

THE IMPROVEMENT OF SEMEN QUALITY AND TESTOSTERONE LEVEL OF BALI CATTLE AFTER PROSTAGLANDIN F2 α ADMINISTRATION

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ABSTRACT

This study aimed to determine the effect of prostaglandin F2 α (PGF2 α) administration on improving semen quality and testosterone levels on Bali cattle. This study used 3 Bali cattle aged 2, 3.5, and 5 years old. The sample used in this study was Bali cattle semen. In this study, the Latin Square Design was used with three different treatments were administered over three time periods. The treatments performed were P1 (5 mL physiological NaCl), P2 (25 mg prostaglandin), and P3 (37.5 mg prostaglandin) while treatment period was three weeks of treatment administration, which were 1st, 2nd, and 3rd week. Blood collected through coccygea vein 30 minutes after treatment to measure testosterone levels using enzyme-linked immunosorbent assay (ELISA) technique. Statistical analysis showed that PGF2 α administration at a dose of 25 mg and PGF2 α at a dose of 37.5 mg had no effect ($P>0.05$) on volume, color, pH, consistency, concentration, and motility of Bali cattle spermatozoa. The volume of semen obtained from P1; P2; and P3 were 6.5 \pm 0.9; 6.3 \pm 0.6; and 6.2 \pm 1.2 mL, respectively. The color of the semen in P1, P2, and P3 were beige and milky white. The pH in groups P1; P2; and P3 were 6.7 \pm 0.6; 7.1 \pm 0.2; and 6.5 \pm 0.2, respectively. Spermatozoa concentration in P1; P2; and P3 were 1,328 \pm 96.43 x 10⁶ cells/mL; 1,354 \pm 102.19 x 10⁶ cells/mL; and 1,353.3 \pm 88.55 x 10⁶ cells/mL, respectively. Spermatozoa motility in P1; P2; and P3 were 73.3 \pm 2.8%; 71.6 \pm 2.8%; and 73.3 \pm 2.8%, respectively. Testosterone levels in P1 and P3 were 5.05 \pm 0.22 and 6.74 \pm 1.38 ng/mL, respectively ($P<0.05$). Based on the results of this study, it was concluded that the administration of PGF2 α does not affect semen quality but can increase the level of testosterone on Bali cattle.

Key words: Bali cattle, PGF2 α , spermatozoa, testosterone

ABSTRAK

Penelitian ini bertujuan mengetahui pengaruh pemberian prostaglandin F2 α (PGF2 α) terhadap peningkatan kualitas semen dan level testosteron sapi bali. Hewan coba yang digunakan adalah sapi bali sebanyak 3 ekor yang berumur 2; 3,5; dan 5 tahun. Sampel yang digunakan pada penelitian ini adalah semen sapi bali. Penelitian ini menggunakan rancangan bujur sangkar latin dengan dengan tiga perlakuan dan tiga periode waktu perlakuan. Perlakuan yang dilakukan adalah P1 (5 ml NaCl fisiologis), P2 (25 mg prostaglandin), dan P3 (37,5 mg prostaglandin), sedangkan periode waktu adalah tiga minggu kegiatan perlakuan yakni minggu ke-1, 2, dan 3. Darah dikoleksi melalui vena coxygea 30 menit setelah perlakuan, untuk pemeriksaan kadar testosteron menggunakan teknik enzyme-linked immunosorbent assay (ELISA). Berdasarkan hasil uji statistik menunjukkan bahwa pemberian PGF2 α dengan dosis 25 mg dan PGF2 α dosis 37,5 mg tidak berpengaruh ($P>0,05$) terhadap pemeriksaan volume, warna, pH, konsistensi, konsentrasi dan motilitas spermatozoa sapi bali. Volume semen yang diperoleh pada P1; P2; dan P3 masing-masing adalah 6,5 \pm 0,9; 6,3 \pm 0,6; dan 6,2 \pm 1,2 ml. Warna semen antara P1, P2, dan P3 adalah krem dan putih susu. Derajat keasaman (pH) pada P1; P2; dan P3 masing-masing adalah 6,7 \pm 0,6; 7,1 \pm 0,2; dan 6,5 \pm 0,2. Konsentrasi spermatozoa pada P1; P2; dan P3 masing-masing adalah 1.328 \pm 96,43 x 10⁶ sel/ml; 1.354 \pm 102,19 x 10⁶ sel/ml; dan 1.353,3 \pm 88,55 x 10⁶ sel/ml. Motilitas spermatozoa pada P1; P2; dan P3 masing-masing adalah 73,3 \pm 2,8%; 71,6 \pm 2,8%; dan 73,3 \pm 2,8%. Level testosteron pada P1 dan P3 masing-masing adalah 5,05 \pm 0,22 dan 6,74 \pm 1,38 ng/ml ($P<0,05$). Dari hasil penelitian ini dapat disimpulkan bahwa pemberian PGF2 α tidak memengaruhi kualitas semen namun dapat meningkatkan level testosteron sapi bali.

Kata kunci: sapi bali, PGF2 α , spermatozoa, testosteron

INTRODUCTION

The rapid development of reproductive biotechnology in animal husbandry is essential to obtain genetic engineering results which can later be used to improve the quality and quantity of livestock products and to support reproductive biotechnology. One way to improve genetic quality and livestock productivity is through the application of reproductive biotechnology, namely artificial insemination (AI).

The percentage of motility and morphology of spermatozoa are important criteria in evaluating sperm quality before it is used for insemination. Motility and morphology of spermatozoa are some of the factors that determine the success of fertilization. If the quality of ejaculation was not properly checked, economic losses

can arise in the regulation and procedures for implementation of artificial insemination (Ostermeier *et al.*, 2000). Directorate General of Animal Husbandry (2006) stated that the quality of frozen semen is influenced by several factors including the quality of males that produce spermatozoa, the method of producing frozen semen, and the handling of frozen semen until the implementation of artificial insemination in the field.

The success of artificial insemination programs in cattle is very much dependent on the quality and quantity of semen produced by male cattle. Efforts to increase the number of spermatozoa will be able to increase the amount of frozen semen. The use of PGF2 α in cattle, sheep, pigs, and horses has been shown to improve the quality of spermatozoa (Hess,

2002). In dogs, administration of PGF2 α does not affect the quality of spermatozoa (Traas and Kustritz, 2004). The difference in results is due to differences in animal types and is also thought to be influenced by differences in breeds or strains (Bard, 1975).

Several researchers claimed that the administration of PGF2 α also provides very significant benefits in increasing testosterone levels. Kissler *et al.* (1976) and Saifudini *et al.* (2005) reported an increase in cow and sheep testosterone levels after being induced with PGF2 α . One function of testosterone is to affect the maturation of spermatozoa. Low testosterone levels can cause a decrease in the quality of spermatozoa because (Cornwall, 2009). To date, there have been no reports of the effect of PGF2 α administration on semen quality and Bali testosterone levels.

MATERIALS AND METHODS

This study was conducted at Regional Artificial Insemination Office (Balai Inseminasi Buatan Daerah/BIBD) Bengkulu Province. The experimental animals used were 3 Bali cattle aged 2, 3.5, and 5 years old. The sample used in this study was Bali cattle semen.

In this study, the Latin Square Design was used with three different treatments were administered over three time periods. The treatments performed were P1 (5 mL physiological NaCl), P2 (25 mg prostaglandin, LutalyseTM, dinoprost tromethamin 5 mg/mL, Pfizer Manufacturing, Belgium NV/SA Puurs-Belgium), and P3 (37.5 mg prostaglandin) while treatment period was three weeks of treatment administration, which were 1st, 2nd, and 3rd week. The treatment activities are presented in Table 1.

Cow in P1 injected with 5 mL of physiological NaCl was the control, whereas cow in P2 and P3 were injected with PGF2 α 30 minutes before sampling. Sampling was conducted twice a week, on Mondays and Thursdays. Semen sampling was performed using an artificial vagina by an experienced collector.

Spermatozoa examination includes macroscopic and microscopic examinations to evaluate volume, concentration, and motility.

Observation of Spermatozoa Motility

The motility of individual spermatozoa can be observed using a microscope with a magnification of 400x. A thin layer of semen was placed on the object glass which was then covered with cover glass. Criteria for sperm motility according to Susilawati (2012) are as follows: 0%= immotile spermatozoa; 50%= rotating spermatozoa, less than 50% move progressively and not wavy; 50-80%= spermatozoa move progressively and produce mass movements; 90%= progressive movements that are nimble and form waves; 100%= very progressive movements and very fast waves.

Observation of Spermatozoa Concentration

The evaluation of spermatozoa concentration was carried out using a hemocytometer pipette and Neubauer chamber. Semen was withdrawn using hemocytometer pipette to a scale of 0.5 then diluted with 3% NaCl solution until it reached 1.01. The solution in the pipette was shaken for 2-3 minutes until homogeneous, and a few drops was discarded. The semen was subsequently dropped onto the Neubauer chamber which was covered with a glass cover, then observed with light microscope at 400x magnification. Spermatozoa contained in five squares with a diagonal direction were counted. The spermatozoa number in five squares which contained 80 small squares in it multiplied by 10⁷ per mililiter (Moussa *et al.*, 2002; Susilawati, 2011).

Blood Sampling

Blood samples were collected just before semen collection. 3 mL of blood was collected without anticoagulants from the jugular vein using a 3 mL syringe. The collected blood was then placed into a centrifuge tube and allowed to stand for 60 minutes until it clots and the serum was subsequently separated.

Table 1. Treatment activities

Week	Treatment		
	5 mL Physiological NaCl (P1)	25 mg PGF2 α (P2)	37.5 mg PGF2 α (P3)
I	A	B	C
II	C	A	B
III	B	C	A

A= Cow 1; B= Cow 2; C= Cow 3

Table 2. The average volume (\pm SD), color, pH, consistency, spermatozoa concentration, spermatozoa motility, and testosterone levels in Bali cattle injected with PGF2 α different doses

Semen criteria	Treatment		
	Control (P1)	25 mg PGF2 α (P2)	37,5 mg PGF2 α (P3)
Volume	6.5 \pm 0.9 ^a	6.3 \pm 0.6 ^a	6.2 \pm 1.2 ^a
Color	Beige	Beige	Milky white
pH	6.7 \pm 0.6	7.1 \pm 0.2	6.5 \pm 0.2
Consistency	Thick	Thick	Thick
Concentration (10 ⁶ cells/mL)	1,328 \pm 96.43	1,354 \pm 102.19	1,353.3 \pm 88.55
Motility	73.3 \pm 2.8	71.6 \pm 2.8	73.3 \pm 2.8
Testosterone concentration (ng/mL)	5.05 \pm 0.22	-	6.74 \pm 1.38

Afterwards, the tube was centrifuged at a speed of 1200 rpm for 10 minutes. Serum was then taken and placed into a microtube and stored in the freezer at a temperature of -20°C (Gholib *et al.*, 2016). Specifically for blood samples, there were only two groups because blood samples prepared from P2 was damaged.

Testosterone Analysis

Hormone analysis was carried out following the procedure from the testosterone catalog. A standard solution of 0.2 ng/mL to 16 ng/mL was prepared. 25 μL of both samples and prepared standard solutions were poured into the microplate wells. Subsequently, 200 μL of the conjugate enzyme was added to each well and the mixtures were incubated for 60 minutes at room temperature. After incubation, the microplate was washed three times using 300 μL of washing solution in each well. Afterwards, 200 μL substrate solution was added to each well. The plates were incubated for 15-20 minutes at room temperature. Enzyme reaction was stopped using stop solutions of 100 μL 0.5 M H_2SO_4 to each well and absorbance reading was obtained using enzyme-linked immunosorbent assay (ELISA) reader (Pratomo and Yudi, 2016).

Data Analysis

Data on motility and spermatozoa concentration were analyzed using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The results of examination on volume, color, pH, consistency, concentration, and motility of spermatozoa from Bali cattle are presented in Table 2. The volume of Bali cattle semen P1, P2, and P3 obtained after ejaculation were 6.5 ± 0.9 , 6.3 ± 0.6 , and 6.2 ± 1.2 , respectively. The results of semen volume examination showed that the control treatment had a higher volume compared to the PGF2 α treatment groups but all volumes obtained in this study were still in the normal range. This was in accordance to Toelihere (1993) who stated that the volume of semen of Bali cattle was 1-15 mL. Feradis (2010) also explained that the semen volume of Bali cattle ranges from 5-8 mL.

The semen color of the three Bali cattle were normal, they were beige, beige and milky white. This was in accordance with the opinion of Nursyam (2007) and Feradis (2010) who stated that normal cow semen is milky white or beige and whitish. Fresh Bali cattle semen has a distinctive odor criteria, the same was conveyed by Toelihere (1993) who stated that male cows produce semen with characteristic odor. Normal color of cow semen is milky white or whitish and turbid. Approximately 10% of cows produce normal semen with a yellowish color caused by riboflavin carried by an autosomal recessive gene and which has no effect on fertility (Feradis, 2010).

The results of acidity examination for P1, P2, and P3 groups were 6.7 ± 0.6 , 7.1 ± 0.2 , and 6.5 ± 0.2 respectively. P2 group with 25 mg PGF2 α treatment had higher result

than other treatments, but the results obtained were still within the normal range. According to Butar (2009), the pH of fresh semen is 6.8-7.8. Toelihere (1993) also explained that the pH can be maintained in a range from 5-10 to maintain partial motility.

The consistency of fresh semen obtained in this study was thick with a concentration of spermatozoa for P1, P2, and P3 groups $1.328\pm 96.43 \times 10^6$, $1,354\pm 102.19 \times 10^6$, and $1,353.3\pm 88.55 \times 10^6$ cells/mL respectively. This was in accordance with the opinion of Feradis (2010) who stated that the consistency of thick cow semen contains 1,000-2,000 spermatozoa cells per mL. Bali cattle spermatozoa concentration in this study was much higher than the results of the examination conducted by BBIB Singosari, which was 946.70 ± 198 cells/mL.

Statistical analysis showed that the administration of 25 mg of PGF2 α and 37.5 mg of PGF2 α had no effect ($P>0.05$) on the volume, color, pH, consistency, concentration and motility of Bali cattle spermatozoa. Azawi *et al.* (2011) reported that the administration of PGF2 α at a dose of 7.5 mg, 3 hours before collecting semen did not improve semen characteristics in Awasi sheep. In line with Hafs *et al.* (1974) and Hashizume and Niwa (1984), they found no effect or improvement in semen characteristics after PGF2 α administration in rabbits, cows and pigs. Capitan *et al.* (1990) concluded that PGF2 α prostaglandin injection resulted in a non-significant increase in semen volume, sperm concentration and sperm count per ejaculation in buffalo.

The administration of PGF2 α significantly increased semen characteristics after 60 or 90 minutes of collection especially by increasing sperm consistency, sperm concentration, sperm count per ejaculation, mass motility, individual motility and percentage of viable sperm. The optimal interval between prostaglandin PGF2 α injection and ejaculation is around 30-60 minutes. This confirmed the report of Olfati *et al.* (2013) which concluded that the best administration interval was 30 minutes while Capitan *et al.* (1990) showed that some semen characteristics of the treatment group and posttreatment group (1 mg prostaglandin PGF2 α) increased significantly at 1 hour compared to the control group.

The study result showed that administration of PGF2 α could increase testosterone levels of Bali cattle. According to the Kissar *et al.* (1976) and Saifudini *et al.* (2005), administration of PGF2 α can increase testosterone levels in cattle and sheep. Titiroongruang *et al.* (2011) stated that PGF2 α injection on Friesian Holstein (FH) male cows could increase the total number of spermatozoa per ejaculation, increase semen production, and slightly increase testosterone levels without a negative effect on spermatozoa quality. Masoumi *et al.* (2011) also stated that PGF2 α injection increased libido and semen quality.

The mechanism of increased testosterone due to PGF2 α administration is likely through direct action on the testes (Haynes *et al.*, 1975), through stimulation of cyclic AMP production in the testes. Cyclic AMP activation then stimulates testosterone synthesis

(Reichard *et al.*, 1978). Azawi *et al.* (2011) stated that the increase in testosterone by administering PGF2 α is thought to be caused by direct stimulation carried out on the testes during steroidogenesis. According to Reichard *et al.* (1978), PGF2 α stimulates cyclic AMP in the seminiferous tubules and interstitial cells in the reproductive testicular organs, which then stimulates testosterone synthesis.

CONCLUSIONS

Based on the results of this study, it can be concluded that the administration of PGF2 α hormone had no effect on semen quality but can increase testosterone level in Bali cattle.

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