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Estradiol Concentrations During Estrous in Dairy Cattle and Its Association with Pregnancy and Genotype Diversity

Y.W. Setyorini, E. Kurnianto, Sutopo, and Sutiyono

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Jl. Prof. H. Soedarto, S.H., Tembalang, Semarang, 50275, Indonesia
Corresponding Author: kurniantoedy17@gmail.com
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ABSTRACT

This study determined estradiol concentrations during estrous in dairy cattle and its association with pregnancy and genotype diversity. A total of fifteen female dairy cows that had been identified with their genotype type using the PCR-RFLP method on follicle-stimulating hormone receptor (FSHR) and prolactin (PRL) genes were used in this study. The parameters measured were serum estrogen concentrations during estrous, estrous intensity, and pregnancy. Estradiol concentrations were analyzed by the enzyme-linked immunosorbent assay (ELISA) method. The data were analyzed using a general linear model procedure in the SAS program. The results showed that the average estradiol concentrations in dairy cows during estrous were 60.06±33.25 pg/ml. There was no difference (P>0.05) in estradiol concentrations in different classifications of age, body condition score (BCS), and estrous intensity. Estradiol concentrations in the group that showed success in post-insemination pregnancy were higher and significantly different (P<0.05) from the non-pregnant group. Differences in genotype variations of the FSHR and PRL genes had no significant effect (P>0.05) on the estradiol concentrations. In conclusion, estradiol concentrations during estrous were not associated with age, BCS, estrous intensity, and genotype diversity of FSHR and prolactin genes but were associated with pregnancy.

Keywords: Estradiol, estrous intensity, PCR-RFLP, pregnancy

INTRODUCTION

Optimal breeding management is an essential factor that must be considered to improve dairy cow's reproductive performance and productivity (Meconnin et al., 2017). Visual observation of signs of estrous is a standard method for detecting estrous (Astuti et al., 2020). Estrous detection and insemination time accuracy are crucial to obtaining a high conception rate in dairy cattle. Cows are polyestrous animals and, if not pregnant, will display estrous behaviour approximately every 21 days. The estrous cycle is regulated by hormonal mechanisms such as gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone, estradiol, prostaglandins, prolactin, both through the feedback system and activation through its receptors (Suartini et al., 2013; Laksmi and Trilaksana, 2020).

Estrogen is a steroid hormone that plays a vital role in the estrous cycle. The animal body naturally produces three primary forms of estrogen, namely estradiol17β (E2), Estrone (E1), and Estriol (E3). In the ovaries, E2 or estradiol is the most physiologically active type of estrogen produced by preovulatory follicular granulosa cells (Çiftci, 2013). Estrogen synthesis occurs in theca cells and ovarian granulosa cells. The primary function of the hormone estrogen is to stimulate estrous, the onset of secondary sex traits, maintain the female reproductive tract system and udder growth, and are responsible for the manifestation of the estrous symptoms (Ramli et al., 2016; Laksmi and Trilaksana, 2020).

Identification of genotype variations in reproductive trait control genes is a consideration for the genetic development of dairy cows, especially in Indonesia. Genomic technology can be done by deoxyribonucleic acid (DNA) isolation, Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and sequencing method to obtain genotype data. Several studies have reported polymorphisms of FSHR and PRL genes associated with reproductive traits in cows and goats (Sharifiyazdi et al., 2018; Gayari et al., 2020; Al-Thuwaini, 2021; Setyorini et al., 2023[a]). Reproductive properties are under the control of hypothalamic-pituitary-gonadal balance (Hardyta et al., 2020). Follicle-stimulating hormone (FSH) is a glycoprotein hormone secreted by the anterior pituitary gland and has a vital role in male and female reproductive physiology (Das and Kumar, 2018). FSH action is activated through binding with FSHR encoded by the FSHR gene. Prolactin is a polypeptide hormone produced in the anterior pituitary gland.

In addition, it is essential for initiating and regulating the milk trait and is also responsible for reproductive traits (Pytlewski et al., 2021). The hormone prolactin in high-production dairy cows will stimulate the pituitary to inhibit FSH production to interfere with follicle development and estrogen production. Conversely, high estrogen concentrations can cause prolactin, which functions in milk synthesis, to decrease, affecting milk production (Pariswara et al., 2021).

One thing that affects the success of insemination and the accuracy of estrous detection is the amount of estrogen hormone concentrations in the serum. Studies have been conducted on estradiol concentrations in dairy cattle in other countries and other ruminant commodities. Still, information on estradiol concentrations in dairy cows that have adapted to the Indonesian environment and are associated with genotype diversity is limited. This study aimed to measure the estradiol concentrations in dairy cows during estrous and identify the factors that influence them, including their relationship to pregnancy and gene diversity. The information related to estradiol concentrations and gene potential as a genetic marker for estradiol concentrations can be helpful in the implementation of reproductive technology, improved management, and selection of dairy cows that have adapted to Indonesia's environment.

MATERIAL AND METHODS

A total of fifteen female *Friesian Holstein* (FH) dairy cows were used in this study, with criteria were born in Indonesia, aged 3-5 years, second parity, non-pregnant cow, clinically healthy and have no reproductive disorders. All animals were reared under the same environmental conditions, feeding, and uniform management at the Baturraden dairy breeding installation in Central Java. The mating system was carried out using artificial insemination.

The cows used in this study had been identified for molecular genotype diversity using the PCR-RFLP method on follicle-stimulating hormone receptor genes (Setyorini et al., 2023[a]) and prolactin genes (Setyorini et al., 2023[b]). Polymerase chain reaction (PCR) was performed on DNA extracted from blood samples using a mini genomic extraction kit (geneaid, Taiwan). Restriction fragment length polymorphism (RFLP) was analyzed using restriction enzymes AluI and RsaI. The types of genotypes identified from PCR-RFLP results of the FSHR and PRL genes are presented in Table 1.

This research was conducted with the preliminary stages of general health examination and per rectal examination of reproductive status. This stage ensured that the cows were healthy, not pregnant, had normal ovaries, and no inflammation of the reproductive organs. Observation of estrous was carried out every 4 hours by visual of the visible symptoms of estrous. The start time of estrous activity was justified as the onset of estrous. The intensity of estrous and blood sampling was observed 12 hours after the onset of estrous.

Estrous intensity was determined based on visual observations and symptoms of estrous that appeared and was scored according to the method of Hafizuddin et al. (2012). Observations include swelling and redness of the vulva, mucus discharge, decreased appetite, restlessness, and acceptance when ridden by other cows. A score of 1 is given if the cow shows no symptoms of estrous or fewer. The vulva is not very swollen, wet, or reddening. Mucous discharge is slight; appetite does not decrease, the cow is not restless, and does not appear to rise on each other. A score of 2 (moderate) is given to cows that show all of the above symptoms of estrous with a moderate degree. A score of 3 is given to cows that clearly show all the symptoms of heat.

Blood sampling was collected through the coccygeal vein of the cow during standing heat before insemination, using an 18 G venoject needle and tube without EDTA. The sample was then allowed to stand at room temperature for 30 minutes and centrifuged at 3,000 revolutions per minute (rpm) for 10 minutes.

Table 1. Genotype type from PCR-RFLP results of FSHR and PRL genes

Gene Locus	Genotype			Allele Frequency		Number of Cows
FSHR/AluI	CC	CG	GG	C	G	15
	5	8	2	0.60	0.40	
Prolactin/RsaI	GG	AG	AA	A	G	15
	10	5	-	0. 167	0. 833	

The serum obtained is collected on a serum cuvet, coded, and stored in a freezer temperature of -20°C. The estradiol concentrations were measured from the serum sample by the enzyme-linked immunosorbent assay (ELISA) method using the estradiol ELISA kit (Calbiotech, USA) in the Faculty of Veterinary Medicine, Gadjah Mada University. The test principle uses a delayed competitive binding mechanism with stages according to instructions from the kit, starting from preparation, analysis, and reading on the ELISA reader.

The cow was carried out by artificially insemination after observation of the estrous intensity and blood sampling. Pregnancy was examined using ultrasonography (USG) 60 days after insemination and rectal palpation examination 90 days after insemination. Age was calculated from the cow's birth date to the sampling date in units of years. The body condition score (BCS) was measured at the time of sampling. The BCS data were obtained from the assessment officer at the study site. The body condition score was measured by scoring a visual assessment of a cow's body on a scale of 1-5.

The data obtained were tabulated and analyzed by a general linear model procedure using the statistical analysis system (SAS) program to determine the effect of age, BCS, estrous intensity, pregnancy, and genotype diversity on estradiol concentrations. The Tuckey test was performed if there was a noticeable difference (P<0.05).

RESULTS AND DISCUSSION

Estradiol concentrations measured from 15 dairy cows during estrous obtained an average of 60.06±33.25 pg/ml. This result is almost the same as the estradiol concentrations during estrous reported in Bali cattle 55.00 ± 4.33 pg/ml (Suartini et al., 2013); 63.16 pg/ml (Laksmi and Trilaksana, 2020); in beef cattle 40.06 – 67.80 pg/ml (Astuti et al., 2020); in SimPO cattle 69.30±27.20 pg/ml; dairy cattle 61.00 ±19 pg/ml (Firdausyia, 2018); and in kaligesing goats 50.12±61.26 pg/ml (Hardyta et al., 2020).

The function of the reproductive hormonal system that plays a role in ovulation, estrous, fertility, and maintaining pregnancy in dairy cows will affect the productivity and production of milk (Zulfa et al., 2022). Estrogen, a steroid hormone produced by follicles in the ovaries, is the primary reproductive hormone that affects growth, development, maturation, and function of the reproductive tract and is responsible for estrous behavior (Çiftci, 2013). Estradiol concentrations during estrous based on age, BCS, estrous intensity, and pregnancy classification were presented in Table 2.

The results of the study in Table 2 showed that the estradiol concentrations in group I (3-3.9 years) were relatively the same and not significantly different (P>0.05) from group II (4-4.9 years). This is likely due to cows being in close age ranges and having equal parity. Observations of estrous intensity in the form of signs of acceptance when mounted by another cow, reddening and swelling of the vulva, and viscosity of mucus discharge were quantified into scoring. The results showed that the cow with an estrous intensity score of 3 had a higher estradiol concentration but did not significantly differ (P>0.05) from the cow with a score of 2. In this study, no cows were found with a score of 1. Cows with an estrous intensity score of 3 showed clearer estrous behaviour than group 2.

Table 2. Estradiol concentrations during estrous in dairy cows

Classification	Category	N	Mean±SD Estradiol Concentrations (pg/ml)	P-value
Age	3-3.9 years	7	59.53±06.82	0.957
	4-4.9 years	8	60.52±32.37	
BCS	2.75-3	4	46.12±10.40	0.283
	3.25-3,5	9	71.28±38.65	
	3.75-4	2	37.44±16.40	
Estrous intensity	Score 1	0	-	0.173
	Score 2	3	36.22±17.34	
	Score 3	12	66.02±34.04	
Pregnancy	Pregnant	10	72.57±34.19[a]	0.033
	Not pregnant	5	35.04±8.09[b]	

N: The number of samples. SD: Standard deviation. P: Probability. Different superscripts in the same classification and column show a significant difference (p<0.05)

Gonadotroin-releasing hormone releases gonadotropins (FSH and LH) from the anterior pituitary, stimulating ovarian follicle development. Follicles that grow and develop will produce estrogen released in the blood circulation so that it shows symptoms of estrous (Suartini et al., 2013). The initiation of estrous in cows occurs after an increase in the circulating concentration of estradiol (Perry et al., 2014). Increased estradiol concentrations will increase the blood supply to the vagina so that the external genital organs experience swelling and redness and increased vaginal mucus secretion (Laksmi and Trilaksana, 2020). Estrogen also affects the nervous system, causing cows to appear restless. Measurement of estrogen concentrations during estrous needs to be done as an essential factor in the estrous behaviour of dairy cows (Meconnin et al., 2017). The results of this study were similar to the research reported by Ramli et al. (2016), that there is no relationship between estrous intensity and estradiol concentration during insemination in Aceh cattle. Astuti et al. (2020) revealed that the hormone estrogen has a moderate correlation with the intensity of estrous. So that by sufficient estrogen concentrations, the quality of estrous will be clearer and facilitate the detection of estrous. The accuracy of heat detection supports an increase in conception rates since the timing of insemination is more precise. De Graaf follicles that grow in the estrous phase produce an increase in estradiol and low concentrations of progesterone, triggering the appearance of estrous behavior.

The statistical analysis showed cows with a range BCS of 3.25-3.5 had a higher estradiol concentration but did not significantly differ (P>0.05) from other cattle groups. In this study, cows with BCS less than 3.25 and above 3.75 had lower average estradiol concentrations. The BCS value is a subjective measure to describe the energy reserves of the body and is used indirectly as an indicator of the energy balance status of a cow, expressed as extremely low or extremely high if the BCS value is lower than 2.0 or higher than 4.0 (Susanto et al., 2018). The value of BCS is related to nutrition. Nutrition is a critical factor that affects reproductive status, including the hormonal system. Unbalanced nutrition will affect the function of the body's glands, one of which is the anterior pituitary, which results in low secretion of hormones, including estradiol. This condition can affect the decrease in reproductive efficiency. In the beginning of lactation and high milk yield, cows have the most energy needs. The inability to digest sufficient amounts of feed in this phase leads to a decrease in body weight and an imbalance of energy (Pariswara et al., 2021).

The average estradiol concentrations in the cows that became pregnant after insemination showed higher estradiol concentrations at estrous and were significantly different (P<0.05) than those that were not pregnant. It relates to the role of estradiol concentration during the preovulatory period, which can affect uterine reception and maintenance of pregnancy (Meconnin et al., 2017), increasing the chances of successful fertilization, sperm transport, and embryo survival (Perry et al., 2014). In addition, sufficient estradiol concentrations will be released into the blood vessels and reach the anterior pituitary, with a positive feedback mechanism that will stimulate LH release, affecting ovulation rates (Ramli et al., 2016). In cows that fail the implantation, it is likely to occur due to failure or delay in ovulation due to hormonal factors, in addition to the unpreparedness of uterine conditions for pregnancy.

The association between the genotypic diversity of FSHR and PRL genes with estradiol concentrations during estrous were presented in Table 3. In this study, genotype diversity of both FSHR and PRL genes was not associated (P>0.05) with estradiol concentrations during estrous. The FSHR gene is a gene that controls reproductive traits.

Table 3. Estradiol concentrations based on genotype diversity of FSHR/AluI and PRL/RsaI genes

Gene Locus	Type of Genotype	N	Mean±SD Estradiol Concentrations (pg/ml)
FSHR/AluI	CC	5	70.37±39.26
	CG	8	56.02±34.39
	GG	2	50.43±13.33
PRL/RsaI	GG	10	60.27±29.43
	AG	5	59.65±43.82

N: The number of samples. SD: Standard deviation. Different superscripts in the same classification and column show a significant difference (p<0.05)

The FSHR gene is polymorphic and associated with service per conception at first parity in Indonesian Holstein cattle (Setyorini et al., 2023[a]) and in dairy cows in Iran (Sharifiyazdi et al., 2018). The FSHR gene polymorphism occurs due to transversion between cytosine (C) and guanine (G), which causes a missense mutation of amino acid threonine into serine; however, the diversity of FSHR genes in this study did not affect estradiol concentrations. This is likely because productive and reproductive traits are quantitative traits controlled by polygenes, and genetic factors are also influenced by the environmental factor and the interaction between genotype and environment.

Prolactin gene polymorphism occurs due to the substitution of adenine (A) for guanine (G), which does not cause amino acid changes (synonymous). The relationship between genotype variation in the prolactin gene and reproductive traits has been revealed in several studies. The prolactin gene is polymorphic with two genotypes in awassi sheep and is associated with progesterone concentration, twinning ratio, fecundity, and prolificacy (Al-Thuwaini, 2021). Prolactin gene polymorphism is also associated with age at sexual maturity and age at first calving in crossbreed cattle in India (Gayari et al., 2020), age at first insemination, age at first calving, and length of pregnancy in dairy cattle in Poland (Pytlewski et al., 2021). The genotype of FSHR and prolactin gene were not related to the estradiol hormone in this study; this may be due to environmental influences and other non-genetic factors, such as feed, temperature, and humidity, and is associated with low heritability in reproductive traits.

Based on the mechanism, the FSHR and PRL genes have the potential to be used as a genetic marker for reproductive traits, including their effect on reproductive hormones. However, no relationship was found between genotype and estradiol concentration in this study. The estrogen mechanism directly influences folliculogenesis by stimulating the proliferation of granulosa cells in follicles and facilitates the action of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Estrogen is also responsible for granulosa cell differentiation, including induction of FSH, LH, and prolactin receptor systems, and may affect post-receptor mechanisms (Çiftci, 2013). The release of FSH and LH stimulates the activity of bovine ovaries, follicle formation, and ovulation and promotes the formation of a normal corpus luteum. The hormone estrogen increases with follicle growth, as it is produced by granulosa cells that form the follicle wall (Ramli et al., 2016; Astuti et al., 2020). The results of this study are similar to research on the Kiss1 gene locus by Hardyta et al. (2020) in kaligesing goats, which found no association between estradiol levels and the Kiss1 gene. Henceforth, studying the association of hormone concentrations in larger samples and at other SNP loci of the FSHR and PRL genes is necessary.

CONCLUSION

Based on the study's results, it can be concluded that the average estradiol concentrations in dairy cows during estrous were 60.06±33.25 pg/ml. Estradiol concentrations during estrous in this study were not associated with age, body condition score, and estrous intensity but were associated with the success of post-insemination pregnancy. The genotype diversity of the FSHR and prolactin genes was not associated with bovine estradiol concentrations during estrous.

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