Research Article

The Effect of Time Interval Differences Between the Prostaglandin F2α Injection and Semen Collection on the Improvement of Spermatozoa and Testosterone Concentrations in Aceh Bull

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Abstract | The injection of prostaglandin F2 alpha (PGF2α) has been adopted as an alternative to improve sperm quality. The difference in sample collection time after administration is assumed to have a positive effect on testosterone concentration. This research aimed to determine the effect of PGF2α injection and the optimal time for sample collection on improving semen quality and testosterone concentrations in Aceh bull. Aceh bull was used in this study and allotted into two treatments. In treatment 1 (P1), the bull received 25.0 mg PGF2α (Lutalyse™, dinoprost, tromethamine) 1 hour before semen collection, while in treatment 2 (P2), the semen collection was carried out 2 hours after PGF2α administration. The treatment was performed five times in alternate weeks. Semen collection was performed using an artificial vagina once a week for ten weeks. Testosterone concentrations were analyzed using the enzyme-linked immunosorbent assay (ELISA) method. The average spermatozoa volume (mL); concentration (10^6 cells/mL); and testosterone concentration (ng/mL) in P1 vs. P2, were 4.82±1.93 vs. 4.92±2.45; 889.0±250.50 vs. 721.0±246.25 ($P<0.05$); and 8.352.34 vs. 9.60±2.24 ($P>0.05$), respectively. It is concluded that the PGF2α injection 1 hour prior to the collection was able to increase the spermatozoa concentration compared to the 2 hours of treatment, while the value obtained for testosterone was not affected.

Keywords | Aceh bull, ELISA, PGF2α, Sperm, Testosterone, Time interval

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INTRODUCTION

The quality of spermatozoa is known to be influenced by testosterone level. This is an essential reproductive steroidal hormone in males derived from cholesterol precursor molecules and secreted by Leydig cells under the influence of luteinizing hormone (L.H.) (Sherwood, 2015). In addition, low amounts of testosterone have been associated with the decline in spermatozoa quality, due to the effect on its maturation (Cornwall, 2009). The research

conducted by Rachmawati *et al.* (2014) proved the existence of a positive correlation of the level of testosterone with libido and sperm quality.

One of the methods used to enhance the volume and concentration of spermatozoa is by administering prostaglandin F2α (PGF2α) hormone. However, the use of PGF2α in increasing the spermatozoa quality is still debatable. Hess (2002) proved that PGF2α administration could improve the spermatozoa quality in cattle, sheep, pigs, and horses. Olfati *et al.* (2013) also reported the PGF2α ability to work directly on muscle tissue contraction in the scrotum and epididymis, which is associated with the increased release of spermatozoa to the ductus deferens. In contrast, Traas and Kustritz (2004) observed that PGF2α injection did not affect the spermatozoa quality in dog.

According to Barwary *et al.* (2013), the optimum interval between the injection of PGF2α and ejaculation is 30-60 minutes. In addition, Capitan *et al.* (1990) collected semen after one hour, and at seven days after the last dose (post-treatment), while Masoumi *et al.* (2011) obtained the sample after 30 minutes. Therefore, the quality of spermatozoa was reported to have not improved (Capitan *et al.*, 1990), which was contrary to the results obtained by Masoumi *et al.* (2011).

The increase in the time interval between the administration of PGF2α and semen collection is assumed to have a positive effect on testosterone concentration. According to Haynes *et al.* (1975), the administration of 30 mg PGF2α enhances the concentration of testosterone in plasma. Kiser *et al.* (1976) also reported tendency for a sustained elevation of testosterone during 300 minutes post-injection of 20 mg PGF2α. Furthermore, Hess (2002) stated that PGF2α administration influenced spermatozoa quality and testosterone hormone 40-50 minutes after injection and lasted for 8 hours, while Armansyah *et al.* (2018) noted an increase in testosterone concentration in Kacang goat two days after PGF2α injection. However, Mulkan (2018) did not observed the increase of testosterone concentration in Aceh bull when the blood was collected 30 minutes after PGF2α injection. To date, the information regarding the optimal time for PGF2α injection before semen collection, which can improve the quality of sperm and testosterone in Aceh bull, is not available. Therefore, the objective of this study was to determine the effect of PGF2α injection and the optimal time for sample collection on improving semen quality and testosterone concentrations in Aceh bull.

MATERIALS AND METHODS

Experimental animals

The animal used in this study was a 3-year-old Aceh bull, which served as a semen donor at the Technical Implementation Unit of Experimental Animals, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia. The bull was allotted into two treatments. In treatment 1 (P1), the bull received 25.0 mg PGF2α (Lutalyse™, dinoprost, thromethamin) 1 hour before semen collection, while in treatment 2, the semen collection was carried out 2 hours after PGF2α administration. The treatment was performed five times on alternate weeks for ten weeks. Semen collection was performed using an artificial vagina once a week for ten weeks (Husnurrizal *et al.*, 2023).

Spermatozoa volume

Semen was collected using an artificial vagina, and the semen volume was immediately evaluated. Semen volume is assessed using a tube that has a size label (mL) (Hafizuddin *et al.*, 2021).

Spermatozoa concentration

Spermatozoa concentrations were calculated using Neubauer counting chamber. The semen was sucked up to a scale of 0.5 and made up to 101 with the addition of 3% NaCl solution, homogenized by swinging it gently for 2-3 minutes. Subsequently, a few drops of the solution were removed, and the sample loaded into the Neubauer counting chamber, mounted with cover glass, and the spermatozoa were counted in 5 squares. The concentration obtained was expressed as Y x 10^6 (Y = number of spermatozoa in 5 chambers) (Sutriana *et al.*, 2022; Syafruddin *et al.*, 2020).

Blood collection

The blood collection was conducted 10 times for 10 weeks with a withdrawal interval was 1 week. A total of 10 mL blood was withdrawn from jugular vein using 10 mL syringe, then transferred to sterile tube without anticoagulant, and left at room temperature for 60 minutes. The blood was then centrifuged for 10 minutes at 3000 rpm, and the blood serum obtained was stored at -20° C until further analysis (Husnurrizal *et al.*, 2021; Panjaitan *et al.*, 2021).

Analysis of testosterone

Testosterone hormone analysis was performed following the procedure in the testosterone catalogue (ELISA DRG testosterone ELISA Kit, DRG Instruments GmbH, Germany). Testosterone measurements were conducted following the manufacturer's instructions, as described by (Hafizuddin *et al.*, 2023). A total of 25-µl of samples and standard solutions were loaded into microplate well, and added with 200-µl of the conjugate enzyme. The mixture was softly homogenized for 10 seconds and incubated for 60 minutes at room temperature. The mixture was washed three times with 300-µl of the washing solution using a microplate strip washer tool, before adding 200-µl- tetra methyl benzine (substrate solution) into each well. The

microplate was covered with cling film and left for 15-20 minutes at room temperature. A total of 100-µl 0.5 M H_2SO_4 was added into each well to stop the reaction. The absorbance was read with ELISA reader at 450 nm wavelength.

Data analysis

All data were presented as mean ± standard deviation. The comparison between treatment groups (P1 vs P2) were analyzed using independent-sample T test, p-value ≤ 0.05 considered significant (Ramadan *et al*., 2023).

RESULTS AND DISCUSSION

Data of semen volume, spermatozoa concentration and testosterone concentration evaluation in Aceh bull after administered with PGF2α with different time of semen collection are presented in Table 1.

Table 1: The mean (± S.D.) of semen volume, spermatozoa concentration, and testosterone concentration in Aceh bull after administered PGF2α with different time of semen collection.

Parameters	Treatments	
	P1	P2
Volume (mL)	4.82±1.93[a]	4.92±2.45[a]
Spermatozoa concentration (10^6cell/mL)	889.0±250.50[a]	721.0±246.25[b]
Testosterone concentration (ng/mL)	8.35±2.34[a]	9.60±2.24[a]

[a, b]Different superscripts in the same row indicate significant differences (P<0.05). P1: Semen was collected 1 hour after PGF2α injection. P2: Semen was collected 2 hours after PGF2α injection.

This study showed that the concentration of spermatozoa in P1 and P2 was significantly different (P<0.05) with the values were 889.0±250.50 (10^6 cells/mL) and 721.0±246.25 (10^6 cells/mL), respectively.

It was reported that the range of normal value for spermatozoa concentration in adult bulls was 800.0-1,200 million/mL (Cornwall, 2009) and 800.0-2,000 million (Nur, 2019). The present finding indicated that the longer interval between PGF2α injection and semen collection, the lower concentration of spermatozoa. Armansyah *et al.* (2018) observed that the PGF2α injection did not have any effect on the spermatozoa concentrations of Kacang goats and it was assumed due to the long interval between injection and collection (two days interval).

Generally, the injection of PGF2α in Aceh bull has been known could improve the quality of spermatozoa, although the differences between PGF2α injection and semen collection time lead to variable results (Sari *et al*., 2021). However, the present study showed that the PGF2α injection did not significantly affect (P>0.05) the semen volume of Aceh bull. Similarly, Sari *et al.* (2019) reported that the PGF2α administration at a dose of 25.0 mg and 37.5 mg did not affect the volume, color, pH, consistency, concentration and motility of spermatozoa in Bali cattle. Capitan *et al.* (1990) also observed the absence of a significant increase in semen volume, sperm concentration and amount per ejaculation in buffalo. In contrast, Estienne and Harper (2004) established a dose dependent correlation between the injection of PGF2α in male animals with early libido, genetic, species, and animal age. The variations results are possibly due to the difference in animal type and breed used in different study.

The mechanism of sperm volume and concentration elevation due to PGF2α administration occurs indirectly or directly. The direct mechanism involves the contraction of smooth muscle around the epididymis. The PGF2α receptors are mostly present in the distal part of epididymis, thus, the changes in PGF2α concentrations will increase its sensitivity (Hafs *et al*., 1974). Furthermore, endogenous PGF2α confer a more significant influence on the caudal epididymis, a storage place for mature spermatozoa, than other segment. When caudal epididymis contracts as a response to PGF2α injection, it will force a mature spermatozoa move towards the ducts deferens for ejaculation. A similar condition has been reported by Hafs *et al.* (1974), which stated that the injection of PGF2α in anaesthetized rabbits cause spermatozoa redistribution from the epididymis to the ductus deferens. PGF2α also stimulates testicular capsule contraction (Henney *et al*., 1990), which plays an important role in elevating the number of spermatozoa (Masoumi *et al*., 2011).

The testosterone concentration in P1 and P2 treatments did not show significant difference (P>0.05), with average values of 8.35±2.34 ng/mL and 9.60±2.24 ng/mL, respectively. These values were lower than the previous finding that was performed on the same cow breed (Sari *et al*., 2021), with the average value before (control) and after PGF2a treatment was 28.07±14.37 and 24.19±6.11 ng/mL, respectively. However, these values were higher than the previous finding that was performed in Bali bull (Sari *et al*., 2019), with the average value before (control) and after PGF2a treatment was 5.05±0.22 and 6.74±1.38 ng/mL, respectively. The differences might be attributed to the cow's condition during treatment and testosterone fluctuations in livestock during the examination, which was affected by the physiological conditions. However, the testosterone concentrations recorded in this research were within the normal range observed in bull serum (6.37 13.70 ng/mL), as reported by Ismaya (1975).

The testosterone concentration in treatment P2 was relatively higher than P1, although not significantly difference (P>0.05). In week I; II; III; IV; and V, the values recorded in P1 were 10.42; 6.4; 6.33; 7.29; and 11.3 ng/mL, while in P2, were 9.98; 11.26; 6.92; 12.11; and 7.7 ng/mL, respectively. The effect of collection time following the administration of PGF2α did not vary greatly. This is in line with the study by Kiser *et al.* (1976), which demonstrated that the increase of testosterone lasts for 300 minutes after PGF2α α injection. However, Mulkan (2018) did not observed the increase of testosterone 30 minutes after PGF2α injection. Moreover, Armansyah *et al.* (2018) reported the occurrence of testosterone elevation within two days after PGF2α injection.

The difference in the increase in testosterone concentration after administration of PGF2α may be related to the difference in the increase in cyclic adenosine monophosphate (cAMP). The PGF2α hormone works directly in the process of forming testosterone in Leydig cells. The PGF2α hormone stimulates the formation of cAMP which is a ring-shaped molecule made from ATP which is a common intracellular signaling molecule (second messenger) in eukaryotic cells, such as in vertebrate endocrine cells. cAMP catalyzes the synthesis of protein kinase A, which is required to carry cholesterol from the cytoplasm to the mitochondria. Steroidogenic acute regulatory protein (StAR) and peripheral benzodiazepine receptor (PBR) carry cholesterol from the outer mitochondrial membrane to its inner membrane (Azhar *et al.*, 2020; Haider, 2004). An increase in cAMP concentration should precede an increase in testosterone concentration. Therefore, the choice of time 1 or 2 hours after administration of PGF2α is thought to not be able to increase testosterone because at the same time the cAMP concentration has not increased significantly.

The results of this study showed that sperm concentration was higher in P1 compared to P2, while testosterone concentration tended to be the same (P>0.05). These results prove that the increase in spermatozoa quality is not influenced by testosterone concentration. These results support the differences in results obtained by previous researchers (Armansyah *et al.*, 2018; Sari *et al.*, 2019). Injection of PGF2α two days before semen collection in Kacang goats can increase testosterone concentration although it is not followed by a significant increase in spermatozoa quality (Armansyah *et al.*, 2018). The same thing was reported by Sari *et al.* (2019) in Bali cattle. However, the opposite happened in Aceh cattle, that administration of PGF2α could improve the quality of spermatozoa which was not accompanied by an increase in testosterone concentration (Sari *et al.*, 2021).

CONCLUSIONS AND RECOMMENDATIONS

Based on the result and discussion, it was concluded that PGF2α injection 1 hour prior to semen collection possesses the capacity to increase the quantity of spermatozoa, compared to those injected 2 hours earlier, while testosterone concentration was not affected by the time interval difference of semen collection after PGF2α injection.

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NOVELTY STATEMENT

This study could provide the new information regarding the optimal time for PGF2Î± injection before semen collection, which can improve the quality of sperm and testosterone in Aceh bull.

AUTHOR'S CONTRIBUTION

BP, H Husnurrizal, VYE and DA: Nishcaya participated in performing, selecting samples, sample collection, and writing the initial manuscript.
TA, MA and AS: Performed manuscript revision.
TNS: Conducted the research, performed practical experiments, and data analysis.
AS and H Hafizuddin: Developed the original idea and protocol and revised the final manuscript.

Conflict of interests

The authors have declared no conflict of interest.

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