

Estrus Synchronization Using Prostaglandin F2 α (PGF2 α) and Combination of PGF2 α and Gonadotropin-Releasing Hormone In Ongole Crossbred

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Abstract. The response of estrus can be enhanced by providing PGF2 α and a combination of PGF2 α -GnRH so that the time of mating using Artificial Insemination (AI) can be held precisely. This study aims to compare the response of estrus synchronization using PGF2 α compare to PGF2 α -GnRH. Twenty female cattle Ongole Crossbreds, 4-6 years, BCS average of 3-4 were used in this research. Cattle were selected based on their luteal phase at the days of 7 to 18 by rectal examination. Then, all of the cattle were injected by cloprostenol® with a dose of 500 ug intrauterine. Ten of the animals were injected by 2.5 mL of Fertagyl® intramuscularly after 48 hours then continued observing and response estrus detection. The onset of estrus using PGF2 α was detected on 75.94 ± 0.78 , while 41.57 ± 28.40 hours for PGF2 α -GnRH. Using statistical, there was a significant difference ($P < 0.05$). Duration of estrus phase using PGF2 α was 24.65 ± 0.49 whereas PGF2 α -GnRH was 10.88 ± 7.45 hours. Using statistical methods, there was a significant difference ($P < 0.05$). It could be concluded that the use of PGF2 α was more effective for the duration and intensity of estrus, and a combination of PGF2 α -GnRH is more effective for stimulating estrus.

Keywords: estrous synchronization, Ongole crossbred, PGF2 α , GnRH.

1 Introduction

The use of prostaglandin F2 α hormone (PGF2 α) and combination of PGF2 α and GnRH for estrus synchronization in cattle are common, but how efficient if they were used only in Ongole crossbred which in luteal phase directly, and then associated with hormonal and estrus quality through scoring, clinical sign, duration and onset of estrus still did not understand scientifically. There are factors involving in the estrus synchronization success rate i.e cattle strain, breeding systems, feeding, and surrounding temperature.

Estrus synchronization has been efficiently benefiting the use of Artificial Insemination (AI). The practice is not only facilitating fixed time AI but also cutting down labor, calving period and estrus detection [1]. Those protocols have been proved able to induce 75 to 95% animals on the cycle to exhibit estrus within the 5 days. Time to Response depends on the stage of the follicular wave. Estrus can be detected by watching the clinical signs of estrus, although cows not always show oblivious clinical signs. This study aims to determine the response of estrus synchronization using preparations of PGF2 α compare to combination PGF2 α and GnRH in

crossbred Ongole regarding return to estrus, quality of estrus based on scoring, periods of estrus, level of estradiol and progesterone on estrus phase.

2 Materials and Methods

2.1 Animals Experimental and Estrus Synchronization

Twenty Ongole crossbred 4-6 years old with the average body weight of 250 – 300 kg and BCS of 3 – 4 (using 5-point scales) were used in this study. All of the livestock were maintained in the housing system at Wedomartani village, Yogyakarta. The cows were fed with around 10 percent body weight of green fodder and 1-1.5 Kg of concentrate. For efficiency time and budget, all of the animals were rectally palpated to determine the luteal phase during the days of 7 to 18. Then, all of the cattle were injected by PGF2 α (cloprostenol® with a dose of 500 ug intrauterine. After PGF2 α , ten of animals were then injected 2.5 mL of GnRH (Fertagyl®) intramuscularly after 48 hours and continued observing and response estrus detection.

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2.2 Clinical Signs of Estrus And Scoring of Estrus

Observation the visual and clinical signs of estrus would be determined by Hafizuddin *et al.* [2] with the criteria such as score 1: a few mucus discharge, lack of swelling, reddening and wet of vulva, being mounted by another female (homosexual behavior); score 2: moderate of restless, swelling, reddening and mucus discharge of vulva, homosexual behavior; score 3: restless, activity of homosexual behavior, swelling, reddening and mucus discharge of vulva were very clear. The scores of a behavioral sign displayed by each individual were then categorized into scores 1, 2 or 3.

2.3 Blood Withdrawn

Blood withdrawn was approximate of 5 mL has been done in a jugular vein when animals showed the estrus sign visually. The whole blood then is centrifuged with a speed of 3000 rpm for 20 minutes, then frozen at the -20°C until the hormone of estradiol and progesterone were assayed. The hormone was assayed by commercial ELISA kit with competitive binding methods.

3 Results and Discussion

3.1 Onset of Estrus

In this experiment, estrus synchronized has been done using two methods namely PGF2 α and PGF2 α -GnRH. The onset of estrus between the two groups has been shown in Table 1. This result showed that the onset of estrus in the intramuscular injection of PGF2 α was 75.94 ± 0.78 hours, whereas injection of PGF2 α -GnRH was 41.57 ± 28.40 hours after injection. Using statistical analysis there was a significant difference in the onset of estrus between two groups ($P < 0.05$). Intramuscular injection of PGF2 α treatment compare to the combination of PGF2 α -GnRH for estrus synchronization has a good response on the onset, clinical signs, and scoring of estrus (data have not to be shown). These results are the same as the previous study where a single prostaglandin injection was sufficient to synchronize both cows and heifers [3]. In detail, the calving interval reduced and estrus highly returns, however, the conception and delivery rates were poor [4]. In cattle, when dominant follicle has been grown up to > 10 mm in diameter after GnRH treatment, LH release and ovulation has occurred directly.

Table 1. Comparison onset of estrus between injection of PGF2 α and combination PGF2 α -GnRH

Estrus Synchronization	The onset of Estrus (hours)
PGF2 α	$75.94 \pm 0.78^*$
PGF2 α and GnRH	41.57 ± 28.40

*indicate a significantly different ($P < 0.05$)

PGF2 α treatment was aimed to induce Corpus Luteum (CL) regression whereas PGF2 α -GnRH were to induce CL regression and also inhibit ovulation following spontaneous CL regression [5]. Based on its application, the PGF2 α can be applied intramuscular or by direct administration to the uterine musculature. Both application methods have a similar effect. Malik et al [6] reported that estrus synchronization of Ongole crossbred using PGF2 α injection through intrauterine route produced similar estrus response and pregnancy rates with those that were synchronized intramuscularly. Corpus luteal regression mechanism by PGF2 α is currently described by abolishing steroid production of LH through blocking the luteal cell's receptor and interruption of multiple LH receptors intracellular mediators [7]. Even though PGF2 α luteolytic mechanism is still unknown, Superoxide Dismutase is likely to involve in CL degradation induced by PGF2 α in cow [8]. During much of pregnancy, the mechanisms that cause PGF secretion from the uterus in response to oxytocin are intact but luteolysis does not normally occur, perhaps due to lack of efficient utero-ovarian transfer of PGF [9].

3.2 Scoring of Estrus

Scoring of estrus has been done using the method of Hafizuddin *et al.* [2]. The result shows that both groups have a good response in a clinical sign of estrus such as discharge, red and swollen of the vulva with the grade of 2 and 3. Many roles of estrogen such as increases endothelial vasodilator function and thickening [10], promotes angiogenesis and modulates autonomic has been proven.

3.3 The length of the estrus phase

Besides the onset of estrus, the comparison length of the estrus phase could be shown in Table 2. Injection of PGF2 α has a longer period in estrus namely 24.65 ± 0.49 hours than the combination of PGF2 α -GnRH which only get 10.88 ± 7.45 hours ($P < 0.05$).

Table 2. Period stage of estrus between injection of PGF2 α and combination PGF2 α -GnRH

Estrus Synchronization	Period of Estrus (hours)
PGF2 α	$24.65 \pm 0.49^*$
Combination of PGF2 α -GnRH	10.88 ± 7.45

*indicate a significantly different ($P < 0.05$)

The PGF2 α -GnRH treatment gets a shorter time in the onset of estrus than PGF2 α ($P < 0.05$). On the other hand, based on the length of the estrus phase, the intramuscular injection of PGF2 α gets a longer period in the estrus phase. Nevertheless, other research showed that Jerseys exhibited a significantly shorter estrus duration and lower estrus strength compared to both Red Danes and Holsteins in a similar age [11,13]. Wenzinger and Bleul [12,14] reported all cows that responded to PGF given on day 5 ovulated on day 9, inducing a synchronized estrus. In the cows that did not respond on day 5, the luteal

cross-sectional area stagnated after treatment, whereas the plasma progesterone concentration continued to increase. During estrus, vulva arterioles expand, blood flow volume increases and caused hyperemia. The surrounding hyperemic part turns scarlet, becomes tense, enlarges and swells. Importantly, the low osmotic pressure of vulva and intravaginal tissue leads to the occurrence of edema due to capillaries dilatation causing blood volume in tissue and intravascular pressure to increase [13]. For dairy cows housed in a tie-stall barn, the highest scores of estrus signs usually appeared around the E₂ peak and the LH surge, and subsequently declined afterward [14].

Table 3. Comparison level of Estradiol, progesterone, and scoring during the stage of estrus

No	Synch-ronized	Progesterone (ng/mL)	Estradiol (pg/mL)	Scoring of estrus
1	PGF2 α	0.16	43.60	2
2	PGF2 α	0.25	50.07	2
3	PGF2 α	0.26	53.77	3
4	PGF2 α	0.29	50.53	3
5	PGF2 α	0.31	56.70	2
6	PGF2 α	0.28	52.67	2
7	PGF2 α	0.30	60.34	2
8	PGF2 α	0.32	63.04	2
9	PGF2 α	0.21	62.68	3
10	PGF2 α	0.36	40.06	2
11	GnRH	0.42	67.80	3
12	GnRH	0.19	40.46	3
13	GnRH	0.21	50.33	2
14	GnRH	0.25	53.78	3
15	GnRH	0.35	60.56	3
16	GnRH	0.16	41.50	2
17	GnRH	0.24	62.07	3
18	GnRH	0.22	43.91	2
19	GnRH	0.30	42.55	2
20	GnRH	0.27	43.59	2

3.4 Level Estradiol and Progesterone on the Estrus Phase

The level of Estradiol during the estrus phase in this study ranged from 40.06 pg/mL up to 67.80 pg/mL. For progesterone, the lowest level was 0.16 ng/mL and the highest level was 0.42 ng/m (Table 3 and Figure 1). There was a moderate coefficient correlation between the level

of estradiol and scoring of estrus with r value was 0.43. There was a weak correlation between the concentration of progesterone during the onset of estrus and the estrus behavior ($r = 0.20$).

Level estradiol and Progesterone on Estrous Phase

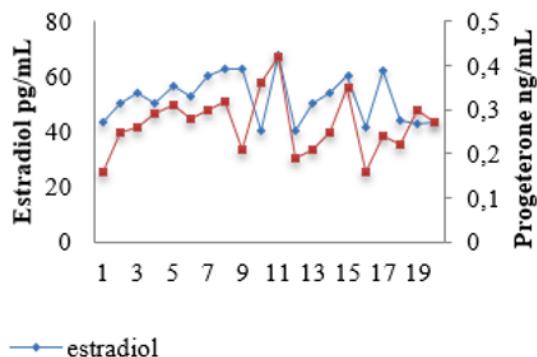


Figure 1. Level of estradiol and Progesterone during the stage of estrus

Mekonnin *et al.* [15] reported the aim of measurement total estrogen and progesterone during the estrus period are essential factors of behavioral manifestation of estrus in crossbred (Zebu and Holstein Friesian) dairy cattle, but then level Estradiol is to determine quality of preovulatory, and measurement of progesterone to predict the ovulation according to earlier research [16].

Furthermore, Sumiyoshi *et al.* [14] reported there was a high correlation between the estrogen concentration and the apparent estrus signs such as mounting and standing. The maximum score of the clinical sign as the same profile as the peak of estradiol and LH surge. Therefore, precise estrus detection and the right timing of insemination are imperative to attain a high conception rate in dairy animals [17]. In this experiment, it has been a similar result which moderate relationship between the level of estradiol and estrus scoring with $r: 0.43$. The correlation is not higher as previous research probably due to the breed of cattle or different types of estrus scoring. There was a slight correlation between level progesterone and estrus scoring with r value: 0.21. This is reinforced by Fortune *et al.* [18] who suggested that progesterone concentration itself is deficient to determine the ovulation period because of a varied timing of progesterone decline relative to ovulation among animals.

4 Conclusion

Injection of PGF2 α and PGF2 α -GnRH were efficient at the onset of estrus. Regarding return to estrus, a combination of PGF2 α -GnRH was a shorter time than PGF2 α ($P < 0.05$), on the other hand, injection of PGF2 α , has a long time of estrus periods than combination PGF2 α -GnRH ($P < 0.05$). Scoring of estrus has a moderate correlation with the level of estradiol probably due to the breed of cattle or different types of scoring which depend on visual appearance.

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