Kafkas Univ Vet Fak Derg
21 (6): 893-898, 2015
DOI: 10.9775/kvfd.2015.13827

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Distributions of *CYP19, ERα* and *PGR* Allele Frequencies between Fertile and Subfertile Holstein-Friesian Heifers [1]

Abdulkadir KESKİN [1] Yasemin ÖNER [2] Gülnaz YILMAZBAŞ-MECİTOĞLU [1]
Barış GÜNER [1] Ebru KARAKAYA [1] Cengiz ELMACI [2] Ahmet GÜMEN [1]

[1] *This work was financially supported by the Scientific Research Council of Uludag University (Project number: KUAP 2013/49)*

[1] Department of Gynecology and Obstetrics, Faculty of Veterinary Medicine, University of Uludag, TR-16059 Bursa - TURKEY

[2] Department of Animal Science Biometry and Genetics, Faculty of Agriculture, University of Uludag, TR-16059 Bursa - TURKEY

Article Code: KVFD-2015-13827 Received: 06.06.2015 Accepted: 20.08.2015 Published Online: 20.08.2015

Abstract

The aim of this study was to investigate the gene and genotype distributions of some mutations in the aromatase cytochrome P450 *(CYP19)*, estrogen receptor α *(ERα)*, and progesterone receptor *(PGR)* genes in fertile and subfertile Holstein-Friesian heifers using the PCR-RFLP method and comparing the distributions between groups. A total of 106 heifers were included the study, and the heifers that became pregnant after the first artificial insemination (n=51) were used as a fertile group. Heifers (n=55) with equal and more than 3 AIs were accepted as a subfertile heifers. Blood samples from all of the heifers were obtained for DNA isolation. While two alleles and three genotypes were found at the *PGR* and *ERα* loci, two alleles and two genotypes were detected at the *CYP19* locus. The A allele and AA genotype, G allele and GG genotype, and C allele and CT genotype were found to be predominant in *CYP19, ERα* and *PGR*, respectively. According to the chi-square test ($\chi 2$), two of the groups investigated were in Hardy-Weinberg equilibrium for all gene loci. There were no differences detected in allele or genotype frequencies between the fertile and subfertile heifers.

Keywords: *Holstein-Friesian, Heifer, Fertility, Subfertilty, Polymorphism, CYP19, PGR, ERα*

Fertil ve Subfertil Siyah Alaca Düveler Arasında *CYP19, ERα, PGR* Allel Frekanslarının Dağılımı

Özet

Bu çalışmanın amacı fertil ve subfertil Siyah Alaca düvelerde aromataz sitokrom P450 *(CYP19)*, östrojen reseptör α *(ERα)*, progesteron reseptör *(PGR)* genlerindeki bazı mutasyonların gen ve genotip frekanslarınınn PCR-RFLP yöntemi kullanılarak incelenmesi ve bu dağılımların fertil ve subfertil Siyah-Alaca düveler arasında karşılaştırılmasıdır. Çalışmaya toplam 106 düve dâhil edilmiş ve ilk tohumlamadan sonra gebe kalan düveler fertil düve grubu (n=51) olarak kullanılmıştır. Üç ve daha fazla tohumlama sayısına sahip düveler (n=55) subfertil olarak kabul edilmiştir. DNA izolasyonu için tüm düvelerden kan alınmıştır. *PGR* ve *ERα* genlerine ait lokuslarda iki allel ve üç genotip bulunurken, *CYP19* geninde incelenen lokusta iki allel ve iki genotip belirlenmiştir. *CYP19, ERα, PGR* lokuslarında sırasıyla A alleli ve AA genotipi, G alleli ve GG, C alleli ve CT genotipi predominat olarak bulunmuştur. Ki- Kare ($\chi 2$) test sonuçlarına göre incelenen her iki grupta tüm lokuslar bakımından Hardy- Weinberg dengesinde idi. Allel ve genotip frekansları bakımından fertil ve subfertil düveler arasında fark bulunamamıştır.

Anahtar sözcükler: *Siyah Alaca, Düve, Fertilite, Subfertilite, Polimorfizm, CYP19, PGR, ERα*

INTRODUCTION

In the dairy industry, most economically important traits depend on reproduction, which determines the economic value of dairy herds [1-3]. Reproduction is influenced by many environmental and genetic components, as are most complex traits. In contrast, long generation intervals and the low heritability of reproductive traits can cause limited success in the selection of these traits [1]. Intensive selection has been applied for 50 years in the dairy industry, resulting in increased milk production while leading to an important decrease in reproduction [3,4]. High producing dairy cows generally require more service per conception, with prolonged calving intervals and

İletişim (Correspondence)
+90 224 2940826
kadirk@uludag.edu.tr

higher culling rates [2,3,5,6]. However, the fertility of heifers is greater than that of dairy cows due to the former's lack of milk production. The rate of pregnancy per artificial insemination (P/AI) is approximately 60-75% in heifers inseminated following estrus detection. Thus, heifers are expected to become pregnant after a maximum two AIs, and heifers with ≥3 AIs can be considered subfertile [7-11]. Subfertile heifers, like repeat breeder cows, cause economic losses in dairy herds because of increased insemination costs, later first calving ages and higher culling rates [5]. Subfertile heifers with increased age of first calving are not suitable for herds [10] because they are culled quickly due to low milk production and some health problems (dystocia, metritis, replacement of abomasum etc.). Subfertility in heifers is a multifactorial condition [7], and there is no accurate method for diagnosing the cause in most individuals. Although there are many causative factors, hormonal imbalance, genetic factors and the uterine environment are important as etiological factors in subfertile heifers [7,8]. Genetic markers might be helpful for selecting more appropriate individuals to keep in breeding herds [12,13].

In addition, long generation intervals and the low heritability of reproductive traits have caused limited success of selection for reproductive traits, such as fertility. Molecular genetics tools, which allow for the detection of genes, have major effects on complex traits, such as reproductive performance, and they can be used as selection criteria for these types of traits for genetic improvement [14]. There have been limited efforts to determine the major genes influencing reproductive traits.

Researchers have focused on the gene-regulated hormones that play critical roles in reproduction, such as aromatase cytochrome P450 enzyme *(CYP19)*, estrogen receptor α *(ERα)*, and progesterone receptor *(PGR),* which have been investigated [15-17] to evaluate their polymorphisms as selection criteria for these types of traits for genetic improvement. However, there have been limited efforts to determine the major genes influencing reproductive traits [14]. It is also important to determine the genes and genotype frequencies of candidate gene for quantitative traits. Various studies have been performed to determinate the gene and genotype frequencies affecting quantitative traits in cattle breeds reared in Turkey [12,13].

The aromatase cytochrome P450 enzyme catalyzes the conversion of androgens into estrogens and the biosynthesis of estrogen by aromatization [16]. This function of the hormone is important for controlling female reproduction. The aromatase cytochrome P450 enzyme is coded by the *CYP19* gene, which located on chromosome 10 in the bovine genome and has tissue-specific expression [18,19]. Placental expression of the *CYP19* gene is regulated by P1.1 promoter, and an A→G mutation at this region has been detected [19]. Subsequently, due to the critical role of this gene region where the mutation occurs, various studies have been undertaken to investigate the associations between alleles at this locus and different economically important traits, including reproductive traits [20-23].

The other important candidate gene is estrogen receptor α *(ERα)*. As with other nuclear receptors, estrogen receptors are transcription factors, and they regulate gene transcription [17]. Estrogens have also many functions in the critical process of the life cycle [24]. Due to their important roles, estrogen receptors and their genes are believed to be candidate markers for reproductive traits. There are two isoforms of estrogen receptor, called ERα and ERβ, which are coded by different genes located on chromosomes 9 and 10 of the bovine genome, respectively. Investigations of the functions of these two receptors have revealed that, while failure of ERα leads to infertility in both male and female mice, failure of ERβ has little effect on fertility [25]. All nuclear receptor genes, including *ER,* have a special structure at their 5'UTR region [26]. In addition to eight exons of *ERα,* there are additional exons encoding tissue-specific transcripts [26]. An A→G transition found at this region [25] has attracted attention for its possible effects on reproduction-related traits [26,27].

Progesterone plays a key role in the establishment and maintenance of pregnancy [15,28]. Because progesterone activity is regulated by progesterone receptor, the progesterone receptor gene *(PGR)* has been considered a good candidate for reproduction [27]. In the *PGR* gene, a G→C transversion and a T→C transition on introns 3 and 4 have been reported, respectively, and some associations have been indicated between these polymorphisms and reproductive parameters [27,29]. These polymorphisms were only recently reported, and there have been only a small number of studies performed on them to date.

Thus, the aim of this study was to evaluate and compare the frequency distributions of some mutations of the *CYP19*, *ERα*, and *PGR* genes with regard to fertility between fertile and subfertile Holstein-Friesian heifers.

MATERIAL and METHODS

Animals, Housing, and Sampling

This study was approved by the Ethics Committee of Uludag University (UÜHADYEK, Approval date: 04.06.2013; No: 2013-11/1). The study was performed at six different lactating dairy farms with an average of 400-750 milking cows, located in the Marmara region of the Turkey, and it was undertaken between January and December 2014. The first insemination age of the heifers was an average of 15 months old. Heifers were inseminated following estrus detection after spontaneous or $PGF_2\alpha$ -induced (one or two doses of $PGF_2\alpha$ apart from 14 days) estrus. Artificial

inseminations (AIs) were performed by farm veterinarians. The heifers were housed in a free-style barn, and they were fed total mixed rations, based on Natural Research Council [30] recommendations, and had unlimited access to water. Heifers (n=106) were selected and included in the study according to their AI numbers, and they were assigned to one of the two groups. Heifers (n=51) that became pregnant after the first AI were used as a fertile heifers group. First and second pregnancy checks were performed at 30 and 60 days after AI in the fertile heifers. If embryonic loss was detected at the 60 d pregnancy check, the heifers were excluded from the study. Subfertile heifers with ≥ 3 AIs (n=55) formed the study group. Blood samples were obtained from the coccygeal vein for DNA isolation.

DNA Isolation and PCR Amplifications

Total DNA was extracted using a genomic DNA purification kit (K0512, Fermentas, Lithuania) according to the instruction manual. Spectrophotometric methods were used to determine DNA quality and quantity. The primers and restriction enzymes used for PCR amplifications are given in *Table 1*.

PCR amplifications were performed in reaction mixtures of 25 µL containing 12.5 µL of 2× PCR Master Mix (K0172, Fermentas), 0.5 µM of each primer, and 25-75 ng of genomic DNA. Amplification was performed using a Techgene Thermal Cycler (Techne, Cambridge, UK). Restriction enzyme cuttings were performed according to the manufacturer's protocols. The restriction fragments were directly analyzed by electrophoresis in 2% and 2.5% agarose gels in 1× TBE buffer, stained with SafeView™ Classic (Applied Biological Materials Inc.) and visualized under UV light. Direct counting was performed to estimate the phenotype and allele frequencies of the genetic variants for all of the loci.

Statistical Analyses

Statistical analyses were conducted by using SAS [32]. Data were evaluated using PROC GLM, PROC COR and PROC REG in SAS. The effects of heifer ages, numbers of AIs, farm factor, *CYP19*, *ERα*, and *PGR* genes were included to the statistical models. The differences in numbers of AIs and ages between the fertile and subfertile heifers were determined using the PROC GLM. The PROC REG and PROC COR procedure were performed to determine the effect of heifer ages, farm factor, *CYP19*, *ERα*, and *PGR* genes on numbers of AIs.

The chi-square test (χ^2) was used to determine whether the populations were in Hardy-Weinberg equilibrium. All of the calculations and the χ^2 analyses were performed using PopGene32 software [33]. Differences in frequency distribution between the fertile and subfertile groups were tested by Fisher's exact test using Minitab software, version 15.0, and SPSS software, version 17.0. To categorize comparisons after the chi-square test (χ^2), the two-proportion z-test was used.

RESULTS

The numbers of AIs and the ages of the heifers were greater (P<0.01) in the subfertile heifers (4.6±0.16 and 19.9±0.42) than in the normal heifers (1±0.19 and 15.5±0.43, respectively). Although high correlation (r = 63.0%; P<0.001) was detected between age and AI numbers, no correlation was found between other factors. According to regression analyses, only age was effect on AI numbers, but farm factor, *CYP19*, *ERα*, and *PGR* genes did not effect on AI numbers (R^2 = 43.2%: AI = - 0.20 + 0.271 Age - 0.0948 Farm + 0.071 *CYP19* + 0.237 *PGR* -*0.702 ERα*).

When gene polymorphisms were evaluated, while two alleles and three genotypes were found at the investigated loci in the *PGR* and *ERα* genes, two alleles and two genotypes were detected at the investigated locus in the CYP19 gene. The GG genotype revealed two bands of 182 bp and 70 bp, while the AA genotype remained uncut for the investigated locus in *CYP19*. At the investigated locus in the *PGR* gene, TT genotype individuals had 515 bp, and CC genotype individuals had 398 bp and 117 bp. A homozygous individuals had only 242 bp uncut fragment, and G homozygous individuals had 182 bp and 60 bp fragments for the investigated locus in the *ERα* gene. Electrophorograms are presented in *Fig. 1*.

The A allele and AA genotype, the G allele and GG genotype, and the C allele and CT genotype were found to be predominant at the investigated loci in the *CYP19, ERα* and *PGR* genes, respectively. The frequencies of these predominant alleles were calculated for the whole population as 0.9623, 0.9198, and 0.6651 for investigated loci in the *CYP19, ERα* and *PGR* genes, respectively. The

Table 1. *Primers sequences and restriction enzymes used in the study*
Tablo 1. *Çalışmada kullanılan primer dizileri ve restriksiyon enzimleri*

Loci	Primers (5′ → 3′)	Enzym	References
PGR	CCCATCCCTTAGCATCTTCC TTACCAACGCTGACCCGAAG	*Eco*321	Yang et al.[28]
ERα	TTTGGTTAACGAGGTGGAG TGTGACACAGGTGGTTTTTC	*Bgl*I	Szreder and Zwierzchowski [29]
*CYP*19	CTCTCGATGAGACAGGCTCC ACAATGCTGGGTTCTGGACT	*Pvu*II	Vanselow et al.[31]

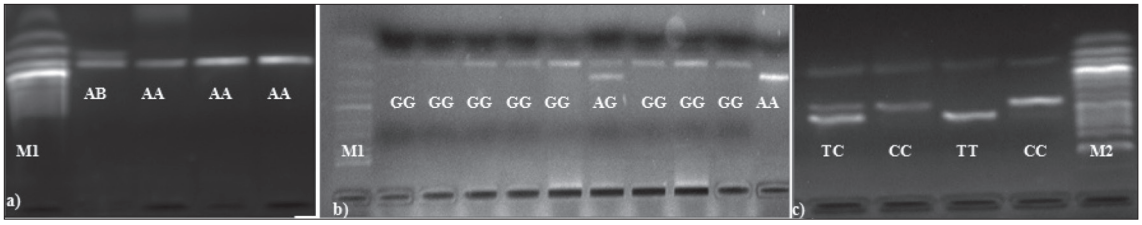

Fig 1. Illustration of PCR-RFLP fragments of loci investigated on agorose gels (a, b, c). **a-** Electroforetic results of CYP19 locus, **b-** Electroforetic results of *ERa*, **c-** Electroforetic results of *PGR;* M1: Bio Basic Inc., MSM 34, 100 bp DNA ladder, M2: BioLab Inc., N3236S, 50 bp DNA ladder

Şekil 1. Lokuslara ait PCR- RFLP parçalarının agoroz jellerdeki görünümü (a, b, c). **a-** CYP19 lokusuna ait elektroforetik sonuçlar, **b-** *ERα* lokusuna ait elektroforetik sonuçlar, **c-** *PGR* lokusuna ait elektroforetik sonuçlar; M1: Bio Basic Inc., MSM 34, 100 bp DNA markır, M2: BioLab Inc., N3236S, 50 bp DNA markır

Table 2. *Distributions of allelle and genotypes of CYP19, ER α and PGR genes in fertile and subfertil groups*
Tablo 2. *Fertil ve subfertil gruplarda CYP19, ERα ve PGR allel ve genotip frekanslarının dağılımları*

Lokus	Groups	n	Allel Frequencies		Genotype Frequencies*			χ^2(HWE)
CYP19	Subfertile	55	A	B	AA	AB	BB	
			0.955	0.045	0.909 (50)	0.091 (5)	-	0.098901^{ns}
	Fertile	51	0.971	0.029	0.940 (48)	0.06 (3)	- -	0.030921^{ns}
ER α	Subfertile	55	A	G	AA	AG	GG	
			0.064	0.936	- -	0.127 (7)	0.873 (48)	0.215877^{ns}
	Fertile	51	0.098	0.902	0.019 (1)	0.157 (8)	0.824 (42)	0.832404^{ns}
PGR	Subfertile	55	C	T	CC	CT	TT	
			0.636	0.364	0.400 (22)	0.473 (26)	0.127 (7)	0.008285^{ns}
	Fertile	51	0.696	0.304	0.431 (22)	0.529 (27)	0.04 (2)	2.992969^{ns}

ns: *non significant; * The numbers of animals carrying of each genotype shown in paranthesis*

allele and genotype frequency distributions between the two groups of heifers are given in *Table 2*.

According to the chi-square test *(χ^2)*, the two groups investigated were in Hardy-Weinberg equilibrium. In addition, no differences were found in allele or genotype frequencies between fertile and subfertile heifers.

DISCUSSION

In cases of the detection of associations between markers and traits of interest, the marker can be used as a selection criterion for decreasing fertility problems. In this study, we investigated and compared polymorphisms in the genes regulating the main reproductive hormones between fertile and subfertile heifers.

Some researchers have been focused on determining the gene and genotype frequencies of candidate gene for subfertile animals [14,15]. However, there have been only limited efforts to determine the major genes influencing reproductive traits [12]. While in previous studies, polymorphisms in the *CYP19* [16,18-21,33], *ERα* [20,21,25,29,34], and *PGR* [15,28,35] genes were evaluated in cows, the present study is the first on these gene polymorphisms in subfertile heifers.

When the results of the present study were compared with those of others, the allele and genotype frequencies were similar *(Table 2)* to the frequencies observed in the previous studies performed in European cattle breeds [15,21,22,28,35,36]. There has been only one study of *ERα* [20] in which the A allele was predominant, in contrast with all the other studies. This contradiction might derive from the differences in nomenclature used in this study. There have been more studies performed on the relationships between *CYP19* and *ERα* polymorphisms [20-23,28] and some reproductive and productive traits, when considering them with *PGR* [28]. However, the results obtained from these studies were not in concordance with each other. While in some previous studies significant relationships have been found between polymorphisms in the *CYP19* and *ERα* genes and calving to conception intervals [20], calving difficulties [22,35] and milk

production, some of them did not find any associations with economically important traits, such as fertility [13] and milk production [36-38]. The gene and genotype frequency distributions for all of the loci did not differ between the fertile and subfertile groups. Although associations have been reported of the *CYP19, ERα* and *PGR* gene polymorphisms with some important traits in other studies [15,20,22,28,35,37], our findings did not support relationships between these mutations and pregnancy per AI. It was emphasized by previous studies that the allele frequency distributions in *CYP19* and *ERα* were not favorable for association analysis, as we found. To ensure the veracity of any associations, larger populations must be studied [14,38].

There have been few studies of the *PGR* gene supporting strong associations between the transferable number of ovaries and *PGR* polymorphisms. These studies have provided markers that can be used in embryo transfer [28,34], despite finding no differences in *PGR* genotypes between the groups' polymorphisms at this gene worth investigating further, because of both the limited numbers of studies of this gene and traits investigated. Association studies should be performed using a larger number of animals and more reproductive parameters.

In this study, we aimed to investigate the differences between fertile and subfertile heifers for polymorphisms in the *CYP19*, *ERα* and *PGR* genes. Despite the unavailable differences between groups, these polymorphisms should be investigated more intensively because they might be important to the improvement of reproductive traits with high heritability and repeatability, based on the critical roles that the genes play in these traits. Among these genes, the *PGR* polymorphism has been evaluated in subfertile heifers for the first time. Further investigations should be conducted with larger groups and more phenotypic data to exhibit more realistic results regarding possible associations.

REFERENCES

1. Pryce JE, Coffey MP, Brotherstone S: The genetic relationship between calving interval, body condition score and linear type and management traits in registered Holsteins. *J Dairy Sci*, 83, 2664-2671, 2000. DOI: 10.3168/jds.S0022-0302(00)75160-5

2. Royal M, Mann GE, Flint AP: Strategies for reversing the trend towards subfertility in dairy cattle. *Vet J*, 160, 53-60, 2000. DOI: 10.1053/tvjl.1999.0450

3. Lucy MC: Reproductive loss in high-producing dairy cattle: Where will it end? *J Dairy Sci*, 84, 1277-1293, 2001. DOI: 10.3168/jds.S0022-0302(01)70158-0

4. Butler WR: Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. *J Dairy Sci*, 81, 2533-2539, 1998. DOI: 10.3168/jds.S0022-0302(98)70146-8

5. Bartlett PC, Kirk JH, Mather E: Repeated insemination in Michigan Holstein-Friesian cattle: Incidence, descriptive epidemiology and estimated economic impact. *Theriogenology*, 26, 309-332, 1986. DOI: 10.1016/0093-691X(86)90150-0

6. Dobson H, Walker SL, Morris MJ, Routly JE, Smith RF: Why is it getting more difficult to successfully artificially inseminate dairy cows? *Animal*, 2, 1104-1111, 2008. DOI: 10.1017/S175173110800236X

7. Båge R, Bosu WTK, Rodríguez-Martínez H: Ovarian follicle apoptosis at the onset of standing estrus in virgin and repeat-breeder dairy heifers. *Theriogenology*, 56, 699-712, 2001. DOI: 10.1016/S0093-691X(01)00601-X

8. Båge R., Gustafson H, Lasson B, Forsberg M, Rodríguez-Martínez H: Repeat-breeding in dairy heifers: Follicular dynamics and estrus cycle characteristics in relation to sexual hormone patterns. *Theriogenology*, 57, 2257-2269, 2002. DOI: 10.1016/S0093-691X(02)00840-3

9. Singh B, Saravia F, Båge R, Rodríguez-Martínez H: Pregnancy rates in repeat-breeder heifers following multiple artificial inseminations during spontaneous oestrus. *Acta Vet Scand*, 46, 1-12, 2005. DOI: 10.1186/1751-0147-46-1

10. Wathes DC, Pollott GE, Johnson KF, Richardson H, Cooke JS: Heifer fertility and carry over consequences for life time production in dairy and beef cattle. *Animal*, 8, 91-104, 2014. DOI: 10.1017/S1751731114000755

11. Sood P, Zachut M, Dube H, Moallem U: Behavioral and hormonal patterns of repeat breeder cows around estrus. *Reproduction*, 149, 545-554, 2015. DOI: 10.1530/REP-14-0598

12. Oner Y, **Pullu M, Akın O, Elmacı C:** Investigation of beta-lactoglobulin (β-lg) and bovine growth hormone (bGH) genes polymorphisms by using HaeIII and MspI restriction enzymes in Brown Swiss and and Holstein breeds reared in Bursa region. *Kafkas Univ Vet Fak Derg*, 17, 371-376, 2011. DOI: 10.9775/kvfd.2010.3501

13. Korkmaz Ağaoğlu Ö, Akyüz B: Growth hormone gene polymorphism in four cattle breeds in Turkey. *Kafkas Univ Vet Fak Derg*, 19, 419-422, 2013. DOI: 10.9775/kvfd.2012.7961

14. Veerkamp RF, Beerda B: Genetics and genomics to improve fertility in high producing dairy cows. *Theriogenology*, 68, 266-273, 2007. DOI: 10.1016/j.theriogenology.2007.04.034

15. Driver AM, Huang W, Gajic S, Monson RL, Rosa GM, Khatib H: Effects of the progesterone receptor variants on fertility traits in cattle. *J Dairy Sci*, 92, 4082-4085, 2009. DOI: 10.3168/jds.2009-2013

16. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito YJ, Fisher CR, Michael MD, Mendelson CR, Bulun SE: Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocrinol Rev*, 15, 342-355, 1994. DOI: 10.1210/edrv-15-3-342

17. Metivier R, Petit FG, Valotaire Y, Pakdel F: Function of N-terminal transactivation domain of the estrogen receptor requires a potential α-helical structure and is negatively regulated by the A domain. *Mol Endocrinol*, 14, 1849-1871, 2000. DOI: 10.1210/mend.14.11.0546

18. Fürbass R, Kalbe C, Vanselow J: Tissue-specific expression of the bovine aromatase-encoding gene uses multiple transcriptional start sites and alternative first exons. *Endocrinology*, 138, 2813-2819, 1997. DOI: 10.1210/endo.138.7.5257

19. Vanselow J, Kühn C, Fürbass R, Schwerin M: Isolation of the bovine CYP19 promoter 1.2 and identification of genetic variants. *Anim Genet*, 31, 337-338, 2000. DOI: 10.1046/j.1365-2052.2000.00659.x

20. Szatkowska I, Grzesiak W, Jędrzejczak M, Dybus A, Zaborski D, Jankowiak D: An analysis of CYP19, CYP21 and ER genotypes in Polish Holstein-Friesian cows with regard to the selected reproductive traits. *Acta Vet Brno*, 80, 65-71, 2011. DOI: 10.2754/avb201180010065

21. Jędrzejczak M, Grzesiak W, Szatkowska I, Dybus A, Muszyńska M, Zaborski D: Associations between polymorphisms of CYP19, CYP21 and ER1 genes and milk production traits in Black-and-White cattle. *Turk J Vet Anim Sci*, 35, 41-49, 2011. DOI: 10.3906/vet-0911-205

22. Zaborski D, Grzesiak W: Detection of heifers with dystocia using artificial neural networks with regard to ERα-BglI, ERα-SnaBI and CYP19-PvuII genotypes. *Acta Sci Pol Zootechnica*, 10 (2): 105-116, 2011.

23. Zaborskia D, Grzesiaka W, Pilarczyka R: Detection of difficult calving's in the Polish Holstein-Friesian Black-and-White heifers. *J App Anim Res*, 2014. DOI: 10.1080/09712119.2014.987293

24. Eng FCS, Lee HS, Ferrara J, Willson TM, White JH: Probing the

structure and function of the estrogen receptor ligand binding domain by analysis of mutants with altered transactivation characteristics. *Mol Cell Biol,* 17 (8): 4644-4653, 1997.

25. Korach SK: Insights from the study of animals lacking functional estrogen receptor. *Science*, 266, 1524-1527, 1994. DOI: 10.1126/science.7985022

26. Kos M, Reid G, Denger S, Gannon F: Genomic organization of the human ERα gene promoter region. *Mol Endocrinol*, 15 (12): 2057-2063, 2001.

27. Gronemeyer H: Transcriptional activation by estrogen and progesterone receptors. *Annu Rev Genet*, 25, 89-123, 1991. DOI: 10.1146/annurev.ge.25.120191.000513

28. Yang WC, Tang KQ, Li SJ, Yang LG: Association analysis between variants in bovine progesterone receptor gene and superovulation traits in Chinese Holstein cows. *Reprod Dom Anim*, 46, 1029-1034, 2011. DOI: 10.1111/j.1439-0531.2011.01780.x

29. Szreder T, Zwierzchowski L: Polymorphism within the bovine estrogen receptor- α gene 5´-region. *J Appl Genet*, 45 (2): 225-236, 2004.

30. National Research Council: Nutrient Requirements of Dairy Cattle. 7th ed., National Academy of Sciences, Washington, DC, USA. 2001.

31. Vanselow J, Kühn C, Fürbass R, Schwerin M: Three PCR/RFLPs identified in the promoter region1.1 of the bovine aromatase gene (CYP19). *Anim Genet*, 30, 232-233, 1999. DOI: 10.1046/j.1365-2052.1999.00404-11.x

32. SAS 2002 User Guides: Version 9.2, Carry NC, USA, SAS Instute, Inc.

33. Yeh F, Yang RC, Boyle T: Popgene (v.1.32), Microsoft Windows-based freeware for Population Genetic Analysis, 2000. http://www.ualberta.ca/*fyeh/Pop32.exe, *Accessed: 13.04.2015*

34. Zahmatkesh A, Rahmani HR, Edriss MA, Sayed-Tabatabaei BE: Estrogen receptor α (ER-α) gene and bovine performance: Is there any relation? *Anim Sci Paper Rep,* 30 (4): 373-381, 2012.

35. Zahmatkesh A, Rahmani HR, Edriss MA, Sayed-Tabatabaei BE: Bovine ER-α polymorphism in relation with production traits. *International Conference on Food Engineering and Biotechnology, IPCBEE,* 9, 250-254, 2011.

36. Jędrzejczak M, Szatkowska I, Zych S, Grzesiak W, Czerniawska-Piątkowska E, Dybus A: Evaluation of associations of the polymorphism in the placenta-specific promoter 1.1 of the CYP19 gene in Black-and-White and Jersey cattle with milk production traits. *Arch Tierz Dummerstorf*, 49, 311-314, 2006.

37. Kowalewska-Łuczak I: Polymorphism of the CYP19 gene and milk production traits of dairy cattle. *Turk J Vet Anim Sci,* 34, 493-496, 2010. DOI: 10.3906/vet-0707-30

38. Kowalewska-Łuczak I, Michniewicz E, Kulig H: Effect of CYP19 SNPS on milk production traits of Jersey cows. *Acta Sci Pol Zootechnica*, 12 (1): 33-40, 2013.