



## RESEARCH ARTICLE

### Comparison of Estrus Synchronization with Application of Prostaglandin F<sub>2</sub>α Intrauterine and Intramuscular in Bali and Crossbred-Ongole Cattle

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#### ABSTRACT

The objective of this study was to compare effects of intramuscular and intrauterine injection of prostaglandin F<sub>2</sub>α (PGF<sub>2</sub>α) on estrous synchronization in Bali and crossbred-Ongole cattle. A total of 80 Bali and 70 crossbred-Ongole (PO) cows were used for the study. The average body weights were 327±9.98 and 355±8.45 kg and were aged 4-8 years (parities 2 to 5) for Bali and PO cattle, respectively. The Bali and PO cows were divided into two treatment groups and to each cow, two injections of PGF<sub>2</sub>α, 14 days apart were administered. Cows in group 1 (Bali, n=40; PO, n=35) were injected with 25 mg Dinoprost® (Glandins, Tad Pharmazeutisches werk GmbH, West Germany) intramuscularly (i.m). Whereas, for the cows in group 2 (Bali, n=40; PO, n=35), 5 mg Dinoprost® was injected intra uterine (i.u) using a modified AI gun. The percentage estrus after first and second injection with i.m and i.u were non significantly different (P>0.05) between Bali and PO cows. The percentage estrus response was significantly different (P<0.05) between first injection and second injection. The pregnancy rate after first injection with i.m and i.u were non significantly different (P>0.05). Whereas, pregnancy rate after second injection with i.m and i.u between Bali and PO cows were significantly different (P<0.05). Thus, differences in breed and method of PGF<sub>2</sub>α administration were not significantly different (P>0.05). It was concluded that injection of PGF<sub>2</sub>α via the i.u route resulted in similar estrus response and pregnancy rates compared with the i.m route of administration. Furthermore, the i.u application of the PGF<sub>2</sub>α or its analogues may reduce the dose requirement of the drug.

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#### INTRODUCTION

There are two breeds of beef cattle in Indonesia namely Bali cattle and the crossbred-Ongole cattle called Peranakan Ongole (PO). The Bali cattle (*Bos sondaicus*), domesticated from *Bos banteng* in Java, have been reported to have higher reproductive performance than other indigenous Indonesian cattle. Bali cattle account for approximately 25% of the total cattle population in Indonesia (Lisson *et al.*, 2010). The population of Bali cattle is declining in most areas of eastern Indonesia because the demand for beef cattle exceeds the local capacity to supply these animals. The PO cattle are found in east and central Java, Indonesia. They were the outcome of cross-breeding between the Sumba-Ongole and Java-Ongole cattle breeds. The white coat color is dominant and they are also considered as a local breed in Indonesia (Martoyo, 2004).

To raise the reproductive efficiency of local cattle breeds, estrus synchronization has become a regular procedure to improve the useful reproductive life of livestock (Ruiz-Gonzalez *et al.*, 2012). Estrus synchronization and artificial insemination can also be used to optimize the reproductive potential of cows to get better pregnancy rate in the modern beef industry (Iqbal *et al.*, 2003). Furthermore, synchronization of estrus in cows facilitates the use of the fixed time artificial insemination (Kasimanickam *et al.*, 2009).

Several methods can be used for estrus synchronization in cattle (de Araujo *et al.*, 2002; Neglia *et al.*, 2003), including the use of Prostaglandin F<sub>2</sub>α (PGF<sub>2</sub>α) (Holm *et al.*, 2008). The use of prostaglandin F<sub>2</sub>α or its analogues as a luteolysin to block estrus and ovulation temporarily has been the most common method of estrus synchronization (Jainudeen *et al.*, 2000). The time taken for treatment with PGF<sub>2</sub>α to induced estrus is inconsistent,

but generally varies from 2 to 5 days in heifers (Wenzel, 1997). PGF<sub>2</sub>α or its analogues can cause regression of the corpus luteum (CL) in cows from day 5-6 until day 15-17 of the estrus cycle and can be used to synchronize estrus cycle from day 7 onwards (Holm *et al.*, 2008). Nevertheless, the effectiveness of PGF<sub>2</sub>α to synchronize estrus is dependent upon the presence of a responsive corpus luteum. The variation in time to estrus is due to the differences in the developmental stage of the pre ovulatory follicle at the time of prostaglandin F<sub>2</sub>α injection (Kastelic and Ginther, 1991; Macmillan *et al.*, 2003) and is not related to the rate of progesterone decrease to basal concentrations (Cirit *et al.*, 2008).

There are several different strategies to use PGF<sub>2</sub>α for estrus synchronization in cows, the strategy used in this study is called the 2-shot prostaglandin protocol. There are numerous reports about using reduced doses of PGF<sub>2</sub>α into various locations of the reproductive tract such as intravenous and intrauterine administration (Neglia *et al.*, 2008; Malik *et al.*, 2009). The PGF<sub>2</sub>α can be administered through intramuscular injection or injected directly into the uterine musculature. It is hypothesized that intrauterine injection of PGF<sub>2</sub>α and analogs for estrus synchronization would improve pregnancy rates. Therefore, the present study was designed to compare the effects of intrauterine and intramuscular injections of PGF<sub>2</sub>α on estrus synchronization pregnancy rate in Bali and PO cattle.

## MATERIALS AND METHODS

**Animals:** A total of 80 Bali and 70 PO cows were used for the present study. The average body weights were 327±9.98 and 355±8.45 kg, and aged 4-8 years (parities 2 to 5) for Bali and PO cattle, respectively. All the cows were 60-90 d postpartum and were cycling normally. Their body condition scores were 5-6 on a scale of 1 to 9 (1=emaciated, 9=obese; Roche *et al.*, 2009). This body condition score was subjectively given to females to describe overall body condition, fat cover and flesh over the ribs, loin and tail head. Non-pregnant status in these cows was confirmed based on farm records and rectal palpation. The ovary was also palpated for the presence of either follicles or an active corpus luteum. All the cows were raised under a similar grazing system (various kinds of grass) and supplemented with mixture of rice bran (byproduct of milling) at the rate of 1.5 kg/head/day.

**Experimental design:** The Bali and PO cattle were divided into two treatment groups for estrus synchronization. The first group was injected with intramuscular and second group was injected with intrauterine. Cows in group 1 (n=40 Bali; n=35 PO) were each given injections PGF<sub>2</sub>α intramuscularly (i.m) with 25 mg Dinoprost® (Glandins, Tad Pharmazeutisches werk GmbH, West Germany). Those in group 2 (n=40 Bali; n=35 PO) were injected with 5 mg Dinoprost® via intrauterine (i.u) by using a modified AI gun. The second injections of prostaglandin F<sub>2</sub>α was given at 14 days after the first injection with same of method.

**Estrus observation:** The cows were observed continuously in the paddocks for estrus onset and behavioral patterns of

the estrus every 6 h for 80 hours. All the cows were visually observed and standing to be mounted by another cow (homosexual mounting) was taken as an indication of estrus (Mattoni and Ouedraogo, 2000). Cows receptive to at least 2-3 mounts were considered to be in estrus. The percentage of estrus response was calculated by:

$$\text{Percentage of estrus} = \frac{\text{Number of cows in estrus}}{\text{Total number of cows synchronized}} \times 100$$

**Artificial insemination and pregnancy diagnosis:** All cows were artificially inseminated twice by an experienced technician using frozen Bali and PO semen obtained from the Artificial Insemination Centre (Singosari, Malang, Indonesia) at 70-75 hours after first injection (Cavalieri *et al.*, 2008). Cows that failed to exhibit estrus were given a second equal dose of prostaglandin F<sub>2</sub>α after 14 days and underwent timed AI after 70 -75 hours. Pregnancy diagnosis was conducted by rectal palpation 60 days after AI.

**Statistical analysis:** The proportions of cows that showed estrus after first and second PGF<sub>2</sub>α injections, as well as pregnancy rates were analyzed by separate Chi-square analyses and Frequency Procedure of SAS software version. 9.1.3 (SAS, 2006).

## RESULTS

The percentage estrus response in Bali and PO cows in groups 1 and 2 following the first i.m and i.u injections were 41% (i.m), 38% (i.u) and 45% (i.m), 43% (i.u), respectively (Fig. 1). The percentage estrus response after first and second injection were non significantly different (P>0.05) between Bali and PO cows. The estrus responses after the second PGF<sub>2</sub>α i.m and i.u injection were 80 & 84% and 83 & 78% in the Bali and PO cows, respectively. Thus, differences in breed and method of PGF<sub>2</sub>α administration were not significantly different (P>0.05). The percentage estrus response was significantly different (P<0.05) between first injection and second injection. The pregnancy rates among the cows that were inseminated after the first injection were non significantly different (P>0.05) irrespective of the method of PGF<sub>2</sub>α administration (i.m or i.u). Whereas, the pregnancy rates among the cows that were inseminated after the second injection were significantly different (P<0.05) between Bali and PO cows (Table 1). Pregnancy rates after first injection in Bali and PO cows were 55% (i.m), 50% (i.u) and 46% (i.m), 53% (i.u), respectively. Thus, route of administration did not result in significant differences between Bali and PO breed. Similarly, the pregnancy rate after the second PGF<sub>2</sub>α injection was non-significantly different (P<0.05) between the i.m and i.u routes of PGF<sub>2</sub>α administration.

## DISCUSSION

The percentage estrus response among Bali and PO cows after the first PGF<sub>2</sub>α i.m or i.u treatment was low compared with the second injection (Fig. 1). The percentage estrus response after the first injection is similar to 46.4% reported by Mattoni and Ouedraogo

(2000) for Indo Brazil and Gyrs cows that were estrus synchronized with PGF<sub>2</sub>α. This lower percentage estrus response was probably because the cows that were used in the experiment were in different phases of the estrous cycle at the time of first injection. It is known that the success of estrus induction with PGF<sub>2</sub>α depends on the presence of a functional corpus luteum. Smith *et al.* (2005) also reported that Prostaglandin F<sub>2</sub>α was effective only in the presence of a responsive corpus luteum (luteal phase). The percentage estrus response among the cows after first injection i.u. was similar compared with the i.m. route. These results are in agreement with Ginther *et al.* (2009), who found that intrauterine PGF<sub>2</sub>α injection in Holstein cows required lower amount of prostaglandin to induce luteolysis. The intramuscular injection of PGF<sub>2</sub>α requires a higher dosage because this hormone will first be absorbed into the circulation before reaching its target organ, which is the ovary. On the other hand, intrauterine PGF<sub>2</sub>α administration requires a lower dose, because the uterus is close to the target organ. Furthermore, the intrauterine route was effective in inducing increased CL blood flow, regardless of whether the treatment induced luteolysis (Malik *et al.*, 2009).

The higher percentage estrus response among cows after the second PGF<sub>2</sub>α injection in both the i.m and i.u routes suggests that most of the cows were in the same luteal phase of the estrus cycle. PGF<sub>2</sub>α or its analogues can be used to synchronize the cows estrus cycle from day 7 onwards (Holm *et al.*, 2008).

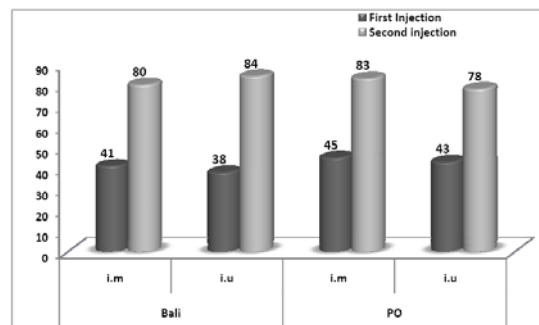
The pregnancy rates among Bali and PO cows after the first i.m and i.u administration were similar. Whereas, the pregnancy rates after the second i.m. and i.u administration in Bali cows were higher than PO cows. The low pregnancy rates after first injection in both cow breeds might be partially explained by the variation in time of ovulation with respect to time of AI. Much of the variation in time to ovulation was probably due to the variation in stage of growth of the preovulatory follicle at the time of PGF<sub>2</sub>α treatment (Olivera-Muzante *et al.*, 2011). The pregnancy rate after the second PGF<sub>2</sub>α injection in Bali cows (77.41%; i.m and 73.33%; i.u) was higher than the pregnancy rate in PO cows (54.54%; i.m and 60 %; i.u). The pregnancy rate following the second PGF<sub>2</sub>α administration in Bali cows was better than the 70.5% reported by Xu *et al.* (1997) in dairy cows. Willamson and Payne (1993) reported that Bali cattle have high adaptive capabilities to the tropical environment and have higher reproductive performance than other indigenous Indonesian cows. Intrauterine prostaglandins application may reduce the dose requirement of the drug. Furthermore, several authors have indicated that PGF<sub>2</sub>α may be transferred directly from the uterine vein into the ovarian artery (Smith *et al.* 2005; McCracken *et al.*, 1999).

**Conclusion:** Based on the data obtained in these experiments, it may be concluded that estrus synchronization with PGF<sub>2</sub>α injection using the intrauterine route resulted in similar estrus response and pregnancy rates to those with intramuscular administration. However, the pregnancy rate in Bali cows was higher than in the PO cows after the second PGF<sub>2</sub>α administration.

**Table 1:** Comparison of pregnancy rate after estrus synchronization in Bali and PO cows

| Breeds    | Application | n  | Dosage of PGF <sub>2</sub> α (mg) | Pregnancy rate (%) |                            |
|-----------|-------------|----|-----------------------------------|--------------------|----------------------------|
|           |             |    |                                   | First injection    | Second injection           |
| Bali Cows | i.m         | 40 | 25                                | (5/9) 55           | (24/31) 77.41 <sup>a</sup> |
|           | i.u         | 40 | 5                                 | (5/10) 50          | (22/30) 73.33 <sup>a</sup> |
| PO Cows   | i.m         | 35 | 25                                | (6/13) 46          | (12/22) 54.54 <sup>b</sup> |
|           | i.u         | 35 | 5                                 | (8/15) 53          | (12/20) 60.00 <sup>b</sup> |

<sup>a,b</sup>values in the same column with different superscripts indicate significant difference (P<0.05).



**Fig. 1:** Percentage response of estrus after estrus synchronization in Bali and PO cattle

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