UDC: 577.218+636.082.4:636.4

STUDY OF POLYMORPHISM OF GENES ASSOCIATED WITH REPRODUCTIVE ABILITY SOWS OF UKRAINIAN MEAT AND WALES BREEDS

M.V. Dragulyan[1], S.A. Kostenko[2], O. Sidorenko[3]

[1]Institute of Molecular Biology and Genetics, NAS of Ukraine

[2]Natsionalny University of Life and Environmental Sciences of Ukraine

[3]Institute of Animal Breeding and Genetics NAAS of Ukraine, p. Chubinskoe

Study the genotypes of genes: *ESR* (estrogen receptor), *NCOA1* (nuclear receptor coactivator A1), *PRLR* (prolactin receptor), *FSHR* (FSH receptor) of sows of Welsh and Ukrainian meat breeds. Found that sows of Welsh breed have frequency allele *B* gene *ESR* equals 0.40 and sows of Ukrainian meat – 0.48. Sows of Welsh breed characterized by a lower frequency of genotype *BB ESR* gene compared with sows of Ukrainian meat breed (0.02 and 0.10, respectively). Study the frequency of genotypes and alleles *NCOA1* gene of pigs Ukrainian meat and Welsh breeds found a fairly high frequency desired from the point of view of increasing multiple births *A1* allele (0.62 and 0.66, respectively). *A1A1* genotype of Ukrainian meat breed sows met by 9% lower than the Welsh breed sows ($p < 0.001$). The frequency of allele *A PRLR* gene of Welsh sows breed equals 0.53, sows of Ukrainian meat breed – 0.58, and genotype *AA* equals 0.34 and 0.52 respectively. Pig breeds were polymorphic in *FSHR* gene. Frequency distribution of desired allele *C FSHR* gene in pigs of Ukrainian meat and Welsh breed found almost identical values (0.75 and 0.73, respectively). Frequency of *CC* genotype *FSHR* gene in sows of Welsh breed is 0.57, Ukrainian meat is 0.56. The actual frequency of genotypes was significantly different from the theoretically expected.

Keywords*: genetic markers, reproductive quality Sus scrofa, the estrogen receptor gene, prolactin receptor gene, the gene A1 coactivator nuclear receptor gene FSH, ESR, NCOA1, PRLR, FSHR, sow, Ukrainian meat breed, Welsh breed.*

INTRODUCTION. Study of population genetic structure of pig breeds beef productivity according to molecular genetic markers remains one of the key programs

in Ukraine [2]. Analysis of polymorphisms genes associated with economically useful traits of pigs provides a significant piece of information, not only about the state of the gene pool, but also species and specific features of the genetic structures of animals. Leading the molecular genetic structure analysis of genes associated with reproduction ability of sows give information not only on the genetic variability, but also causes that causing it.

The most commonly used marker genes affecting multiple births of sows: a gene (ESR), the estrogen receptor that stimulates the development of secondary sexual characteristics [9, 10], gene (NCOA1) coactivator A1 nuclear receptor that interacts with estrogen receptors and stimulates its transcriptional activity [6]; gene (PRLR) receptor prolactin, which stimulates lactation of sows [3] gene (FSHR) which stimulates follicle hormone receptor that regulates the growth and maturation of follicles [8]. At this stage of Genetics biological differences between Welsh and Ukrainian meat breeds are insufficiently studied. Ukrainian meat breed was created by reproductive crossbreeding Great White, Mirgorod, Landrace, Welsh, P'yetren, Wessex-seudlbek and Ukrainian Steppe White breeds. Application of these marker genes will explore the biological and genetic potential of pig breeds in practice.

The aim of research is to explore particular interbreed polymorphisms genes: *ESR* (estrogen receptor), *NCOA1* (nuclear receptor coactivator A1), *PRLR* (prolactin receptor), *FSHR* (follicle stimulating hormone receptor).

MATERIALS AND METHODS. Experimental studies were carried out in the Department of Genetics in Institute of Animal Breeding and Genetics NAAS of Ukraine and in the Department of Genetics, breeding and reproductive biotechnology animals named M.A. Kravchenko NUBiP Ukraine. The total volume of the studies is presented in Table 1.

Table 1

Materials research

Investigated breeds	**Sum of study sows**
Gene the estrogen receptor (ESR)	
Welsh breed	123 individuals
Ukrainian meat breed	73 individuals
Gene coactivator nuclear receptor (NCOA1)	
Welsh breed	123 individuals
Ukrainian meat breed	74 individuals
Gene hormone prolactin receptor (PRLR)	
Welsh breed	120 individuals
Ukrainian meat breed	73 individuals
Follicle stimulating hormone (FSHR)	
Welsh breed	125 individuals
Ukrainian meat breed	72 individuals

Biological material of small breed sows Ukrainian meat and Welsh breeds were studied. These breeds are reproducing in state enterprise "Hontarivka" Volchansky district, Kharkiv region. Genomic DNA was extracted from hair follicles using reagents "DNA Sorb B" on the recommendations of the manufacturer. The study of gene polymorphism ESR, NCOA1, PRLR, was performed by PCR - RFLP. FSHR gene polymorphism was performed by Bi-Passa method (without restriction). For gene amplification using primers presented in Table 2.

Optimized parameters for the restriction sites of genes ESR, NCOA1, PRLR using endonucleases - PvuII, RsaI, AluI. Visualization of restriction fragment length are performed by electrophoresis in 2% agarose gels.

Table 2

Sequences of primers

Locus	Sequences of primers		Author
FSHR	F	GCA ACA AAT CTA TTT TAA GGC AAG A	8
	R	GAT GCT CAC CTT CAT GTA GCT G	
NCOA1	F	AGG GGC TAC CCT CCT GTA AG	12
	R	CTT CTC TGC CAG TTC TCC AGT C	
ESR	F	CCT GTT TTT ACA GTG ACT TTT ACA GAG	11
	R	CAC TTC GAG GGT CAG TCC AAT TAG	
PRLR	F	CGT GGC TCC GTT TGA AGA ACC	7
	R	CTG AAA GGA GTG CAT AAA GCC	

Statistical data processing was performed by standard methods using Excel 2003.

RESULTS AND DISCUSSION. As a result of molecular genetic testing of sows of Ukrainian meat and Welsh breeds were detected gene polymorphism ESR, NCOA1, PRLR, FSHR.

Sows of Welsh breed compared with sows of Ukrainian meat breed are characterized by a lower frequency of genotype BB ESR gene. The frequency of the desired allele *B* ESR gene in sows of Welsh breed was 0.40 ± 0.021, which is 0.08 less than the Ukrainian meat breed sows (p <0.001) (Table 3). In general researched breeds were characterized by a lower frequency of desirable allele *B* and genotype BB in the ESR gene compared with literature data polymorphism's pig of breeds meat productivity [4, 13, 14].

Table 3

The frequencies of alleles and genotypes of ESR gene in pigs of Ukrainian meat and Welsh breeds

Breed	Quantity of stock	Frequency								χ^2
		genotypes						allele		
		BB		***AB***		*AA*		***B***	*A*	
Welsh	123	C	0,02± 0,019 ***	C	0,76± 0,059	C	0,22± 0,057	0,40± 0,021 ***	0,60± 0,026	28,02
		E	0,160± 0,051	E	0,480± 0,069	E	0,360± 0,067			
Ukrainian meat	73	C	0,10± 0,047	C	0,75± 0,068	C	0,15± 0,056	0,48± 0,028	0,52± 0,027	18,15
		E	0,230± 0,067	E	0,499± 0,079	E	0,270± 0,070			

Note: The desired allele are in bold;

*** P <0.01 (between sows of Ukrainian meat compared with sows of Welsh breed).

Analysis of the important indicators of genetic variation, calculated on the basis of the distribution of the respective genotypes showed an excess of heterozygotes. Analysis was close in value in both researched breeds, indicating their genetic consolidation for genome ESR (Fig. 1).

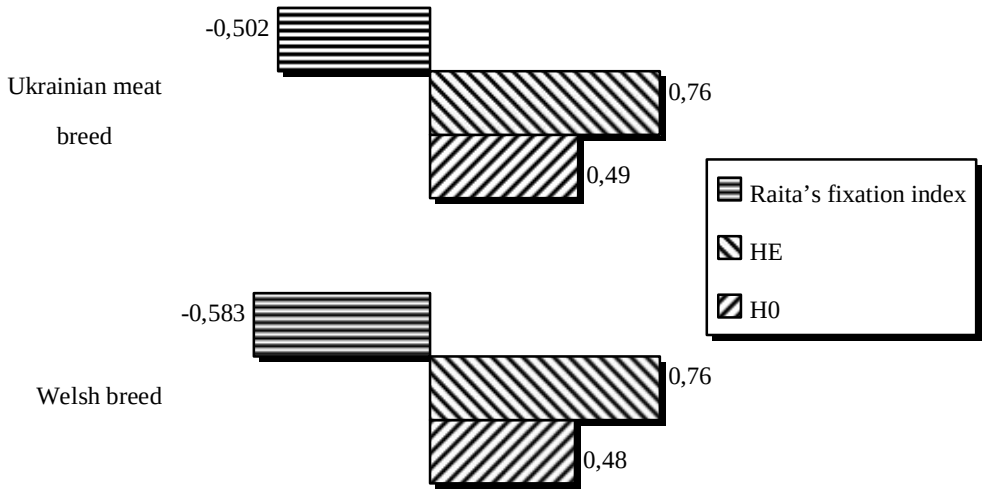

Fig.1 Sows heterozygosity of Welsh and Ukrainian meat breed (ESR gene)

Research frequencies of genotypes and alleles of a NCOA1 gene in pigs of Ukrainian meat and Welsh breeds found relatively high frequencies, as desired in terms of increased multiple births of genotype A1A1, and so the desired allele A1. Genotype A1A1 in the Ukrainian meat breed sows recorded by 9% less than in the Welsh breed sows ($p < 0.001$) (Table 4). It should be noted that in the researched breeds we found the highest frequency of genotype A2A2, compared with the results of other researchers [1, 12].

Table 4

The frequencies of alleles and gene genotypes NCOA1 in pigs Ukrainian meat and Welsh breeds

Breed	Quantity of stock	Frequency								χ^2
		genotypes						allele		
		A1A1		***A1****A2*		*A2A2*		***A1***	*A2*	
Welsh	123	C	0,57± 0,069 ***	C	0,20± 0,065	C	0,23± 0,058	0,66± 0,020	0,34± 0,027	35,99
		E	0,44± 0,069	E	0,45± 0,069	E	0,12± 0,043			
Ukrainian meat	74	C	0,46± 0,079	C	0,36± 0,076	C	0,18± 0,062	0,62± 0,020	0,38± 0,030	3,69
		E	0,38± 0,079	E	0,47± 0,078	E	0,14± 0,052			

Note: The desired allele are in bold;

*** P <0.01 (between sows of Ukrainian meat compared with sows of Welsh breed).

Analysis of accordance obtained by us of frequencies which had distribution of according to Hardy-Weinberg's law indicates that sows of Welsh breeds have significantly higher frequency of heterozygotes (Fig. 2).

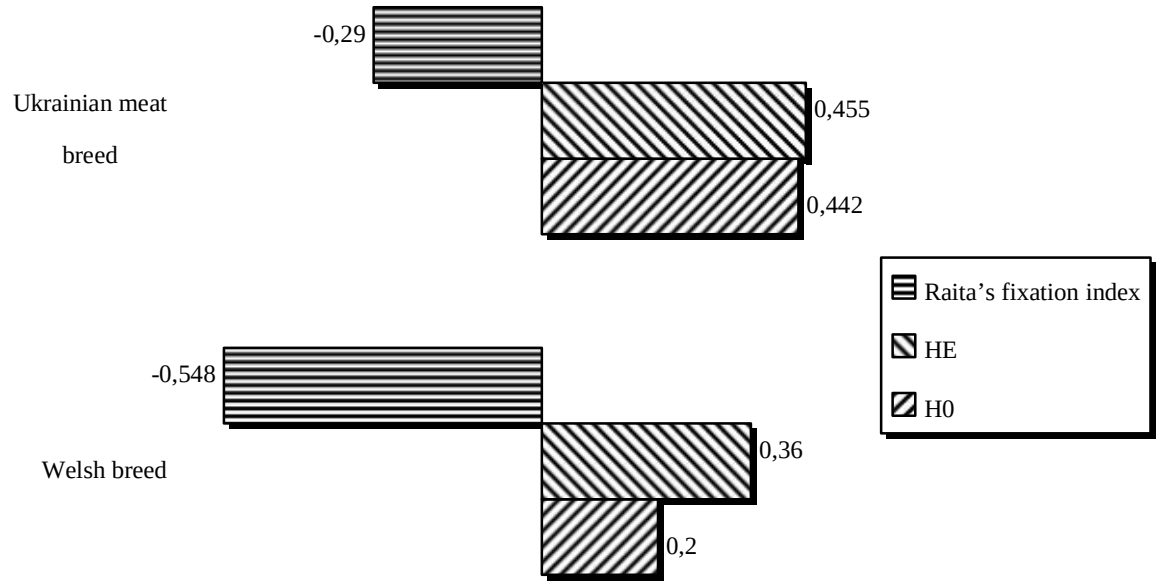

Fig.2 Sows heterozygosity of Welsh and Ukrainian meat breed gene NCOA1

The high rate of AA genotype in pigs Ukrainian meat breed, while in Welsh breed AA genotype frequency was 0,34 ± 0,043 (Table 5).

Table 5

The distribution of genotype frequencies PRLR gene in pigs of Ukrainian meat and Welsh breeds

Breed	Quantity of stock	Frequency								χ^2
		genotypes						allele		
		AA		*AB*		*BB*		*A*	*B*	
Welsh	120	C	0,34± 0,043 ***	C	0,38± 0,044	C	0,28± 0,041	0,53± 0,016	0,47± 0,017	26,71
		E	0,28± 0,041	E	0,22± 0,038	E	0,50± 0,046			
Ukrainian meat	73	C	0,52± 0,058	C	0,13± 0,039	C	0,35± 0,056	0,58± 0,019	0,42± 0,022	11,03
		E	0,33± 0,055	E	0,18± 0,045	E	0,49±0, 059			

Note: The desired allele are in bold;

*** P <0.01 (between sows of Ukrainian meat compared with sows of Welsh breed).

Analysis of genetic variation, calculated on the basis of the distribution of the respective genotypes showed no excess of heterozygotes (Fig. 3).

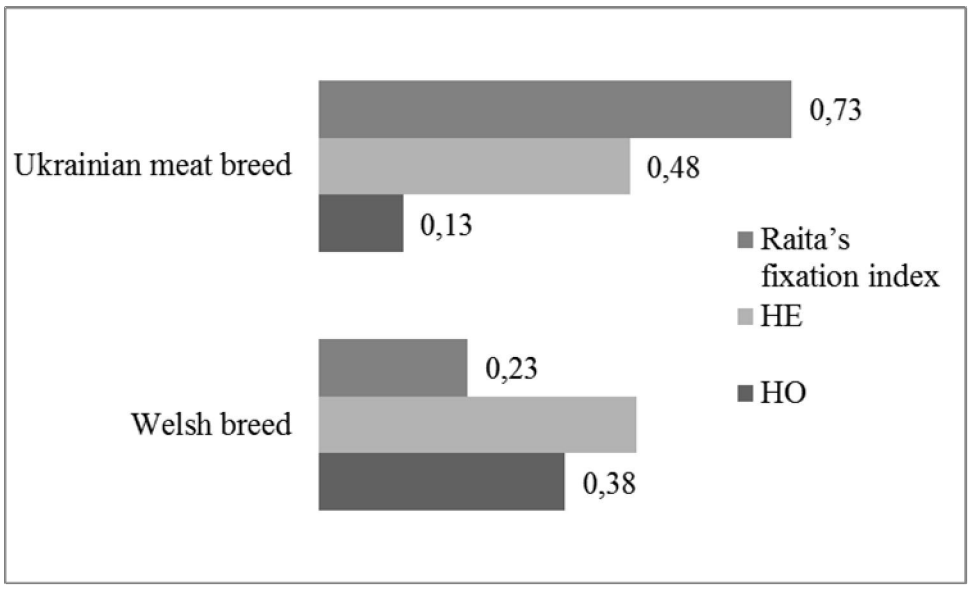

Fig.3 Sows heterozygosity of Welsh and Ukrainian meat breed PRLR gene

Breeds of pigs appeared polymorphic for FSHR gene. The distribution of frequencies of alleles and genotypes FSHR gene in pigs of Ukrainian meat and Welsh breeds showed almost the same values (Table 6). Data analysis of foreign researchers as to efficiency alleles FSHR gene on multiple births sows suggests that gene is displayed differently in environment of alleles in each breed [5].

Table 6

The distribution of genotype frequencies FSHR gene in pigs of Ukrainian meat and Welsh breeds

Breed	Quantity of stock	Frequency								χ^2
		genotypes						allele		
		CC		***CT***		*TT*		***C***	*T*	
Welsh	120	C	0,57± 0,069	C	0,33± 0,065	C	0,10± 0,042	0,75± 0,015	0,25± 0,026	70,03
		E	0,563± 0,069	E	0,375± 0,067	E	0,063± 0,034			
Ukrainian meat	73	C	0,56± 0,069	C	0,34± 0,065	C	0,10± 0,042	0,73± 0,018	0,27± 0,030	47,98
		E	0,533± 0,078	E	0,394± 0,047	E	0,073± 0,075			

Note: The desired allele are in bold;

*** P <0.01 (between sows of Ukrainian meat compared with sows of Welsh breed).

Study our population characterized by high actual heterozygosity FSHR gene compared with the expected. This confirms the calculated Raita's fixation index. Average value *Fis* in both breeds was the same (~ - 0.13) (Fig. 4).

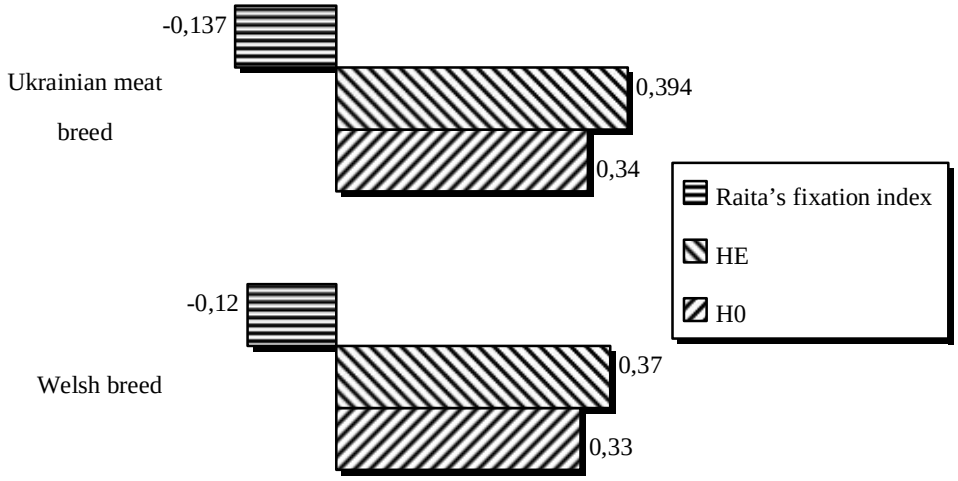

Fig.4 Sows heterozygosity of Welsh and Ukrainian meat breed FSHR gene

These total data from four genes show that researching breeds meat productivity have minor specific particularity genetic structure.

CONCLUSIONS. The result of research show that breeds appeared polymorphic by ESR, NCOA1, PRLR, FSHR genes. The frequency of desired allele *B* ESR gene in sows of Ukrainian meat breed equals 0.09 ± 0.026 and Wales breed – 0.09 ± 0.023. The frequency of desired alleles A1 NCOA1 gene in sows of Ukrainian meat breed equals 0.65, Welsh breed – 0.67. The frequency of genotype A1A1 in sows of Ukrainian meat breed equals 0.46 ± 0.079 and Welsh breed – 0.57 ± 0.069. The frequency of the desired allele A and genotype AA PRLR gene in sows of Ukrainian meat breed equals 0.47 and Wales breed – 0.42 and frequency of genotype AA – 0.28 ± 0.041 and 0.35 ± 0.056, respectively. The frequency of desired allele C FSHR gene in sows of Ukrainian meat breed equals 0.73 and Wales breed 0.75 and the frequency of genotype CC – 0.56 ± 0.069 and 0.57 ± 0.069, respectively.

REFERENCES

1. Адаменко В. А. Роль комплекса полиморфных маркеров в характеристике генетического потенциала свиней: автореф. дис. На соискание научной степени канд. биол. наук: спец. 03.02.21 – «Биотехнология» / В.А.Адаменко. — М., 2005. — 24 с.

2. Інститут тваринництва Української академії аграрних наук (УААН) м. Харкова [Електронний ресурс]. – Режим доступу: http://www.animal.kharkov.ua/index.htm

3. Епишко О.А. Гены, детерминирующие воспроизводительную функцію свиноматок / О.А. Епишко // Весці нацыянальнай акадэміі навук Бэларусі – 2008. – № 2. – С. 81 – 85.

4. Епишко О.А. Полигенный характер детерминации репродуктивных признаков свиней мясной породы / О.А. Епишко, Т.И. Епишко, Л.А. Калашникова // Доклады Российской академии сельскохозяйственных наук. – 2009. – № 2. – С. 42 – 44.

5. Введение в молекулярную генную диагностику сельскохозяйственных животных / [Зиновьева Н.А., Гладырь Е.А., Эрнст Л.К., Брем Г.] – М.: ВИЖ, 2002. – 112 с.

6. Костюнина О.В. Полиморфизм гена *NCOA1* у свиней различных пород / О.В. Костюнина, Н.А. Зиновьева // Международная школа-конференция молодых ученых «Биотехнология будущего». В рамках Международного Симпозиума «ЕС-Россия: перспективы сотрудничества в области биотехнологии в 7-ой Рамочной Программе». — М.: Авиаиздат, 2006. — С. 41–43.

7. Drogemuller C. Candidate gene markers for litter size in different German pig lines / C. Drogemuller, H. Hamann, O. Dist // J. Anim. Sci. — 2001. — № 79. — P. 2565–2570.

8. A missense mutation in the follicle stimulating hormone receptor (*FSHR*) gene shows different allele effects on litter size in Chinese Erhualian and German Landrace pigs / Z. Jiang, O. J. Rottmann, O. Krebs [et all] // Anim. Breed. Genet. — 2002. — № 119. — P. 335–341.

9. Kaminski S. Short communication Relation between Ava I polymorphism within the estrogen receptor gene (*ESR*) and meatiness in Polish Large White boars / Stanislaw Kaminski, Anna Ruoe, Pawel Brym // J. Appl. Genet. — 2003. — Vol. 44, №4. — P. 521–524.

10. Downregulation of estrogen receptor alpha and beta expression in carcinogen-induced mammary gland tumors of rats / Jin Seok Kang, Na Jin Jung, Seyl Kim [et all] // Exp Oncol. — 2004. — Vol. 26., № 1. — P. 31–35.

11. Kmieć M. Study on a relation between estrogen receptor (*ESR*) gene polymorphism and some pig reproduction performance characters in Polish Landrace breed / M. Kmieć, J. Dvořák, I. Vrtková // Czech J. Anim. Sci., 2002. — Vol. 47, № 5. — P. 189–193.

12. A meishan positive QTL for prolificacy trails found at the *NCOA1* locus on SSC3 / [Melville J.S., Gibbins1 A.M. V., Robinsonl J. A.B., Gibson J.P. at al.] // 7th World Congress on Genetics Applied to Livestock Production, August 19–23. — 2002. — P. 15–30.

13. Estrogen receptor polymorphism in Landrace pigs and its association with litter size performance / J. L. Nogueraa, L. Varonaa, L. Gomez-Rayaa [et all] // Livestock Production Science. — 2003. — № 82. — P. 53–59.

14. Simultaneous Detection of Malignant Hyperthermia and Genetic Predisposition for Improved Litter Size in Pigs by Multiplex PCR-RFLP / R. Omelka, D. Vašieek, M. Martiniakova, [et all] // Folia biologica (Krakow). — 2004. — Vol. 52, №1-2. — P. 214-220

ВИВЧЕННЯ ПОЛІМОРФІЗМУ ГЕНІВ, АСОЦІЙОВАНИХ З РЕПРОДУКТИВНОЮ ЗДАТНІСТЮ СВИНОМАТОК УКРАЇНСЬКОЇ М'ЯСНОЇ ТА УЕЛЬСЬКОЇ ПОРІД

М.В. Драгулян, С.О. Костенко, О.В. Сидоренко

Досліджено генотипи генів: ESR (рецептора естрогена), NCOA1 (коактиватора А1 ядерних рецепторів), PRLR (рецептора пролактину), FSHR (рецептора фолікулостимулюючого гормона) свиноматок уельської і української

м’ясної порід. Виявлено, що у свиноматок уельської породи частота алеля В гена ESR сстановить 0,40, у української м’ясної - 0,48. Свиноматки уельської породи характеризувались нижчою частотою генотипу ВВ гена ESR порівняно зі свиноматками української м′ясної породи (відповідно 0,02 та 0,10). Дослідження частот генотипів та алелів гена NCOA1 у свиней української м’ясної та уельської порід виявили досить високі частоти бажаного з точки зору підвищення багатоплідності алеля А1 (відповідно 0,62 та 0,66). Генотип А1А1 у свиноматок української м'ясної породи зустрічався на 9% рідше, ніж у свиноматок уельської породи ($p<0,001$). Частота алеля А гена PRLR у свиноматок уельської породи - 053, української м’ясної - 0,58, а генотипу АА відповідно 0,34 та 0,52. Породи свиней за геном FSHR виявились поліморфними. Розподіл частот бажаного алеля С гена FSHR у свиней української м’ясної та уельської порід був майже однаковим (відповідно 0,75 та 0,73). Частоти генотипу СС гена FSHR у свиноматок уельської породи становили 0,57, української м'ясної – 0,56. Фактичні частоти генотипів статистично вірогідно відрізнялися від теоретично очікуваних.

Ключові слова: *генетичні маркери, репродуктивні якості, свиня свійська, ген рецептора естрогена, ген рецептора пролактину, ген коактиватора А1 ядерних рецепторів, ген фолікулостимулюючого гормона, ESR, NCOA1, PRLR, FSHR, свиноматка, українська м’ясна порода, уельська порода.*

ИЗУЧЕНИЕ ПОЛИМОРФИЗМА ГЕНОВ, АССОЦИИРОВАННЫХ С РЕПРОДУКТИВНОЙ СПОСОБНОСТЬЮ СВИНОМАТОК УКРАИНСКИЙ МЯСНОЙ И УЭЛЬСКОГО ПОРОД

М.В. Драгулян, С.А. Костенко, О.В. Сидоренко

Исследованы генотипы генов: ESR (рецептор эстрогена), NCOA1 (коактиватор А1 ядерных рецепторов), PRLR (рецептор пролактина), FSHR (рецептор ФСГ) свиноматок уэльской и украинской мясной пород. Выявлено, что частота аллеля В гена ESR у свиноматок уэльской породы составляет 0,40, у украинской мясной – 0,48. Свиноматки уэльской породы характеризовались

низкой частотой генотипа ВВ гена ESR по сравнению со свиноматками украинской мясной породы соответственно 0,02 и 0,10. Исследование частот генотипов и аллелей гена NCOA1 у свиней украинской мясной и уэльской пород обнаружили довольно высокие частоты желаемого с точки зрения повышения многоплодия аллеля А1 (0,62 и 0,66). Генотип А1А1 у свиноматок украинской мясной породы встречался на 9% реже, чем у свиноматок уэльской породы (р <0,001). Частота аллеля А гена PRLR у свиноматок уэльской породы была 053, украинской мясной - 0,58, а генотипа АА соответственно 0,34 и 0,52. Породы свиней оказались полиморфными по гена FSHR. Распределение частот желаемого алеля С гена FSHR у свиней украинской мясной и уэльской пород было почти одинаковым (0,75 и 0,73). Частоты генотипа СС гена FSHR у свиноматок уэльской породы - 0,57, украинской мясной - 0,56. Фактические частоты генотипов статистически достоверно отличались от теоретически ожидаемых.

Ключевые слова: *генетические маркеры, свинья домашняя, украинская мясная порода, уэльская порода ген рецептора эстрогена, ген рецептора пролактина, ген коактиватора А1 ядерных рецепторов, ген фолликулостимулирующего гормона, ESR, NCOA1, PRLR, FSHR свиноматка.*