, as evidenced by lower sperm production in boars treated with aromatase inhibitors compared to control boars [10]. Because estrogen receptors are important for the hormone to exert its effects, its gene (*ESR*), emerges as a strong candidate molecular marker to explore association with boar traits.

Between the two receptors, ESR1 received considerable attention because an SNP in its gene (*ESR1*) recognizable by PvuII restriction enzyme was seen to be associated with litter size [11]. Large White-based sow lines with the favorable allele B showed higher numbers of piglets born and born alive compared to AA genotypes [12]. Although *ESR1* was reported to be associated with semen characteristics [13], there is paucity of information on the distribution of *ESR1* genotypes in local dam line breeds of boars, and its effect on indicators of boar fertility including testes morphometry and litter traits of its mate Thus, the objectives of this study are to determine *ESR1* distribution in local dam line breeds of boars; and to determine the effect of breed and *ESR1* genotypes on boar traits and performance within the conditions and limitations of the study in selected swine breeder farms.

2. Materials and Methods

2.1 Ethical Statement

This study was reviewed and approved by the University of the Philippines Los Baños Institutional Animal Care and Use Committee (UPLB IACUC) under protocol review number CAFS-2019-006.

2.2 Animal Population

Landrace and Large White boars used for artificial insemination and production of breeder stocks from two commercial breeding farms (Farm A located in Tarlac and Farm B located in Batangas) were used in this study. A total of 27 boars were included in the study (Farm A: Landrace = 8, Large White = 11 and in Farm B: Landrace = 4, Large White = 4). These service boars were regularly collected two to three times a week in both farms.

2.3 Sample Collection and Genotyping

At least 30 pieces of hair strands with intact follicles were plucked from each boar. Samples were cleaned, air-dried, wrapped and labeled using paper cards, and stored in an airtight container at room temperature. Hair samples were subsequently submitted to the Swine Genetics Analytical Service Laboratory of the Department of Agriculture-Bureau of Animal Industry, Quezon City for *ESR1* genotyping. The laboratory employed similar primers, restriction enzyme, and procedures as previously described [12].

Genotypes for each boar were determined based on agarose gel electrophoresis images of PCR products digested with the PvuII restriction enzyme. Homozygous animals for A allele have uncut PCR fragment of 120 base pairs, while those homozygous for B allele have two bands, 65 and 55 base pairs each, and heterozygous genotypes have all three bands; 120, 65, and 55 base pairs. Allelic and genotypic frequencies were computed per breed and for all the samples collected.

2.4 Boar Records

Testicular length and width were recorded using a measuring tape following routine semen collection from 16 boars (nine boars from Farm A and seven boars from Farm B). Testicular measurements were obtained from the scrotum externally using a tape measure. Testicular length was measured vertically top to bottom covering the tail to the head of the epididymis; while the testicular width was measured horizontally on the widest portion of the testes. Farm records of boar performance were obtained. Of the 27 boars included in the study, 25 (19 boars from Farm A and six boars from Farm B) had data on semen volume, while 19 boars from Farm A had records on sperm concentration. A total of 988 (622 Landrace and 366 Large White) artificial insemination service records, including subsequent litter performance traits such as litter size born alive (LSBA), total litter size (LS), and average birth weight (ABW) were also provided for 13 of the 19 boars from Farm A.

2.5 Statistical Analysis

Statistical analysis of data was evaluated using SAS on Demand for Academics. Fisher's exact test was done to test if the proportion of *ESR1* genotypes differ in the two breeds. PROC MIXED was used to determine the effects of breed and *ESR1* genotype on reproductive traits, with the following linear models used:

For morphometric traits,

$$\boldsymbol{Y_{ijkl} = \mu + a_i + b_j + c_k + age_l + e_{ijkl}}$$

where Y_{ijkl} is the morphometric measurements of the testes, μ is the mean, a_i is effect of genotype, b_j is the boar breed effect, c_k is the farm effect, age_l at the time of measurement as a covariate, and e_{ijkl} is the residual error.

For semen characteristics,

$$\boldsymbol{Y_{ijkl} = \mu + a_i + b_j + age_k + boarID_l\left(a_i b_j\right) + e_{ijkl}}$$

where Y_{ijkl} is semen volume or concentration, μ is the mean, a_i is fixed effect of genotype, b_j is the fixed effect of boar breed, age_k at the time of collection as covariate, $boarID_l$ within breed*genotype as a random effect, and e_{ijkl} is the residual error.

For litter traits of mated sows,

$$\boldsymbol{Y_{ijklmn} = \mu + a_i + b_j + s_k + y_l + p_m + boarID_n + e_{ijklmn}}$$

where $Y_{ijklmno}$ is LSBA, LS, or ABW, μ is the mean, a_i is the fixed effect of genotype, b_j is fixed effect of the boar breed, s_k is the fixed effect of the sow breed, y_l is the fixed effect of service year, p_m is the fixed effect of parity, $boarID_n$ as a random effect and e_{ijklmn} is the residual error.

3. Results and Discussion

3.1 Distribution of *ESR1* Genotypes in Landrace and Large White Purebred Boars

Figure 1 shows a sample gel image of the banding patterns used to determine the genotype of the boars. Out of 27 boars, 56% and 44% were

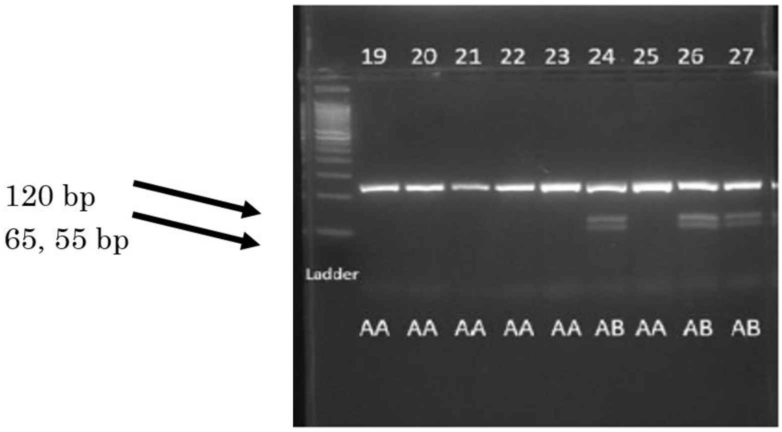

Figure 1. Representative gel image showing genotypes of boars for the *ESR1*-PvuII gene polymorphism. Banding patterns correspond to AA (120 bp) and AB (120, 65, and 55 bp) genotypes. The first lane contains the 50 bp MW ladder, while the succeeding lanes show the sample ID on top and genotype result at the bottom.

AA and AB genotypes, respectively (Table 1). No BB genotype was found. Across breed and farm, majority (0.78) of the *ESR1* allele was A while the remaining small portion (0.22) was the B. Locally, allele A has a frequency ranging from 0.67 to 0.73 in various breeds of pigs [14,15,16]. Similarly, in other countries, frequency of allele A in different pig populations ranges from 0.62 to 1.0 [13,17,18,19].

Table 1. Genotypic and allelic frequency of *ESR1* genes.

Breed		Genotype			Allele	
		AA	AB	BB	A	B
Landrace	Farm A	6	2	0	14	2
	Farm B	4	0	0	8	0
	Total (Frequency)	10 (0.83)	2 (0.17)	0	22 (0.92)	2 (0.08)
Large White	Farm A	4	7	0	15	7
	Farm B	1	3	0	5	3
	Total (Frequency)	5 (0.33)	10 (0.67)	0	20 (0.67)	10 (0.33)
Combined	Total (Frequency)	15 (0.56)	12 (0.44)	0	42 (0.78)	12 (0.22)
Fisher's Exact Test P-value		0.0185				

Interestingly, for both farms, Landrace breeds are mostly AA genotype while Large White breeds are mostly of the AB genotype. This is supported by the results of the Fisher's exact test (p=0.0185), wherein the distribution of the genotypes differs between purebred boars. Others have reported the absence [17,20] or low frequency [13] of the BB genotype in Landrace boars screened for *ESR1*, whereas both AB and BB genotypes are more frequently seen in Large White boars [13]. The results of the present study demonstrate breed-specific differences in genotypic frequencies, with the favorable B allele predominantly observed in Large White.

3.2 Morphometric Characteristics of the Testes

The left and right testes lengths of boars involved in the study ranged from 22.9 to 58.4 cm; while testes width ranged from 12 to 36 cm. Mean lengths of left and right testes were 42.97 and 41.57 cm, respectively; while mean width was 21.88 cm.

Testicular measurements such as weight and size are indicators of reproductive performance of boars [21]. The weight and size of the testes are shown to be positively correlated with sperm output. No significant differences between breeds or genotypes were seen (Table 2). Similar to a previous report, 24-month-old Landrace and Large White boars have comparable testes length and width [22].

Table 2. Least Square Means (± SEM) of testis length and width of purebred genotyped boars.

Breed	**Left Testicle Length (cm)**	**Right Testicle Length (cm)**	**Testicle Width (cm)**
Landrace	44.04 ± 4.22	41.21 ± 3.62	24.33 ± 1.50
Large White	43.64 ± 3.25	42.68 ± 3.62	20.62 ± 1.35
P-value	0.9877	0.6133	0.0530
Genotype			
AA	41.61 ± 3.18	39.97 ± 2.73	22.0 ± 1.39
AB	46.08 ± 4.32	44.09 ± 3.71	22.96 ± 1.49
P-value	0.2358	0.3610	0.4650

3.3 Semen Characteristics

Semen volume of boars involved in the study ranged from 103 mL to 603 mL, while concentration ranged from 1.7 million to 168 million sperm per mL. Mean semen volume and concentration were 315 mL and 57 million sperm per mL, respectively. It can be seen that semen characteristics of the genotyped purebred boars follow the standard volume of 100 to 500 mL and concentration of ten to 100 billion sperm per ejaculate [23]. With these volume and concentration, and a dose of three billion sperm per AI, approximately three to 33 sows can be served in a single ejaculate.

Table 3 shows no significant difference in semen volume and concentration between breeds, consistent with the lack of differences observed in testes length and width. Conversely, higher semen volume and sperm concentration were seen in Landrace than Large White boars aged seven to nine months of age [24]. As semen was collected from boars with a mean age of 30 months, differences in age between study populations may account for the contrasting results.

Table 3. Least square means (± SEM) of semen volume and concentration per breed and genotype.

Breed	Semen Volume (mL)	Semen Concentration (sperm/mL)
Landrace	319.31 ± 36.20	66,070,081 ± 8,327,538
Large White	312.42 ± 29.52	50,848,835 ± 6,783,879
P-value	0.8465	0.2547
Genotype		
AA	316.22 ± 30.52	57,522,561 ± 7,014,265
AB	315.51 ± 34.97	59,396,355 ± 8,044,145
P-value	0.9481	0.9881

In the present study, no genotype effects were detected across breeds (Table 3). Similar to a study which evaluated representative breeds of boars coming from the sire, dam, and terminal lines, it was shown that boars homozygous for the allele A polymorphism (exhibits 120-bp bands) had similar ejaculate volume and semen concentration with the heterozygous genotype (exhibits three bands of 120-, 65- and 55 bp) [13]. A more recent study reported that AA and AB of both Landrace and Yorkshire breeds of boars had significantly higher semen volume compared to BB boars [25]. These reports are in agreement with the results of the present study.

3.4 Reproductive Traits of *ESR1* Genotyped Boars

Litter size born alive ranged from one to 21 piglets, with LS ranged slightly higher from two to 23 piglets. Average birth weight ranged from 0.67 to 2.92. Mean LSBA, LS, and ABW were 10.88 piglets, 11.58 piglets, and 1.47 kg, respectively.

The LSBA, LS, and ABW of Landrace-sired litters were no different than Large White-sired litters (Table 4). When comparing boar breeds, sows mated with Landrace or Large White boars had similar number born alive (10 piglets) and average birth weight (1.7 kg) [26]. This is expected, since Landrace and Large White breeds are recognized for their excellent reproduction traits, and are therefore commonly used as dam lines. Further, the comparable characteristics between Landrace and Large White boars from testes morphometry, semen volume, and sperm concentration, are consistent with the lack of

Table 4. Least square means (±SEM) of litter traits at birth in the progenies of purebred genotyped boars.

Breed	Litter Size Born Alive	Total Litter Size	Average Birth Weight (kg)
Landrace	10.97 ± 0.19	11.75 ± 0.13	1.48 ± 0.012
Large White	11.31 ± 0.22	11.90 ± 0.17	1.47 ± 0.015
P-value	0.2483	0.5069	0.8027
Genotype			
AA	11.29 ± 0.23	11.85 ± 0.18	1.47 ± 0.015
AB	10.99 ± 0.18	11.81 ± 0.14	1.49 ± 0.012
P-value	0.2937	0.8361	0.3280

significant differences in the litter size and birth weight found. This may be explained by the genetic correlation among testes size, semen characteristics (volume and sperm concentration), and litter size born alive [27,28].

While the effect of *ESR1* genotypes on litter size has long been studied in sows [14,29,30,31, 32,33], information on the effect of boar genotype on these traits remains limited. A similar approach was reported wherein differences in the number born alive and weaned between Pietrain boars genotyped for ryanodine receptor (*RYR1*) were determined [34]. No significant differences were seen in the litter traits measured between AA and AB genotyped boars included in the study (Table 4). Given the limited sample size, unbalanced number of genotypes, and absence of BB genotype, boar fertility (indirectly measured by litter size and average birth weight) between the two genotypes were similar.

4. Conclusions

In summary, the distribution of *ESR1*/PvuII genotypes were different between breeds, wherein AB was predominantly seen in Large White. Indicators of fertility or prolificacy in Landrace and Large White boars such as testes morphometry and semen characteristics were comparable. This is consistent with the similar litter size and average birth weight between offspring sired by Landrace and Large White boars. At the gene level, no significant associations between boar traits and *ESR1* were detected under the conditions and limitations of the study.

Further studies may include using a cohort of junior boars with a more balanced distribution of genotypes that will be monitored for a longer period of time to establish the impact of *ESR1* on the boar's lifetime productivity. As litter size is a quantitative trait influenced by the combined effects of multiple genes, investigating other candidate genes particularly those of hormones and receptors in the steroidogenic pathway may be considered.

Availability of Data and Materials

Included as part of the submitted paper.

Author Contributions

Conceptualization, N.G.R.A., and C.A.S.E.; Methodology, N.G.R.A., and C.A.S.E.; Validation, N.G.R.A., and C.A.S.E.; Formal analysis, N.G.R.A.; Investigation, N.G.R.A.; Resources, N.G.R.A., and C.A.S.E.; Data curation, N.G.R.A., and C.A.S.E.; Writing – original draft, N.G.R.A.; Writing – review and editing, N.G.R.A., C.A.S.E., A.J.S., and M.C.C.M; Visualization, N.G.R.A; Supervision, C.A.S.E.; Project administration, N.G.R.A.; Funding acquisition, N.G.R.A.

Ethics Approval and Consent to Participate

This study was reviewed and approved by the University of the Philippines Los Baños Institutional Animal Care and Use Committee (UPLB IACUC) under protocol review number CAFS-2019-006.

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Conflict of Interest

The authors declare no conflict of interest.

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