

**A STUDY ON EFFECTS OF ESR GENE POLYMORPHISM ON REPRODUCTIVE TRAITS OF F1 HYBRID WILD BOAR (♂WILD BOAR X ♀VCNMS15) IN FARM HOUSEHOLDS.**

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**ABSTRACT**

Studying on the effect of ESR gene polymorphism on the reproductive traits of F1 hybrid wild boars (♂R Wild Boars x ♀VCNMS15) determined the correlation between ESR gene polymorphism with reproductive capacity of sows which were monitored directly more than 20 F1 sows (♂Wild Boars x ♀VCNMS15) raised on the households. The data was processed and analyzed on statistical software, the correct sampling method, the process of separation, extraction and analysis of ESR gene polymorphism on modern equipments, the results showed:

Successfully analyzed ESR gene polymorphism from 20 individual F1 sows (♂Wild Boars x ♀VCNMS15) identified 3 genotypes AA; AB; BB. The results obtained the difference in genotype, as follows: 8 individuals with genotype AA accounted for 40%, 11 individuals with genotype AB accounted for 55.00% and 1 individual with genotype BB accounted for 5, 00%. The difference in the A and B allele frequencies of the studied samples was clearly shown, the A allele frequency of 0.67 accounts for a higher percentage than the B allele frequency of 0.33.

Initially the researchers determined the correlation between genotype ESR polymorphism for the reproductive traits of F1 wild boars (♂Wild Boars x ♀VCNMS15). Wild boars carrying genotype AB in ESR gene showed higher reproductive performance than genotypes AA and BB with (P <0.05).

**Keywords:** Wild boars F1 (♂Wild boars x ♀VCNMS15); genetic polymorphism; gene ESR; reproductive traits; genotype.

**I. INTRODUCTION**

Wild boars and F1 hybrid wild boars were raised in some northern mountainous provinces of our country, with many advantages such as quality of fragrant meat, high lean rate, high nutritional value, no antibiotic residues and the ability to withstand miserable farming conditions. However, there were limitations such as the number of children born per litter, the growth and development slowly. Therefore, in order to exploit and develop this breed of wild boar, it is necessary to have researches and solutions to increase productivity in livestock production.

Nowadays, genetic technology has been widely used in practical production, the development of molecular genetic engineering, many genes associated with reproductive traits have been studied to improve productivity. breeding in sows, bringing high economic efficiency in breeding. In order to enhance pig reproductive traits by breeding selection was difficult due to the low genetic ability of these traits, researchers have selected livestock breeds based on molecular markers in order to increase the primary ability accuracy, shorten time and increase selectivity. In particular, research on the correlation of genetic polymorphism with reproductive traits was very important in the conservation of varieties. According to research by Drogemuller et al. (2001) [6]; Omelka et al. (2008) [9] showed that the estrogen receptor gene (ESR) was related to reproductive performance of the sow. The pig's ESR gene was closely related to pregnancy and its receptor function, which had a characteristic allele associated with the number of offspring born.

With the aim of applying modern techniques of genetic engineering and molecular genetic engineering in pig production to bring the highest economic efficiency to the breeder while retaining the outstanding advantages of the breed, we conducted this study to investigate the correlation between the ESR gene polymorphism with the reproductive traits of F1 hybrid wild boar (♂wild boar x ♀VCNMS15) raised in the farm households.

## **II. RESEARCH MATERIALS AND RESEARCH METHODOLOGY**

### **2.1. Research Materials**

The study was conducted on 20 F1 sows (♂wild boar x ♀VCNMS15) raised at the Indigenous Plant and Animal Research and Development Branch, Tuc Tranh commune, Phu Luong district, Thai Nguyen province.

Pigs ♂Vietnam Wild Boar; Pigs ♀VCNMS15: is the Meishan pig breed imported from China by the National Institute of Livestock of Vietnam and purebred with the name VCNMS15.

Research period: The study was conducted from January 1, 2019 to December 31, 2020

### **2.2. Research methodology and some monitoring indicators**

### ***Research content***

ESR gene polymorphism analysis of F1 sows (♂ wild boar x ♀ VCNMS15): Total DNA extraction from obtained ear tissue samples; PCR cloning specific gene PRLR; Cut reaction with corresponding restriction enzyme.

Determination of genetic polymorphism correlation of ESR genes to reproductive traits of F1 sows (♂ Rung x ♀ VCNMS15).

### ***Sampling method***

Using specialized pliers to click a sample of the ear tissue of the experimental pig, then put it into an eppendorf tube containing 70% alcohol. The sample was stored at -200C and then used to separate total DNA.

### ***Methods of DNA extraction***

Total DNA extraction using proteinase K method.

DNA quality testing, using the NanoDrop 2000 machine to check the purity and concentration of DNA after extraction at 260 nm and 280 nm.

Principle: The NanoDrop 2000 purity tester worked on the strong absorption of ultraviolet light at 260 wavelengths of purine and pyrimidine bases. Values of the optical density at 260 nm of these samples allowed the determination of the DNA concentration in the samples based on the following correlations:

One unit OD<sub>260</sub> corresponds to a concentration of 50 µg / ml for a double strand DNA solution. In which a unit OD<sub>260</sub> corresponded to the concentration of DNA = 50 (µg / ml).

To test the purity of DNA in solution, measure the OD value at 280 (OD<sub>280</sub>). The wavelength of 280 was the wavelength at which proteins had the highest absorption, but proteins also absorbed light at 260 just as nucleic acid molecules could thus distort the true concentration value of the nucleic acid.

To ensure the correct concentration of CDNA, we used value  $A_{260} / A_{280}$ . When the value  $A_{260} / A_{280} = 1.8 - 2.0$ , the DNA extract was considered purified (Khuat Huu Thanh, 2012) [2].

### ***PCR method***

Ingredients for a reaction include: Enzyme Taq-polymerase, buffer Taq-polymerase (buffer), corresponding primer, MgCl<sub>2</sub>, dNTP, purified water and template DNA. ESR primers were designed by Short et al. 1997.

#### ***Limit enzyme cutting method***

After amplification, a 120 bp ESR gene segment was cut with PvuII enzyme by overnight incubation at 37°C.

#### ***Analysis of genetic polymorphism***

The genotype frequency and allelic forms were summarized of the PRLR gene with the following proportions of the cut-outs: Allel A: 85bp - 59bp - 19bp; Allel B: 104bp - 59bp. And so the corresponding frequencies of the corresponding genes would be: Genotype AA: 85bp - 59bp - 19bp; Genotype AB: 104bp - 85bp - 59bp - 19bp; Genotype BB: 104bp - 59bp

Monitoring some sow fertility indicators including indicators of number of offspring / litter; number of children alive after 24 hours; number of children weaned; birth / baby weight; the weight of weaned piglets.

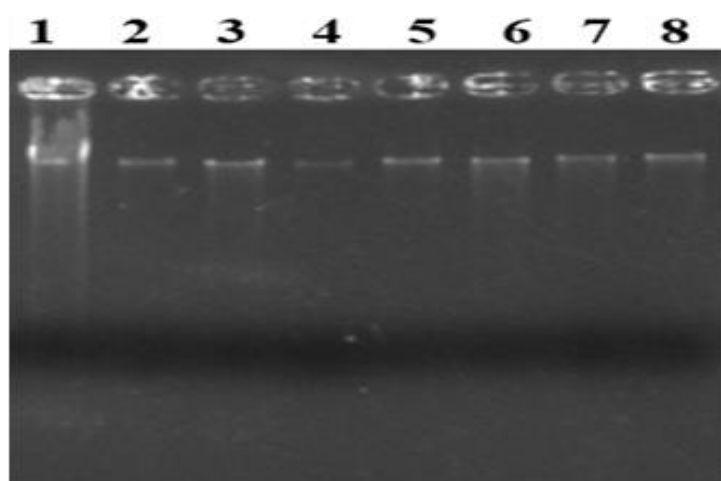
Data were collected, processed and analyzed statistically on Minitab.16 software.

### **III. RESULTS AND DISCUSSION**

#### **3.1. Results of analysis of ESR gene polymorphism (Estrogen Receptor)**

##### ***3.1.1. Results of total DNA extraction from F1 sow ear samples (♂wild boar x ♀VCNMS15)***

The results of DNA extraction for a total of 8 F1 sow ear samples (♂wild boar x ♀VCNMS15) are represented as shown in Figure 3.1



**Figure 3.1: Results of DNA extraction for a total of 8 F1 sow ear tissue samples (♂wild boar x ♀VCNMS15)**

(Track from 1-8: total ear tissue DNA product of F1 sow samples (♂wild boar x ♀VCNMS15))

From the results of electrophoresis of the above DNA separating products, the total DNA samples obtained had relatively good quality. DNA was less prone to fracture, the bands were brightly. The concentration and purity of the obtained DNA samples were shown in Table 3.1

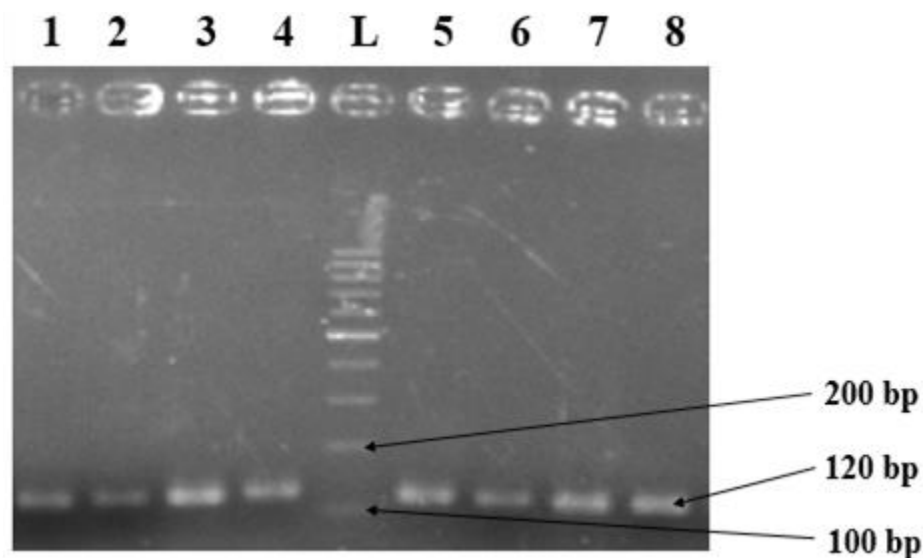
**Table 3.1: Results of DNA purity and concentration measurement**

Research sample	OD <sub>260</sub> /OD <sub>280</sub> ratio	DNA concentration (ng / µl)
1	1,92	35,4
2	1,81	40,5
3	1,89	36,5
4	1,98	35,8
5	1,90	36,8
6	1,95	38,7
7	1,87	39,5
8	1,97	38,8

From the above results, it shows that the ratio of OD260 / OD280 in the studied samples was relatively uniform. The results ranged from 1.80 to 1.99 within the permissible limits (1.80 - 2.00). Therefore, the obtained DNA samples were fully qualified for PCR reaction and further studies.

### **3.1.2. PCR results amplify ESR gene with specific primers**

The PCR product of the ESR gene segment was multiplied from primers designed by Short et al. 1997 [10] with an expected size of 120 bp. The product was finished after multiplying electrophoresis on 1% agarose gel in 35 minutes.



**Figure 3.2: PCR product multiplied from ESR primers**

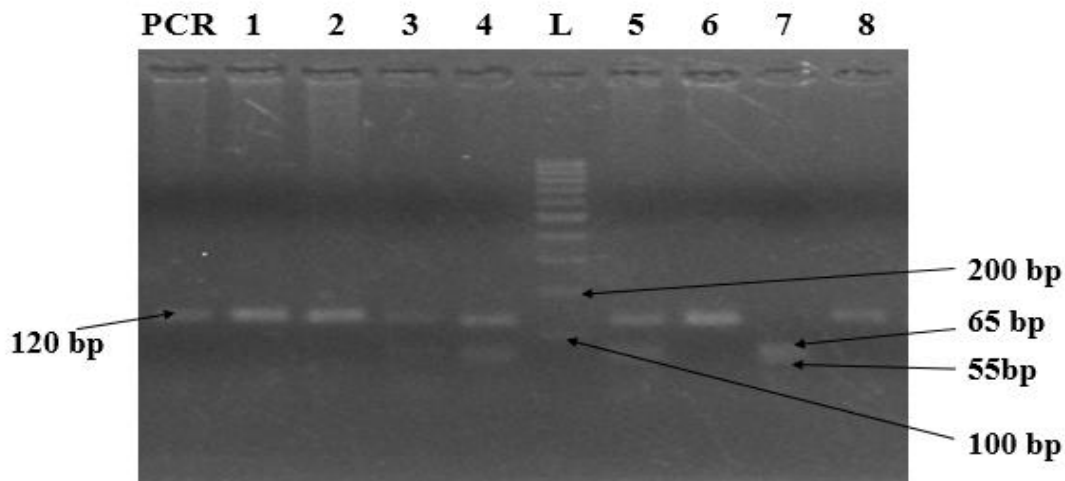
(L: GeneRuler™ 100bp; Track 1 - 8: PCR product of ESR gene segment of F1 sow (♂ wild boar x ♀ VCNMS15))

The PCR results of the ESR gene segment are shown in Figure 3.2, showing that PCR is a single, bright, 120bp band as expected, eligible for the next steps.

## **3.2. Results of determining the correlation of genetic polymorphism of ESR gene with reproductive traits of F1 sows (♂ wild boar x ♀ VCNMS15)**

### **3.2.1. Results of ESR gene segment polymorphism analysis by enzyme *PluII***

ESR gene polymorphism analysis was based on the cutting ability of the restriction enzyme *PluII* on PCR 120 bp. The product cut electrophoresis on 3.5% agarose gel in 40 minutes. Results of ESR gene polymorphism analysis was shown in Figure 3.3.



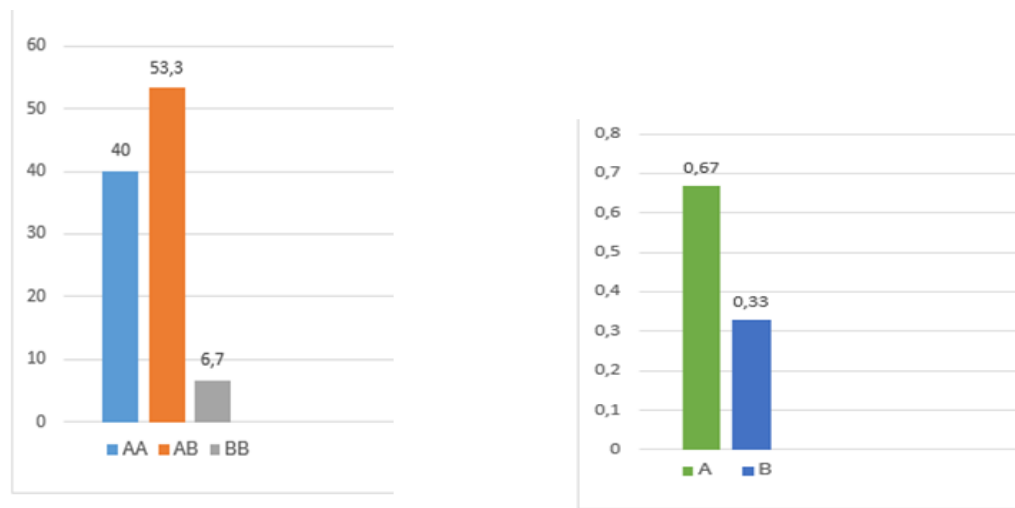
**Figure 3.3: Results of ESR gene segment polymorphism analysis by *PluII* enzyme**

(L: GeneRuler™ 100 bp; PCR: product PCR gene ESR; Genotype AA: 1,2,6,8 (120 bp); Genotype AB: 3,4,5 (120 bp, 65 bp, 55 bp) Genotype BB: 7 (65 bp, 55bp))

The picture above shows that the results of cutting the PCR product of ESR gene with *PluII* enzyme electrophoresis on the 3.5% gel for the bar band had been clearly separated. ESR gene segment after cleavage into DNA fragments of different sizes in which wells 1 and 6 were for genotype AB; wells 2,4,5 for genotype AA; well 3 for the BB genotype. Genotypes of the ESR gene segment in F1 sows (♂ wild boar x ♀VCNMS15) were determined, from which the genotypes rate and allele frequency were also calculated based on the total number of individuals carrying that genotype. The results were shown in Table 3.2 and Figure 3.4 as follows:

**Table 3.2. ESR genotype rate of F1 sows (♂ wild boar x ♀VCNMS15)**

Number of individuals (n)	Genotype AA		Genotype AB		Genotype BB	
	Number of individuals	Ratio %	Number of individuals	Ratio %	Number of individuals	Ratio %
20	8	40,00%	11	55,00%	1	5,00%



A) Percentage of genotypes ESR

(B) Allele frequency of the ESR gene

### Figure 3.4. Rate of genotype and frequency of the ESR allele in the studied pig population

The results in Table 3.2 and Figure 3.4 show that, when using *PluII* enzyme, analysis of ESR gene segment polymorphism in 20 pigs had the appearance of different genotypes. The F1 studied sows ( $\sigma$ wild boar x  $\text{♀}$ VCNMS15) had 3 genotypes AA and AB and BB with the rates of 40%, 55% and 5%, respectively. In which, genotype AB was more dominant than genotypes AA and BB. The difference in the A and B allele frequencies of the studied samples was clearly shown in Figure 3.4 B, where the A allele frequency of 0.67 accounted for a higher proportion than the B allele frequency of 0.33.

By PCR technique Nguyen Van Cuong et al (2003) [1]; Nguyen Thi Dieu Thuy et al (2003; 2004) [3; 4] also identified genotypes related to number of offspring / parity through FSH gene analysis. Research by Rothschild et al (1996) [8]; Baskin et al. (1997) [5] showed that the B allele was closely related to the number of offspring born per litter. According to research by Suwanasopee et al (2010) [11], the genotype frequencies of ESR were 0.6965 respectively; 0.2601 and 0.0434 for genotypes AA, AB and BB, genotype AA had the highest frequency followed by AB and BB.

R. Omelka et al. (2008) [9] studied on 155 heads of the Large White breed (LW), 134 heads of the White Meaty breed (WM) and 132 heads of the Landrace breed (L). The results obtained in the LW pig breed, the AA genotype frequency was 42.58; AB was 48.39 and BB was 9.03. In the WM breed, the genotype frequency AA was 52.99; AB was 44.78 and BB was 2.23. In the L



pig breed, genotype frequency AA was 84.09; AB was 15.91 and BB was 0. The results on this LW pig breed were almost similar to the results obtained by the research topic.

**3.2.2. Results of research on effects of ESR gene polymorphism on the reproduction of F1 sows (♂Wild boar x ♀VCNMS15)**

**Table 3.3: Some indicators of reproductive performance of F1 sows (♂Wild boar x ♀VCNMS15)**

No	Targets	Unit	$\bar{X} \pm m\bar{x}$	Cv %
1	Number of piglets born	head / litter	8,07 ± 0,34	18,58
2	Number of piglets alive after 24 hours	head / litter	7,88 ± 0,30	17,38
3	Newborn weight	Kg/ head	0,88 ± 0,03	14,86
4	Total litter weight	Kg/ litter	7,10 ± 0,20	12,28
5	Number of piglets weaned	head / litter	7,28 ± 0,28	16,75
6	The weight of piglets weaned	Kg/ head	4,40 ± 0,10	14,43
7	Total weaning weight	Kg/ head	32,03 ± 0,60	11,80

The results in Table 3.3 show that the reproductive productivity of 20 F1 sow pigs (♂Wild boar x ♀VCNMS15) raised in a farmer household in Phu Luong district, Thai Nguyen province had an average number of offspring of 8.07. , with an average birth weight of 0.88 kg / head, weight of the whole nest reached 7.10 kg / litter. The average number of animals alive after 24 hours was 7.88 / litter; The number of piglets weaned was 7.28 heads / litter with an average weight of 4.06 kg / head and total weaning weight was 29.56 kg / head. These are synthetic indicators, reflecting reproductive performance of sows, Legault C. (1985) [7].

**3.2.3. The sow's reproductive performance corresponds to the genotypes of the ESR gene**

After analyzing the genotypes and statistics of the number of sows corresponding to each genotype of ESR in the survey, the results showed that 20 F1 sows (♂Rung x ♀VCNMS15) had 8 sows with genotype AA, 11 a sow with genotype AB, and a sow with genotype BB, were shown in table 3.4.

**Table 3.4: Reproductive performance corresponding to genotypes in F1 sows (♂Wild boar x ♀VCNMS15)**

Target	Genotype	n	average $\bar{x}$	Standard deviation S	Standard error $\frac{\bar{m}}{x}$	F value	P value
Number of piglets born(head / litter)	AA	8	6,50	1,20	0,42	6,20	P<0,05
	AB	11	8,40	1,40	0,42		
	BB	1	6,0	0	0		
Number of piglets alive after 24 hours (head / litter)	AA	8	6,40	1,37	0,56	5,80	P<0,05
	AB	11	8,20	1,43	0,50		
	BB	1	6,00	0	0		
Newborn weight (kg/head)	AA	8	0,84	0,09	0,04	5,11	P<0,05
	AB	11	1,00	0,14	0,05		
	BB	1	0,75	0,12	0,04		
Total litter weight(kg/litter)	AA	8	6,00	0,60	0,20	5,78	P<0,05
	AB	11	8,20	1,22	0,43		
	BB	1	5,50	0	0		

The analysis results on 20 F1 sows (♂Forest x ♀VCNMS15) showed that the criteria for the number of piglets born and the number of piglets alive after 24 hours in genotype AB were higher than genotypes AA and BB. respectively: 8.40 - 8.20 head/litter and 6.50 - 6.40 head/litter; 6.00 - 6.00 each. Similar to the target of birth weight in sows with genotype AB higher than sows with genotypes AA and BB, respectively: 1.00 kg / head and 0.840 to 0.75 kg / head with (P <0.05).

Thus, the average values of some reproductive indicators of F1 wild sows (♂wild boar x ♀VCNMS15) corresponding to the genotypes had a statistically significant difference with (P <0.05). From there, it showed that there was an influence by genotype factors on reproductive performance of sows.

There were many studies related to genetic polymorphism of ESR genes such as Rothschild et al. (1996) [8] analysis of ESR gene polymorphism of pigs revealed that there was a characteristic

allele associated with the number of newborn babies. Short et al. (1997) [10] investigated the effect of ESR gene loci on reproductive and production traits of commercial pig lines revealed a correlation of total live offspring with beneficial alleles of the ESR gene. Goliasova and Wofit (2004) [12] analysis of the ESR gene polymorphism of pigs showed that the ESR gene locus significantly influenced the number of newborns at parity 1 and litter. Analysing of genotypes ESR showed that pigs with genotype BB had a superior number of live offspring compared to genotypes AA and AB in the first and subsequent parities.

Improving genetics by genetic technology in breeding livestock has been applied by many countries around the world to increase the productivity and quality of livestock. Through the study of ESR gene polymorphism of F1 sows (♂Wild Boar x ♀VCNMS15), it could be initially confirmed that there was a correlation between the ESR gene polymorphism to the reproductive performance of F1 sows (♂Wild Boar x ♀VCNMS15). However, the sample was not large enough to be able to draw conclusions about exactly which genotype had the greatest effect on reproductive performance. The study also paved the way to conduct further studies on the effect of ESR genotype polymorphism on the fertility of F1 hybrid forest sows (♂Wild Boar x ♀VCNMS15).

#### **IV. CONCLUSION**

The results of analysis and investigation of the effect of the relationship between the ESR gene polymorphism with the reproductive traits of F1 hybrid wild boar (♂Wild boar x ♀VCNMS15) showed that:

Successfully analyzed ESR gene polymorphism from 20 individuals of F1 sow (♂Wild boar x ♀VCNMS15) and identified 3 genotypes AA; AB; BB. The results obtained the difference in genotype specific as follows: 8 individuals with genotype AA accounted for 40%, 11 individuals with genotype AB accounted for 55.00% and 1 individual with genotype BB accounted for 5, 00%. The difference in the A and B allele frequencies of the studied samples was clearly shown, the A allele frequency of 0.67 accounted for a higher percentage than the B allele frequency of 0.33.

Initially, the correlation between ESR genotype polymorphism was determined for the reproductive traits of F1 pigs (♂Wild boar x ♀VCNMS15). Pigs carrying genotype AB in ESR gene showed higher reproductive performance than genotypes AA and BB with ( $P < 0.05$ ).

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