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Estrous characteristics and steroid concentrations in Aceh cows (*Bos indicus*) after estrus synchronization using prostaglandin F2α

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Abstract. *Hafizuddin, Husnurrizal, Thasmi CN, Herrialfian, Salsabil NL, Siregar TN, Sutriana A. 2024. Estrous characteristics and steroid concentrations in Aceh cows* (Bos indicus) *after estrus synchronization using prostaglandin F2α. Biodiversitas 25: 322-328.* This study aims to determine the estrous characteristics and steroid concentrations during estrus in Aceh cattle after estrus synchronization using prostaglandin F2α. This study involved 9 Aceh cows with good reproductive status, not pregnant, and has shown at least 2 regular cycles, which were divided into 3 treatment groups. The first group (GI) received a single injection treatment, the second group (G2) received a double injection treatment with an interval of 10 days, and the third group (G3) was also treatment double injection with an interval of 12 days. Symptoms and responses to estrus were reported descriptively, and the data collected on onset, intensity, and duration of estrus were analyzed using one-way analysis of variance followed by Duncan's test. The results showed that estrus symptoms were almost similar and estrus response was 100% in all three treatment groups. The onset of estrus in G1, G2, and G3 did not differ significantly (P>0.05) with values of 68.00±18.33, 60.00±0.00, and 72.00±24.00 hours, respectively. The intensity of estrus in G1, G2, and G3 showed significant differences (P<0.05) with scores of 3.00±0.00, 5.00±0.00 and 4.33±1.15, respectively. The duration of estrus showed no significant difference in G1, G2, and G3 (P>0.05) with durations of 32.00±18.33, 72.00±0.00, and 52.00±38.57 hours, respectively. The concentrations of estradiol and progesterone obtained also showed no significant differences (P>0.05) in G1, G2, and G3, with respective concentrations of 91.67±24.77, 132.00±23.15, 89.47±24.19 pg/mL and 0.98±0.59, 0.86±0.18, 1.00±0.17 ng/mL. In conclusion, estrus synchronization with PGF2α single injection and double injection in Aceh cattle was found to have 100% estrus response. However, PGF2α with double injection showed higher estrus intensity compared to single injection. In Aceh cows, the recommended protocol for estrus synchronization using PGF2α is double injection.

Keywords: Beef cattle, dinoprost, estradiol, PGF2α, progesterone

INTRODUCTION

Aceh cattle are one type of Indonesia's local beef cattle and are generally farmed in Aceh Province (Abdullah et al. 2012; Sutarno et al. 2015, 2019). Aceh cattle farming patterns are generally still carried out traditionally and semi-intensively. In a semi-intensive system, Aceh cattle farming requires adequate technology application, including Artificial Insemination (AI) (Siregar et al. 2016a; Syafruddin et al. 2021). One obstacle in AI implementation is the proper timing for insemination. According to Consentini et al. (2021), determining of AI in cows could be achieved by performing Estrus Synchronization (ES) prior to AI implementation.

ES is an attempt to standardize the occurrence of estrus and ovulation symptoms in livestock by manipulating the female reproductive organs using hormone preparations. The principle of ES is to shorten the length of the Corpus Luteum (CL) or luteal phase or to extend the length of the CL. The ES method by shortening the luteal phase usually uses the hormone Prostaglandin F2 alpha (PGF2α) which acts by lysing the CL and initiates the estrus. PGF2α administration will be effective in the mid-luteal phase, where in this phase the CL is very sensitive to prostaglandins (Roza et al. 2019; Bihon and Assefa 2021). According to Jinks et al. (2013) and Abebe and Alemayehu (2021), ES using prostaglandins and followed by AI has become the most prominent technology available to small-scale cattle farms for genetic improvement, reproductive management, and cattle mating in almost the same time. ES using PGF2α is usually carried out in two methods, single injection and double injection method. The single injection method is usually effective for synchronizing cattle estrus if the estrous cycle is known to be in the luteal phase with a functional CL, while the double injection method can be applied in both the follicular and luteal phases (Islam 2011; Jainudeen and Hafez 2013; Bihon and Assefa 2021).

Recent studies in cows and other livestock have reported different characteristics of estrus and steroid levels after synchronization with PGF2α or its combination

(Mahmoud and Hussein 2019; Roza et al. 2019). The sheep synchronized with PGF2α + male effect had the faster estrus onset, the higher progesterone concentration and similar estradiol concentration compared with the sheep (*Ovis aries*) synchronized with PGF2α alone (Mahmoud and Hussein 2019). The injection of GnRH combined with PGF2α in buffalo (*Bubalus bubalis kerebau vs. Bubalus bubalis bubalis*) obtained 100% estrus response (n = 21), and progesterone concentrations in pregnant and non-pregnant buffalo were 5.32-8.69 ng/mL and 1.11-2.68 ng/mL, respectively) (Roza et al. 2019). Recent studies in dairy cows (*Bos taurus*) showed that cows receiving double injections of PGF2α had greater estrus rates and lower progesterone concentrations than those receiving single PGF2α (Tschopp et al. 2022). The study on Holstein Friesian cows showed that the success rate in ES was higher in the double injection method compared to single injection (Balumbi et al. 2019). Single injection of PGF2 α in Bali cattle showed 100% estrous response (n = 26) (Mukkun et al. 2021). However, Suastiningsih et al. (2020) reported that single injection of PGF2α intramuscularly showed an estrus response of 75.00%, an estrus onset of 79.83 hours and an estrus intensity score of 1.67, while double injection method (injection of 5 mL PGF2α on day 0, then the second injection on days 11 after the first injection) showed the estrus response of 62. 50%, an estrus onset 67.40 hours and an estrus intensity score of 2.20.

Apart from the success of implementing ES using PGF2α which has been carried out massively in different livestock, there has been no comprehensive report on the success rate of ES in Aceh cattle specifically. Data related to the response and characteristics of estrus, as well as steroid concentrations after synchronization using PGF2α, are important to know as a basis for determining the implementation of sustainable reproductive technology in Aceh cattle and other local livestock in Indonesia. Therefore, this research was conducted to know the estrus characteristics of Aceh cows and steroid concentration as a basis for determining the best timing for AI implementation.

MATERIALS AND METHODS

Animal samples

The sample used was Aceh cows owned by Animal Experiments Unit, and the utilization of the treated animals in this study was ethically ratified by the Veterinary Ethics Committee, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, number: 274/KEPH/III/2021.

Research treatment

This research used 9 Aceh cows of 3-5 years of age with good reproductive status and which were not pregnant. These cows were divided into three groups. All treatment groups were injected with PGF2α (Enzaprost-T®, Dinoprost 5 mg) regardless of reproductive status, whether they were in the follicular or luteal phase. The first group (G1) was treated with a single injection of 5 mL of PGF2α intramuscularly. The second treatment group (G2) underwent double injections of 5 mL of PGF2α intramuscularly with an interval of 10 days from the first injection with the same dose. The third treatment group (G3) underwent double injections of 5 mL of PGF2α intramuscularly with an interval of 12 days from the first injection with the same dose. The parameters observed in this study were estrus characteristics (symptoms, response, onset, intensity, and duration) and steroid levels (estradiol and progesterone).

Estrus characteristic assessment

Estrus detection was carried out every day until the onset of estrus was observed using an observation technique for 30 minutes. Assessment of estrus onset, duration, and intensity of estrus was carried out three times a day at 07.00 AM, 12.00 PM, and 5.30 PM for 5 consecutive days. Cows are considered to have estrus when they show symptoms such as signs of a red, swollen vulva and transparent cervical mucus (Rosmaidar et al. 2021). The onset of estrus was defined as the first time of estrus symptoms and duration of estrus was defined as the time between the first and last estrus symptoms (Randi et al. 2018). Assessment of estrus intensity used the criteria of Sönmez et al. (2005). Cows with primary and secondary signs of heat such as standing heat, mounting another cow, restlessness, red and swollen vulva, discharge of cervical mucus, and decreased appetite were scored on a scale of 0-5 (5 = Excellent: A cow displaying all these characteristics: standing heat, mounting other cows, restlessness, red and swollen vulva, cervical mucus discharge, and decreased appetite; 4 = good: standing heat, mounting other cows, red and swollen vulva, and cervical mucus discharge; 3 = Normal: Red and swollen vulva, discharge of cervical mucus, and decreased appetite; 2 = Fair: Red and swollen vulva and decreased appetite; and 1 = Poor: Decreased appetite; and 0 = No heat) as described previously by Sönmez et al. (2005).

Blood sample collection

To determine the concentration of steroid hormones, 5 mL of blood samples were collected from the jugular vein on the day of estrus (Day 0). Samples were stored at 4°C for 4-6 h, then centrifuged at 3000 RPM for 20 min to separate the serum, and stored at -20°C for subsequent analysis.

Estradiol concentration measurement

The procedure for analyzing estradiol concentrations in this study was carried out using Enzyme-Linked Immunosorbent Assay (ELISA) using an estradiol kit (DRG, International Inc., Germany). Prior to use, all reagents must be left at room temperature (18-25°C), then standard solutions with concentrations of 25, 50, 100, 250, 500, 1000, and 2000 pg/mL and QC (quality control) solution were prepared. The next step was to put 25 µL of standard, sample, and QC (Quality Control) into each microplate well, add 200 µL of HRP Estradiol enzyme conjugate to each microplate well, shaked gently for approximately 10 seconds, then incubate at temperature room for 120 minutes. After incubation, the solution on the microplate was discarded and washed with washing solution with a volume of 300 µL per well. The microplate was dried gently on absorbent paper. Next, 100 µL of substrate solution (TMB substrate) was added to each microplate

well, and then incubated for 20 minutes at room temperature. After incubation with the substrate solution, the enzymatic reaction was stopped by adding 50 µL stop solution into each well. The absorbance value was read at a wavelength of 450 nm using an ELISA reader equipped with the MPM6 program. Readings were recorded no more than 10 minutes after adding the stop solution (Adam et al. 2019).

Progesterone concentration measurement

Hormone analysis to measure the concentration of the hormone progesterone was carried out using the ELISA method. The progesterone hormone concentration measurement was carried out according to the progesterone ELISA kit manual (DRG, International Inc., Germany). Before use, all reagents were left at room temperature (18-25°C), then standard, sample, and QC (quality control) solutions was put into each microplate, incubated for 5 minutes, then added with 200 µL of enzyme conjugate to in each well. The mixture was stirred thoroughly for approximately 10 seconds, and incubated at room temperature for 60 minutes. After incubation, the microplate was washed with washing solution with a volume of 400 µL per well 3 times and dried on absorbent paper. Next, 200 µL of substrate solution (TMB substrate) was added to each well and then incubated for 15 minutes at room temperature. After incubation with the substrate solution, the enzymatic reaction was stopped by adding 100 µL stop solution into each microplate well. After that, the absorbance value was read at a wavelength of 450 nm using an ELISA reader equipped with the MPM6 program. Readings are taken no more than 10 minutes after adding the stop solution (Panjaitan et al. 2021).

Data analysis

Data on symptoms and estrus response were reported descriptively, while data on estrus onset, intensity, and duration of estrus were analyzed using a one-way analysis of variance and followed by the Duncan's multiple range test.

RESULTS AND DISCUSSION

Characteristics of estrous

Symptoms of estrus

The results of visually observing estrus symptoms in various groups of Aceh cows after single and double PGF2α injections can be seen in Table 1 and Figure 1.

The signs of estrus that appeared were relatively similar in cows from the three groups, namely swollen and red vulvas, discharge of cervical mucus, and standing when mounted by a bull. These signs of estrus were the same as those previously observed in Aceh cattle (Ramadhana et al. 2022). According to Gugssa (2015), the administration of PGF2α hormones in the luteal phase plays a role in regressing CL, so that the levels of hormone progesterone in the blood decrease. The low level of progesterone hormone will trigger an increase in the FSH hormone at the pituitary gland which will stimulate the maturation of Graafian follicle, resulting in a rapid increase in estradiol. Visually, in this phase, the cows will show symptoms of estrus which will be followed by ovulation. The presence of estradiol stimulates thickening of the vaginal walls, increased vascularization, increased vaginal secretions, and the external genitals become swollen and reddish in color (Siregar et al. 2016a; Laksmi and Trilaksana 2020).

Table 1. Estrus symptoms observed in Aceh cows after single and double PGF2 α injections

Symptoms of estrus	G1 (single injection of PGF2α)	G2 (double injection of PGF2α interval 10 days)	G3 (double injection of PGF2α interval 12 days)
Nervousness	√	√	√
Decreased appetite	-	√	√
Discharge of cervical mucus	√	√	√
Red vulva and swollen	-	√	√
Mounting other cows	√	√	√
Standing to be mounted by other cattle	√	√	√

Note: √: Shows the response to the symptoms of estrus, -: Does not show a response to the symptoms of estrus

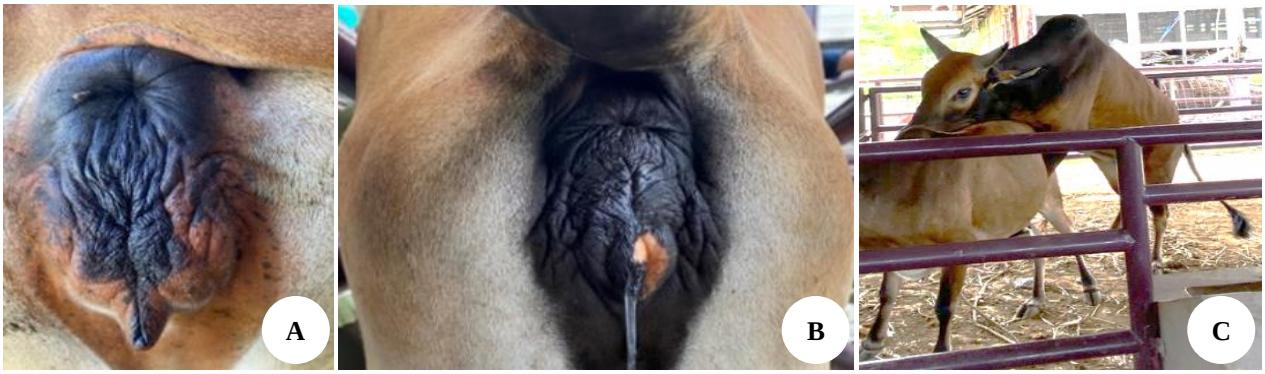

Figure 1. Symptoms of estrus found in Aceh cows in this study: A. Swollen vulva, B. The discharge of cervical mucus, and C. Standing to be mounted by the bull

Estrus response

The estrus response data showed that 100% of Aceh cows showed estrus after being injected with PGF2α using both single injection and double injection. This data indicates that PGF2α injection treatment produces a good response in Aceh cows. This was probably because all the Aceh cows used in this study had their estrous cycles in the luteal phase (functional CL). The visible signs of estrus are in accordance with observations of estrus in Aceh cows by Hafizuddin et al. (2012) and Siregar et al. (2019). Bihon and Assefa (2021) stated that PGF2α can only work during the luteal phase of the estrous cycle and the CL is very sensitive to PGF2 α in this phase resulting in the regression of CL and initiating the return of estrus.

The results obtained in this study were similar to the estrus response reported in Bali cattle after administration of PGF2α (Mukkun et al. 2021). However, the double injection of PGF2α resulted in a better estrus response compared to single injection in Holstein Friesian cows 90% vs 70% (Balumbi et al. 2019), and 95.90% vs 72.20% (Ribeiro et al. 2012). Meanwhile, ES using single injection of PGF2α in Bali cattle produced a better estrus response (75%) as compared to double injection (62.5%) (Suastiningsih et al. (2020). Research on Holstein Friesian x Zebu crossbreed cows in Ethiopia also found better estrus response between double and single injection (84.20% vs 93.30%). In the latter study, it was concluded that applying ES with PGF2α injection to Ethiopian cows was able to improve the estrus performance and increase the estrus intensity scores and estrus duration (Gugssa 2015).

Estrus onset, estrus intensity, and estrus duration.

The results of the research in the form of estrus performance in Aceh cattle based on the average *onset* of estrus (hours), intensity and duration of estrus (hours) are shown in Table 2.

Estrus onset is the time interval observed from treatment until the onset of estrus symptoms (Randi et al. 2018; Reith and Hoy 2018; Roelofs and Van Erp-van der Kooij 2018). In this study, observations of the speed of estrus onset (hours) were carried out from the last injection until the symptoms of estrus appeared. The results of observing the onset of estrus in Aceh cows (Table 2) in G1, G2, and G3 showed no significant difference (P>0.05). The onset of estrus for Aceh cows using the single and double synchronization method after injection of PGF2α on the first day was an average of 68 hours in group 1 (G1), 60 hours in group 2 (G2) and 72 hours in group 3 (G3).

The onset of estrus in 10-day interval double injections (G2) was relatively shorter and more uniform compared to single injections (G1) and 12-day interval double injections (G3). The results of this study were similar to research on Bali cattle which also shows the onset of estrus with no significant difference between single injection and double injection (79.83 hours vs 67.40 hours) (Suastiningsih et al. 2020). Research on Holstein Friesian cows given PGF2α using the single injection method also showed the same range as our research, with the onset of estrus was 70.70±01.90 hours (Putro and Kusumawati 2014). However, a longer onset of estrus was found after administration of PGF2α in Ongole crossbreed cattle, around 75.94±0.78 hours (Astuti et al. 2020).

Intensity scores of Aceh cows between G1, G2 and G3 showed significant differences (P<0.02). Therefore, the results of this study indicated that there was an influence of the injection method on the estrus intensity score. These results provided an interesting depiction that double injection (G2 and G3) was able to make the symptoms of estrus in Aceh cows more clearly visible compared to single injection (G1), with intensity scores of 5.00±0.00 and 4.33±0.67 vs 3.00±0.00, respectively. In addition, the double injection method which is conducted 10 days after the first injection is a new recommendation for the ES protocol in cattle. This is because so far, the repetition of the double injection method is generally 14 days, and some 12 days after the first injection. Therefore, the high estrus intensity score found in Aceh cattle indicates good estrus performance, because the clearer the estrus symptoms, the more accurate the detection of estrus and the more precise the timing of AI implementation.

The duration of estrus or the length of estrus was calculated from the first time the cow shows symptoms of estrus after hormone injection until the end of estrus symptoms. In this study, the duration of estrus found in G1, G2, and G3 was 32.00±10.58, 72.00±0.00, and 52.00±22.57 hours (P>0.21), respectively. Even though the data found was not significantly different, the long duration of estrus was consistently similar to the other characteristics of estrus, the optimal criteria were observed in G2.

The duration of estrus is closely related to the pharmacological and biochemical properties of the PGF2α hormone which activates vaginal smooth muscle. Prostaglandin hormones are very effective in activating smooth muscles, besides having inflammatory effects, vasodilating blood vessels, and elucidating fluid (Setiawati et al. 2021).

The duration of estrus in each cow in this study was not influenced by PGF 2α (P>0.21). The influence of the PGF2α hormone is assumed to only extend to the CL regression process because the hormone quickly undergoes total deactivation in the lungs and liver, so its effect on the length of estrus in livestock is no longer there. Furthermore, the length of estrus lasts naturally by the presence of the high estradiol hormone produced by the ovaries. PGF2α with the active substance dinoprost can reduce progesterone concentrations in cows within 72 hours (Stevenson and Phatak 2010; Esterman et al. 2016; Montaser and El-Desouky 2016; Anton et al. 2019).

Steroid concentration

The steroid concentrations analyzed in this study were estradiol and progesterone. The concentrations of these two steroids found in Aceh cows after ES are presented in Table 3.

Table 2. Mean *onset*, intensity, and duration Aceh cow estrus synchronized with different methods (mean±SE)

Treatment	Estrus performance		
	Onset (hours)	Intensity (score)	Duration (hours)
Single injection (G1)	68.00±10.58[a]	3.00±0.00[a]	32.00±10.58[a]
Double injection interval 10 days (G2)	60.00±0.00[a]	5.00±0.00[b]	72.00±0.00[a]
Double injection interval 12 days (G3)	72.00±13.86[a]	4.33±0.67[b]	52.00±22.57[a]

Note: [a,b] Different superscripts in the same column indicate significant differences (P<0.05)

Table 3. Aceh cattle steroid concentrations synchronized with single and double injection (mean±SE)

Treatment	Estradiol (pg/mL)	Progesterone (ng/mL)
Single injection (G1)	91.67±24.77[a]	0.98 ±0.59[a]
Double injection interval 10 days (G2)	132.00±23.15[a]	0.86±0.18[a]
Double injection interval 12 days (G3)	89.47±24.19[a]	1.00±0.17[a]

All cows subjected to ES showed typical symptoms such as swollen and red vulvas, mucus discharge, standing heat, restlessness, and decreased appetite. Physical changes in the vulva may be related to estradiol which tends to increase in the estrous phase although it is not statistically different (P>0.05). The aim of measuring total estradiol and progesterone during estrus is an important factor in the behavior of estrus manifestations in cows. Estradiol levels are measured to determine preovulatory quality, and progesterone measurements are conducted to predict ovulation (Mekonnin et al. 2017).

The concentration of estradiol on the day of estrus in Aceh cows was almost similar to those found in Bali cows with estradiol level was 69.80 pg/mL (Laksmi and Trilaksana 2020) and dairy cows with estradiol value was 60.06±33.25 pg/mL (Setyorini et al. (2023). However, Siregar et al. (2016b) reported that the concentrations of progesterone and estradiol in Aceh cows on the day of estrus were 0.12+0.02 ng/mL and 223.13±9.50 pg/mL respectively. Meanwhile, Setiawati et al. (2021) observed that Pasundan cattle injected with PGF2α showed an average plasma concentration of the hormone progesterone of 2.68±0.19 ng/mL and estradiol of 26.65±2.09 pg/mL. In other study, Siregar et al. (2017) reported that the progesterone concentration in Aceh cattle on day 5 after synchronization with PGF2α was 2.4±0.42 ng/mL

According to Astuti et al. (2020), estradiol levels in the estrus phase in the study after synchronization with PGF2α ranged from 40.06 pg/mL to 63.04 pg/mL. For progesterone, the lowest level was 0.16 ng/mL and the highest level was 0.32 ng/mL. In addition, research on other breeds of cows (Holstein) showed that estradiol and progesterone levels after administration of PGF2α were 16.14±15.6 pg/mL and 1.81±0.3 ng/mL, respectively (Kaya et al. 2017).

The administration of PGF2α, causes CL regression, thereby reducing progesterone followed by rapid maturation of the dominant follicle to increase estradiol levels, which causes an LH surge for ovulation (Siregar et al. 2016a; Bihon and Assefa 2021). These data provided information that steroid concentrations are not influenced by ES method factors with PGF2α, but are more influenced by factors such as breed, parity, nutrition, season, and others.

The relationship between estradiol concentration and estrus intensity in Aceh cattle has been studied by Ramli et al. (2016). The results of this study showed that there was no significant relationship between estrus intensity and estradiol concentration in Aceh cattle during AI. However, research on other cattle conducted by Astuti et al. (2020), revealed a moderate correlation between estradiol levels and estrus intensity with an R-value of 0.43. The results of other studies reported a high correlation between estrogen concentrations and visible signs of estrus such as rising and standing (Sumiyoshi et al. 2014). Rodrigues et al. (2018) stated that estrous expression is mainly determined by circulating estradiol concentrations, which trigger the hypothalamus to initiate estrous behavior. In turn, the intensity of estrus expression has been associated, albeit weakly, with preovulatory estradiol concentrations in lactating dairy cows (Madureira et al. 2015), while estrus expression is considered a biomarker for estradiol concentrations in cows (Larimore et al. 2015).

For the relationship of other steroids (progesterone) with estrus intensity, lower progesterone concentrations on the day of estrus were associated with increased intensity and duration of estrus expression (Madureira et al. 2021). Likewise, cows with lower intensity and shorter duration of estrous expression have higher progesterone concentrations and lower estradiol concentrations in estrus compared to cows with greater activity and longer duration of estrous behavior (Madureira et al. 2021). Meanwhile, Astuti et al. (2020) found a weak correlation between progesterone concentration during the onset of estrus and estrus behavior (r = 0.20). They were added by Madureira et al. (2021) that the concentration of progesterone around the time of AI is important for the expression of estrus for determining the timing of AI. Therefore, estrus intensity is associated with greater pregnancy per AI.

The mechanism of action of progesterone in its involvement in the expression of estrus is through increasing or decreasing regulation in the hypothalamus of several genes involved in estrus behavior through the estradiol receptor (Liu and Shi 2015). This suggests that

lower progesterone concentrations during diestrus are associated with lower fertility. Progesterone can block the estrus-stimulating action of estrogen and plays an important role in priming the cow's brain for estrogen function (Kommadath et al. 2013). Increased expression of estrus in timed AI protocols that include progesterone supplementation also suggests that progesterone may act as a primer for hypothalamic responsiveness to estrogen (Madureira et al. 2021). The limitation in our study was the small number of sample we used in this study. This was due to the limited number of samples in our experimental unit that met the desired criteria such cows with a good reproductive status, were not pregnant and had at least two regular cycles. This criteria is a common standard criteria for the synchronization of estrus in cattle with the use of PGF2α (Bihon and Assefa 2021).

In conclusion, administering PGF2α using the double injection method can increase the intensity of estrus in Aceh cows. However, both methods had no effect on estrus symptoms, estrus response, estrus onset, estrus duration, and steroid concentration. From the results of this study, it is recommended that PGF2α injection be carried out as a double injection to obtain clear estrus intensity so that AI timing will be more precise.

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REFERENCES

Abdullah MAN, Martojo H, Noor RR, Solihin DD. 2012. Genetic characterization of the Aceh cattle using phenotypic, mitochondrial DNA of D-loop region and microsatellite DNA analyses. Reprod Domest Anim 47 (1): 15-17. DOI: 10.1111/j.1439-0531.2011.01959.x.

Abebe B, Alemayehu M. 2021. Challenges and opportunities on estrus synchronization and mass artificial insemination in dairy cows for Smallholders in Ethiopia. Intl J Zool 2021: 9914095. DOI: 10.1155/2021/9914095.

Adam M, Gholib G, Hafizuddin H, Zamzami RS, Bahi M. 2019. Characteristic of ovarian and estradiol concentrations in the follicular fluid of slaughtered Aceh cattle. Jurnal Kedokteran Hewan -Indones J Vet Sci 13 (4): 71-75. DOI: 10.21157/j.ked.hewan.v13i4.12890.

Anton A, Dharmawan NS, Mahardika IG, Bebas W, Trilaksana IGNB, Bidura IGNG. 2019. Profile of estrogen and progesteron hormones in Bali cows that are exposed in the natural grazing filed. Intl J Fauna Biol Stud 6 (6): 28-33.

Astuti P, Airin CM, Widiyanto S, Sarmin S, Hana A, Maheshwari H, Sjahfirdi L. 2020. Estrus synchronization using prostaglandin F2α (PGF2α) and combination of PGF2α and gonadotropin-releasing hormone in Ongole crossbred. E3S Web Conf 151: 01009. DOI: 10.1051/e3sconf/202015101009.

Balumbi M, Supriatna I, Setiadi MA. 2019. Response and characteristics of estrous after estrous synchronization with cloprostenol in Friesian Holstein cow. Acta Vet Indonesiana 7 (1): 29-36. DOI: 10.29244/avi.7.1.29-36. [Indonesian]

Bihon A, Assefa A. 2021. Prostaglandin based estrus synchronization in cattle: A review. Cogent Food Agric 7 (1): 1932051. DOI: 10.1080/23311932.2021.1932051.

Consentini CEC, Wiltbank MC, Sartori R. 2021. Factors that optimize reproductive efficiency in dairy herds with an emphasis on timed artificial insemination programs. Animals 11 (2): 301. DOI: 10.3390/ani11020301.

Esterman RD, Alava EN, Austin BR, Hersom MJ, Rae DO, Elzo M, Yelich JV. 2016. Cloprostenol sodium and dinoprost tromethamine result in similar artificial insemination pregnancy rates in *Bos taurus*, *Bos indicus*, and *Bos indicus* × *Bos taurus* cattle synchronized with a Select Synch and CIDR plus timed–artificial insemination protocol. Prof Anim Sci 32 (5): 636-646. DOI: 10.15232/pas.2015-01493.

Gugssa T. 2015. Effects of prostaglandin administration frequency, artificial insemination timing and breed on fertility of cows and heifers in eastern zone of Tigray Region, Ethiopia. [Thesis]. Mekelle University, Mekelle.

Hafizuddin H, Siregar TN, Akmal M, Melia J, Rizal H, Armansyah T. 2012. Comparison of oestrous intensity between prostaglandin F2 alfa synchronized and the natural oestrous ones among Aceh cattle. Jurnal Kedokteran Hewan 6: 81-83. DOI: 10.21157/j.ked.hewan.v6i2.296. [Indonesian]

Islam R. 2011. Synchronization of estrus in cattle: A review. Vet World 4 (3): 136-141. DOI: 10.5455/vetworld.2011.136-141.

Jainudeen M, Hafez E. 2013. *Cattle and buffalo*. In: Hafez ESE, Hafez B (eds). Reproduction in Farm Animals. John Wiley & Sons, Hoboken.

Jinks EM, Smith MF, Atkins JA, Pohler KG, Perry GA, MacNeil MD, Roberts AJ, Waterman RC, Alexander LJ, Geary TW. 2013. Preovulatory estradiol and the establishment and maintenance of pregnancy in suckled beef cows. J Anim Sci 91 (3): 1176-1185. DOI: 10.2527/jas.2012-5611.

Kaya S, Kaçar C, Polat B, Çolak A, Kaya D, Gürcan IS, Bollwein H, Aslan S. 2017. Association of luteal blood flow with follicular size, serum estrogen and progesterone concentrations, and the inducibility of luteolysis by PGF2α in dairy cows. Theriogenology 87: 167-172. DOI: 10.1016/j.theriogenology.2016.08.022.

Kommadath A, Te Pas MFW, Smits MA. 2013. Gene coexpression network analysis identifies genes and biological processes shared among anterior pituitary and brain areas that affect estrous behavior in dairy cows. J Dairy Sci 96 (4): 2583-2595. DOI: 10.3168/jds.2012-5814.

Laksmi DNDI, Trilaksana IGNB. 2020. The change in external genital and estrogen level of Bali cattle during estrus. J Vet Anim Sci 3: 40-50. DOI: 10.24843/jvas.2020.v03.i01.p05.

Larimore EL, Amundson OL, Bird SL, Funnell BJ, Kruse SG, Bridges GA, Perry GA. 2015. Influence of estrus at fixed-time artificial insemination on early embryonic development in beef cattle. J Anim Sci 93 (6): 2806-2812. DOI: 10.2527/jas.2015-8892.

Liu X, Shi H. 2015. Regulation of estrogen receptor α expression in the hypothalamus by sex steroids: Implication in the regulation of energy homeostasis. Intl J Endocrinol 2015: 949085. DOI: 10.1155/2015/949085.

Madureira AML, Silper BF, Burnett TA, Polsky L, Cruppe LH, Veira DM, Vasconcelos JLM, Cerri RLA. 2015. Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cows. J Dairy Sci 98 (10): 7003-7014. DOI: 10.3168/jds.2015-9672.

Madureira AML, Burnett TA, Borchardt S, Heuwieser W, Baes CF, Vasconcelos JLM, Cerri RLA. 2021. Plasma concentrations of progesterone in the preceding estrous cycle are associated with the intensity of estrus and fertility of Holstein cows. PLoS One 16 (8): e0248453. DOI: 10.1371/journal.pone.0248453.

Mahmoud GB, Hussein H. 2019. Ram effect on estrus behavior, ovarian structure and steroid hormone levels in Ossimi ewes treated with prostaglandin F2α for estrus synchronization. Egypt J Anim Prod 56 (2): 87-92. DOI: 10.21608/ejap.2019.93001.

Mekonnin AB, Howie AF, Riley SC, Gidey G, Tegegne DT, Desta G, Ashebir G, Gebrekidan B, Harlow CR. 2017. Serum, milk, saliva and urine progesterone and estradiol profiles in crossbred (Zebu x Holstein Friesian) dairy cattle. Anim Husb Dairy Vet Sci 1 (3): 1-10. DOI: 10.15761/ahdvs.1000118.

Montaser AM, El-Desouky AM. 2016. Effect of dinoprost tromethamine, cloprostenol and d-cloprostenol on progesterone concentration and

pregnancy in dairy cattle. IOSR J Agric Vet Sci 9 (2): 64-67. DOI: 10.9790/2380-09216467.

Mukkun RRL, Yusuf M, Toleng AL, Sonjaya H, Hasrin. 2021. Effectiveness of estrous synchronization using prostaglandin (PGF2α) in Bali cows. IOP Conf Ser: Earth Environ Sci 788: 012138. DOI: 10.1088/1755-1315/788/1/012138.

Panjaitan B, Siregar TN, Hafizuddin H, Sayuti A, Adam M, Armansyah T, Syafruddin S. 2021. Comparison of the effectiveness of pregnancy diagnosis in Aceh cattle through measurement of interferon-tau and progesterone concentrations. Biodiversitas 22 (4): 1712-1716. DOI: 10.13057/biodiv/d220414.

Putro PP, Kusumawati A. 2014. Ovulatory follicular dynamics after estrus synchronization using prostaglandin F2α in dairy cows. Jurnal Sain Veteriner 32 (1): 22-31. DOI: 10.22146/jsv.5419. [Indonesian]

Ramadhana Q, Riady G, Hafizuddin H, Thasmi CN, Rahmi E, Sutriana A. 2022. Determination of estrus in Aceh cows based on the van Eerdenburg method. Ovozoa: J Anim Reprod 11 (3): 103-108. DOI: 10.20473/ovz.v11i3.2022.103-108.

Ramli M, Siregar TN, Thasmi CN, Dasrul D, Wahyuni S, Sayuti A. 2016. Relation between estrous intensity and estradiol concentration on local cattle during insemination. Jurnal Medika Veterinaria 10 (1): 27-30. DOI: 10.21157/j.med.vet.v10i1.4032. [Indonesian]

Randi F, McDonald M, Duffy P, Kelly AK, Lonergan P. 2018. The relationship between external auditory canal temperature and onset of estrus and ovulation in beef heifers. Theriogenology 110: 175-181. DOI: 10.1016/j.theriogenology.2018.01.001.

Reith S, Hoy S. 2018. Behavioral signs of estrus and the potential of fully automated systems for detection of estrus in dairy cattle. Animal 12 (2): 398-407. DOI: 10.1017/s1751731117001975.

Ribeiro ES, Bisinotto RS, Favoreto MG, Martins LT, Cerri RLA, Silvestre FT, Greco LF, Thatcher WW, Santos JEP. 2012. Fertility in dairy cows following presynchronization and administering twice the luteolytic dose of prostaglandin F2α as one or two injections in the 5-day timed artificial insemination protocol. Theriogenology 78 (2): 273-284. DOI: 10.1016/j.theriogenology.2012.01.012.

Rodrigues AD, Cooke RF, Cipriano RS, Silva LGT, Cerri RLA, Cruppe LH, Meneghetti M, Pohler KG, Vasconcelos JLM. 2018. Impacts of estrus expression and intensity during a timed-AI protocol on variables associated with fertility and pregnancy success in *Bos indicus*-influenced beef cows. J Anim Sci 96 (1): 236-249. DOI: 10.1093/jas/skx043.

Roelofs J, Van Erp-van der Kooij E. 2018. Estrus detection tools and their applicability in cattle: Recent and perspectival situation. Anim Reprod 12 (3): 498-504. DOI: 10.1016/j.theriogenology.2017.01.037.

Rosmaidar R, Handayani M, Fadillah F, Armansyah T, Siregar TN, Hafizuddin H, Husnurrizal H. 2021. The effect of red betel leaf (*Piper crocatum*) and moringa leaf extracts on endometritis levels in Aceh cows. Trad Med J 26 (3): 161-168. DOI: 10.22146/mot.64626.

Roza E, Aritonang SN, Susanti H, Sandra A. 2019. Synchronization of GnRH and PGF2α on estrus response, pregnancy, progesterone hormones in crossing of swamp buffalo and water buffalo in West Sumatra, Indonesia. Biodiversitas 20 (10): 2910-2914. DOI: 10.13057/biodiv/d201019.

Setiawati EN, Sumaryadi MY, Armelia V. 2021. Profile of progesteron, estrogen and pregnancy level of Pasundan cattle after estrus synchronization with prostaglandin and releasing gonadothropin hormone. Intl J Environ Agric Biotechnol 6 (1): 75-80. DOI: 10.22161/ijeab.61.10.

Setyorini YW, Kurnianto E, Sutopo S, Sutiyono S. 2023. Estradiol concentrations during estrous in dairy cattle and its association with pregnancy and genotype diversity. Jurnal Sain Peternakan Indonesia 18 (3): 120-126. DOI: 10.31186/jspi.id.18.3.120-126.

Siregar TN, Armansyah T, Panjaitan B, Gholib G, Herrialfian H, Sutriana A, Abidin Z, Reynaldi MA, Razak F, Artaliani Y, Yuswar Y. 2019. Changes in cervical mucus as an indicator of fertility in Aceh Cattle. Adv Anim Vet Sci 7 (4): 306-314. DOI: 10.17582/journal.aavs/2019/7.4.306.314.

Siregar TN, Hafizuddin H, Akmal M, Sayuti A, Aliza D, Melia J, Armansyah T, Syafruddin S, Panjaitan B, Adani DL. 2016a. Follicle dynamics of Aceh cattle during estrous cycle. Glob Vet 17 (5): 424-429. DOI: 10.5829/idosi.gv.2016.424.429.

Siregar TN, Melia J, Rohaya, Thasmi CN, Masyitha D, Wahyuni S, Rosa J, Nurhafni, Panjaitan B, Herrialfian. 2016b. Determining proportion of exfoliative vaginal cell during various stages of estrus cycle using vaginal cytology techniques in Aceh cattle. Vet Med Intl 2016: 3976125. DOI: 10.1155/2016/3976125.

Siregar TN, Wajdi F, Akmal M, Fahrimal Y, Adam M, Panjaitan B, Sutriana A, Daud R, Armansyah T, Meutia N. 2017. Embryonic death incidents due to heat stress and effect of therapy with gonadothropin releasing hormone (GnRH) in Aceh cattle. Vet Med Zoot 75 (97): 70-74.

Sönmez M, Demirci E, Türk G, Gür S. 2005. Effect of season on some fertility parameters of dairy and beef cows in Elazığ Province. Turk J Vet Anim Sci 29 (3): 821-828.

Stevenson JS, Phatak AP. 2010. Rates of luteolysis and pregnancy in dairy cows after treatment with cloprostenol or dinoprost. Theriogenology 73 (8): 1127-1138. DOI: 10.1016/j.theriogenology.2010.01.014.

Suastiningsih IGAK, Trilaksana IGNB, Puja IK. 2020. Evaluation of one shot and two shot prostaglandin injection on estrus response in Bali cattle. Intl J Vet Sci 9 (3): 409-412. DOI: 10.37422/ijvs/20.040.

Sumiyoshi T, Tanaka T, Kamomae H. 2014. Relationships between the appearances and changes of estrous signs and the estradiol-17β peak, luteinizing hormone surge and ovulation during the periovulatory period in lactating dairy cows kept in tie-stalls. J Reprod Dev 60 (2): 106-114. DOI: 10.1262/jrd.2013-119.

Sutarno S, Setyawan AD, Lymbery AJ. 2015. Genetic diversity of five Indonesian native cattle breeds at microsatellite loci. Asian J Anim Sci 9 (2): 57-64. DOI: 10.3923/ajas.2015.57.64.

Sutarno S, Zahrah S, Astirin OP, Herawati E, Setyawan AD. 2019. Genetic diversity of Ongole Grade, Aceh, and Sumbawa cattle based on polymorphism on ND-5 fragment mitochondrial DNA using PCR-RFLP technique. Biodiversitas 20 (3): 783-788. DOI: 10.13057/biodiv/d200324.

Syafruddin S, Adam M, Roslizawaty R, Siregar TN, Hafizuddin H, Panjaitan B, Armansyah T, Wahyuni S, Sayuti A. 2021. Utilization 2% lugol's solution to overcome the repeat breeding case of cows in Cot Madhi Village, Aceh Besar. Bull Community Serv 1 (2): 59-62. DOI: 10.24815/bulpengmas.v1i1.20108. [Indonesian]

Tschopp JC, Macagno AJ, Mapletoft RJ, Menchaca A, Bó GA. 2022. Effect of the addition of GnRH and a second prostaglandin F2α treatment on pregnancy per artificial insemination in lactating dairy cows submitted to an estradiol/progesterone-based timed-AI protocol. Theriogenology 188: 63-70. DOI: 10.1016/j.theriogenology.2022.05.019.