Amelioration of Seminal Plasma Testosterone Concentration in Gembrong Goats after In Vivo Administration of PGF2α

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Abstract

The semen quality of Gembrong goats is lower than other goats and may be related to the low concentration of testosterone hormone. Implementation of reproductive technology using prostaglandin F2α (PGF2α) hormone is beneficial to increase the testosterone hormone in Gembrong goats. This study aimed to determine the effect of PGF2α injection on increasing testosterone levels in Gembrong goats. Male Gembrong goats (n=4), aged 2.5–4 years with similar body condition scores (BCS=3) were used in this study. Goats were divided into two treatment groups (n=2). Goats in group 1 (G1) were injected intramuscularly with 1 ml PGF2α (75 µg), while those in group 2 (G2) were injected with 1 ml physiological NaCl. Semen collection was carried out 30 minutes after treatment using an artificial vagina. Testosterone levels were measured using the enzyme-linked immunosorbent assay (ELISA). The collected data was tabulated and analyzed descriptively. The results showed that the average testosterone concentration of G1 was higher than G2 with respective concentrations of 6.41 ± 0.70 and 2.81 ± 1.75 ng/ml. It was concluded that administration of PGF2α in vivo could increase testosterone concentration in Gembrong goats.

Keywords: Gembrong goat, prostaglandin F2α, seminal plasma, testosterone

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INTRODUCTION

Gembrong goats are part of the germplasm of Indonesian goats. This goat has distinctive morphological characteristics and has been developed and cultivated in Karangasem Regency, Bali Province. In 1993, the Board of Agriculture National Study Council determined the Gembrong goat population to have critical status. Reports from the Gembrong goat breeding in Tumbu Village, Karangasem District, Karangasem Regency, Bali in 2013 revealed that the population of Gembrong goats was only 20 individuals. After intervention through the National Innovation System Study Incentive Program (INSINAS), there was an increase in Gembrong goat population to more than 40 individuals (Zein *et al*., 2016).

Many factors are causing the decline in the Gembrong goat population. Initially, Gembrong goat breeders ran their livestock business using semi-intensive and even extensive methods. This causes many Gembrong goats to be attacked by predators such as coyotes. Other factors causing the decline in the population of Gembrong goats include cross-breeding, the belief that males will experience hair loss if they are used as a breeder, difficulty in detecting the goat estrus symptoms, and sales factors (Dyantari *et al*., 2015).

Husnurrizal *et al*. (2023) reported that the semen volume (ml) and sperm concentration ($x10^6$ cells/ml) of Gembrong goats were lower than Boerka goats ($p < 0.05$) with respective

values of 0.50 ± 0.00 compared to 1.00 ± 0.20; and 1557 ± 712.00 compared to 4500 ± 317.65. When compared with other goat breeds, it can be seen that the semen volume of Gembrong goats is relatively lower. Hafizuddin *et al*. (2020) report that semen volume in Anglo-Nubian x Peranakan Etawah (Anpera) crossbred goats in the age groups of 24 months, 30 months, 36 months, and more than 48 months of age groups were 0.60 ± 0.08 ml, 0.78 ± 0.05 ml, 0.84 ± 0.18 ml, and 0.75 ± 0.03 ml, respectively. The semen volume of Saburai goats was 0.78 ± 0.40 ml (Saputra *et al*., 2019) and Boer goat semen volume was 0.78 ± 1.54 ml (Greyling and Grobbelaar, 1983 as reported by Saputra *et al*., 2019). Meanwhile, the volume of Kejobong goat semen was 0.60 ± 0.20 ml (Syamyono *et al*., 2014).

Improving the quality of spermatozoa can be achieved by various ways. Recent studies reported that the addition of prostaglandin F2α (PGF2α) can improve the quality of spermatozoa which can be administered in vivo (Armansyah *et al*., 2018, Husnurrizal *et al*., 2021; Sari *et al*., 2021) and in vitro (Prestiya *et al*., 2020; Aswadi *et al*., 2021) because PGF2α can work directly or indirectly in improving the quality of spermatozoa. Directly, PGF2α can improve semen quality by increasing the hormone testosterone.

Saifudini *et al*. (2005) and Masoumi *et al*. (2011) reported that PGF2α induction was able to increase testosterone levels in local sheep and Holstein cattle. Testosterone is an important hormone during the spermatogenesis process. The mechanism by which PGF2α influences testosterone secretion has not been further studied. However, it is assumed that PGF2α acts directly on the testes to increase testosterone secretion. Prostaglandins stimulate the production of cyclic adenosine monophosphate (cAMP) which then stimulates testosterone synthesis (Hess, 2002). This study aims to determine the testosterone concentration in Gembrong goats after in vivo administration of PGF2α. It is expected that the results of this study can be used as recommendations for increasing testosterone concentrations in the production of frozen semen from Gembrong goats.

MATERIALS AND METHODS

Samples

This study was performed at Goat Study Station, Agricultural Study and Development Agency, Ministry of Agriculture of the Republic of Indonesia, Galang, North Sumatra. The goats used were adult Gembrong goats, aged 2.5-4 years with body condition score (BSC) 3. Goats were divided into two treatment groups (n=2). Group 1 (G1) received 75 µg (1 ml) PGF2α injection while Group 2 (G2) received 1 ml physiological NaCl injection intramuscularly. The limitation of the study was the low number of experimental animals used. This was due to the total number of Gembrong males in the study location being only six and of the six animals, only four of their semen could be collected using an artificial vagina. Plans to change the study design to use Latin squares are limited by technical constraints and permits from related parties.

Collection and Preparation of Seminal Plasma

Testosterone levels were measured using seminal plasma from male goats. Semen collection was carried out using the artificial vagina 30 minutes after treatment. A total of 1 ml of goat semen was taken and then centrifuged at a speed of 3500 rpm for 10 minutes. The seminal plasma (supernatant) obtained was transferred into an Eppendorf tube and then stored in a freezer.

Testosterone Concentration Examination

Testosterone levels were measured using the enzyme linked immunosorbent assay (ELISA) (DRG ELISA kit EIA-1559, DRG Instruments GmbH, Germany). Testosterone concentrations were measured following the manufacturer's instructions (DRG diagnostics) as outlined by Syafruddin *et al*., (2020) and Hafizuddin *et al*., (2021). Seminal plasma and standard solutions were added 50 µl each into microplate wells, followed by 50 µl of HRP-conjugate solution added to each well and 50 µl of antibody solution. The wells were covered with plastic wrap,

homogenized, and incubated for 60 minutes at 37ºC. The solution was then discarded and washed three times (1x using wash solutions) and left for 10 seconds. At each step, all the liquid was removed. After the last wash, the remaining wash buffer was removed by aspiration. The microplate was inverted and cleaned with absorbent paper. Next, 50 µl of substrate A was added into each well, followed by the addition of 50 µl of substrate B, then covered and incubated for 15 minutes at 37ºC. Next, 50 µl of stop solution was added to each well, homogenized, and the absorbance was read on an ELISA reader with optical density (OD) wavelength at 450 nm.

Data Analysis

All study data were displayed in graphical form and analyzed descriptively.

RESULTS AND DISCUSSION

In vivo administration of PGF2α at a concentration of 75 µg showed an effect on increasing testosterone concentration in the seminal plasma of Gembrong goats. The results of measuring testosterone concentration levels are presented in Figure 1. The results showed that the testosterone concentration in G1 was higher than G2 with respective concentrations of 6.41 ± 0.70 ng/ml and 2.81 ± 1.75 ng/ml.

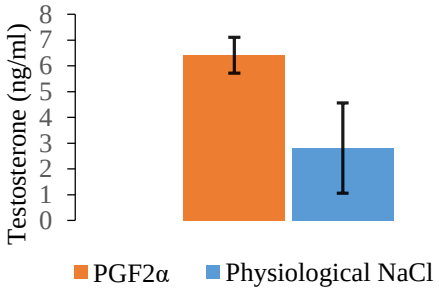

Figure 1. Testosterone levels in Gembrong goats after PGF2α and physiological NaCl injection.

The results of this study were in line with Armansyah *et al*. (2018) on local goats. Administration of PGF2α to local goats can increase testosterone concentration to 18.51 ± 19.46 ng/ml compared to the control group given physiological NaCl (10.27 ± 5.42 ng/ml). Similarly, Saifudini *et al*. (2005) reported an increase in local sheep testosterone levels after administration of PGF2α one week before sample collection. Kiser *et al*. (1976) also reported that administration of PGF2α 90 minutes before sample collection could increase the cattle testosterone levels. In contrast, Siregar *et al*. (2014) observed different findings in which the application of PGF2α could not increase testosterone levels in white mice. The varying results may be caused by differences in animal species used and differences in collection intervals and treatments.

Data regarding the concentration of Gembrong goats has never been reported before. In this study, all aspects other than treatment that can influence testosterone levels were relatively similar to each individual Gembrong goat, thus the testosterone concentration of goats in the G2 could be used as a reference for normal testosterone concentration in Gembrong goats. The testosterone concentration of Gembrong goats in G2 was lower compared to other goat breeds. The testosterone concentration of white goats was 4.30 ± 0.47 ng/ml (Polat *et al*., 2011), Etawah crossbreed goats were 6.82 ± 4.18 ng/ml, Kejobong goats were 12.00 ± 6.56 ng/ml, and Bligon goats was 9.23 ± 4.73 ng/ml (Rachmawati *et al*., 2013). These differences may be influenced by genetic and environmental factors (Sukaryana *et al*., 2011).

There are two mechanisms for increasing testosterone levels by PGF2α, direct and indirect mechanisms. Directly, PGF2α will provide the same effect as the mechanism of steroids and also the effect of local contraction of the lumen muscles in the reproductive system of male animals (Capitan *et al*., 1990). Indirectly, it is known that PGF2α plays a role in the secretion of luteinizing hormone (LH) (Haynes *et al*., 1977). The PGF2α hormone stimulates the hypothalamus to produce gonadotropin releasing hormone (GnRH) to further stimulate the pituitary to produce interstitial cells stimulating hormone (ICSH) or LH. Furthermore, LH will stimulate Leydig cells to increase testosterone production (Rachmawati *et al*., 2014).

The PGF2α hormone induces directly in the process of testosterone formation in Leydig cells. The hormone PGF2α stimulates the formation of cyclic adenosine monophosphate (cAMP) which is a ring-shaped molecule made from ATP which is a common intracellular signaling molecule (second messenger) in eukaryotic cells, for example in vertebrate endocrine cells. In Figure 2 it can be seen that cAMP catalyzes the synthesis of protein kinase A, which is needed 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 (Haider, 2007).

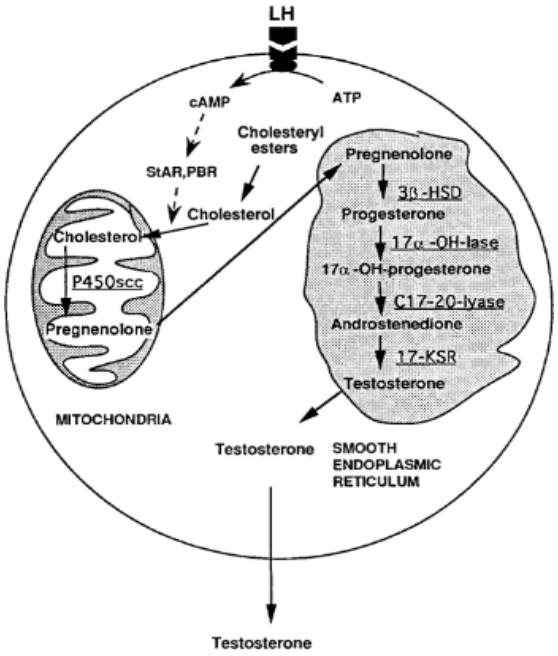

Figure 2. Testosterone hormone biosynthesis (Chen and Zirkin, 2000).

Cholesterol transport is initiated by StAR and then by PBR across the mitochondrial cell membrane gate. Then, the p450scc enzyme (side-chain cleavage) located in the inner mitochondrial membrane matrix will convert cholesterol to pregnenolone. The pregnenolone is then brought to a smooth state endoplasmic reticulum (SER) where testosterone will be formed through stages that require steroidogenic enzymes (Haider, 2007; Chen and Zirkin, 2000).

CONCLUSION

In conclusion, the injection of PGF2α could increase testosterone levels in Gembrong goats.

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