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# Testosterone Concentration, Libido and Spermatozoa Quality in Gembrong Goats after *in vivo* and *in vitro* PGF2α Administration

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**Abstract:** The Gembrong goat population is currently in a critical state. The preliminary study results show that one of the factors causing the population decline is the low fertility of male goats characterized by low sperm quality. The improvement of the spermatozoa quality can be performed with the administration of PGF2α. This study aims to determine the difference in semen quality in Gembrong goats after *in vivo* and *in vitro* administration of PGF2α. The study was divided into two phases. Phase I involved *in vivo* testing of PGF2α administration, while phase II involved *in vitro* PGF2α administration in both groups from phase I. In phase I, goats were separated into two groups (n = 3): Group 1 (G1) was treated with 75 µg (1 mL) PGF2α, while group 2 (G2) was treated with 1 mL physiological NaCl. Assessment of libido, semen collection for semen quality examination and blood collection for testosterone concentration examination were performed 30 min after treatment. The collected semen in Phase I was divided into two groups for dilution and freezing, resulting in four treatment groups in Phase II: G1a, G1b, G2a and G2b. Semen from groups G1a and G2a were diluted with Andromed diluent which was supplemented with 75 µg of PGF2α, while groups G1b and G2b were diluted with AndroMed® without the administration of PGF2α. The results indicated that *in vivo* PGF2α administration increases the libido ($p<0.05$), with scores in G1 and G2 being 5.00±1.00 and 8.67±1.15, respectively. However, testosterone concentration and all variables related to spermatozoa quality did not show significant differences ($p>0.05$). *In vitro* administration of PGF2α enhanced pre-freezing motility in G1a, G1b, G2a and G2b, with motility values were 75.04±5.43, 55.98±0.74, 70.21±0.04 and 52.19±18.53%, respectively ($p<0.05$). In conclusion, *in vivo* administration of PGF2α can enhance libido, while *in vitro* administration can increase pre-freezing spermatozoa motility in Gembrong goats.

**Keywords:** Gembrong Goats, PGF2α, PRE-Freezing, Semen

## Introduction

The Gembrong goat population is currently in a state of crisis (Ridho and Putra, 2021). To improve their reproduction, Artificial Insemination (AI) technology can be applied. The success of AI depends significantly on the semen quality used. Initial studies revealed that the semen quality of Gembrong goats is relatively lower compared to Boer goats with the semen volume and sperm concentration showing significantly lower ($p<0.05$) in Gembrong goats (Husnurrizal *et al*., 2023a). It has also been shown that Gembrong bucks have a lower volume of semen when compared to other breeds (Hafizuddin *et al*., 2021; Pinto *et al*., 2023; Saputra *et al*., 2019; Syamyono *et al*., 2014). The quality of spermatozoa is correlated with testosterone concentration, while testosterone concentration greatly determines the libido of animals (Hafizuddin *et al*., 2020).

The quality of sperm can be elevated through several treatments, one of which is Prostaglandin F2α treatment.

Prostaglandin F2α (PGF2α) treatment has been shown in recent research to enhance sperm quality both *in vivo* and *in vitro* (Husnurrizal *et al*., 2021; Sari *et al*., 2021) and *in vitro* (Aswadi *et al*., 2021; Prestiya *et al*., 2020). PGF2α improves sperm quality by raising testosterone levels.

Injection of PGF2α before semen collection in Kacang goats (Armansyah *et al*., 2018) and Bali cattle (Sari *et al*., 2019) increased testosterone concentration, although it may not significantly improve sperm quality. However, the opposite effect has been observed in Aceh cattle, where PGF2α administration improved sperm quality without a significant increase in testosterone concentration (Sari *et al*., 2021). Although there are still inconsistent results, all the research agrees that there is an increase in sperm quality in certain parameters, specifically sperm concentration and motility, due to increased testosterone concentration. This is further supported by Aswadi *et al*. (2021) who stated that *in vivo* PGF2α administration increased the frozen sperm motility of Nubian goats post-thawing. It is assumed that differences in animal types and breeds influence the response to *in vivo* PGF2α administration. So far, there are no reports available on the effects of PGF2α administration in Gembrong goats.

Improvement of sperm quality due to *in vitro* administration in diluent media has been previously reported in Nubian goats (Prestiya *et al*., 2020) and waring in sheep (Husnurrizal *et al*., 2021). The mechanism of sperm quality improvement is due to PGF2α acting on contractile elements of spermatozoa, resulting in increased sperm motility (Şen and Akcay, 2015). Kowalczyk *et al*. (2021) explained that prostaglandin E2 EP3 (PTGER3) and prostaglandin E2 EP1 (PTGER1) receptors, modulating sperm motility, acrosomal reaction, sperm capacitation and increased fertilization ability by facilitating the increased of intracellular calcium concentration. There has never been any research that reveals the low fertility of male Gembrong goats in relation to low sperm quality, testosterone and libido. In addition, the attempt to improve sperm quality through the administration of PGF2α *in vivo* and in vitro has never been reported simultaneously in the same breed, therefore this combination treatment is expected to improve the quality of Gembrong goat semen.

## Materials and Methods

### *Samples*

This study was performed at an Indonesian instruments standardization testing station for small Ruminants, Deli Serdang, North Sumatra. The semen and blood samples used in this study were collected from six Gembrong bucks, weighed between 30 and 40 kg and aged between 2.5 and 4 years old with a body condition Score (BCS) of three. The animals were given a daily feed of 3-4 kg of fodder, 0.4-0.5 kg of concentrate and provided with water ad libitum. The low number of experimental animals used was one of this research's limitations. This was due to the fact that there were only six Gembrong bucks in the study area that fit the criteria to be utilized as research samples.

### *Research Design*

This research was divided into two phases. Phase I involved the *in vivo* PGF2α administration in the goat, while phase II involved the *in vitro* trial of PGF2α administration on the two goat groups from phase I.

### *Phase I: In vivo Treatment*

The study involved two treatment groups of bucks (n = 3 each). Group 1 (G1) was administered with 1 mL (75 µg) PGF2α (Sincrovall, Mevet), while Group 2 (G2) received an injection of 1 mL NaCI. The injections were administered intramuscularly, following the dosage and injection method as directed by Aswadi *et al*. (2021).

### *Phase II: In vitro Treatment*

The semen collected from phase I was divided into two groups for dilution and freezing. Thus, in phase II, there were four treatment groups: G1a, G1b, G2a and G2b. Groups G1a and G2a were diluted with AndroMed® extender (Minitube), to which 75 µg of PGF2α were added, following the standard procedure (Prestiya *et al*., 2020). Meanwhile, groups G1b and G2b were diluted with AndroMed® without PGF2α supplementation.

### *Semen Collection and Evaluation*

Semen collection was performed 30 min after treatment, following the guidelines by Aswadi *et al*. (2021). Semen collection was conducted using artificial vagina methods and the collected semen was immediately evaluated for its quality both macroscopically and microscopically. The macroscopic evaluation included semen volume, pH, color and consistency. The microscopic evaluation included spermatozoa concentration, motility, viability and abnormalities. These evaluations were conducted following Arifiantini (2012) procedure.

### *Libido Measurement*

Goat libido was assessed during semen collection, based on a scoring system ranging from 0-10 over a 10 min period. The scores were as follows: 0, not interested; 1, interested only once; 2, interested more than once; 3, interested throughout the test; 4, mounting or trying once, no ejaculation; 5, mounting or trying twice, no ejaculation; 6, mounting or trying more than twice, no ejaculation, no further interest; 8, one ejaculation followed by interest; 9, two ejaculations, no further interest;

10, two ejaculations followed by interest (including mounting and/or ejaculation) (Hafizuddin *et al*., 2020).

### *Semen Dilution*

Semen was diluted using commercial extender AndroMed® and distilled water at a ratio of 1:4 in a 50 mL measuring glass. The mixture was homogenized and placed in a 38°C water bath (Susilawati, 2011). AndroMed® extender was divided into four parts and placed in labeled tubes, G1a, G1b, G2a and G2b. Groups G1a and G2a received extenders to which 75 µg of PGF2α were added according to Prestiya *et al*. (2020), while groups G1b and G2b received extenders without PGF2α supplementation. The collected semen from Gembrong goats was added to each respective tube and mixed with the extender according to the required volume. The volume of extender added followed the formula recommended by Pandur and Pacala (2012).

### *Semen Freezing Phase*

Each sample of semen in all groups was loaded into plastic straws. Semen samples were then placed in a 4°C refrigerator for 2 h. After pre-freezing, the straws were put on a rack, 8 cm above of liquid nitrogen surface at -96°C for 10 min. After pre-freezing was completed, the straws were kept in a container containing liquid nitrogen for 24 h, with additional liquid nitrogen added as needed (Husnurrizal *et al*., 2023b).

### *Blood Collection and Testosterone Concentration Examination*

Blood collection was performed 30 min after the *in vivo* treatment. The collected blood was placed in blood collection tubes and kept in a thermos filled with ice. Afterward, the blood was transported to the laboratory. Prior to serum extraction, the blood was allowed to stand for 30 min. Centrifugation was conducted at 3000 rpm for 15 min. The measurement of testosterone concentration was performed using the Enzyme-Linked Immunosorbent Assay (ELISA) technique with a testosterone kit (Cat. no. EIA-1559, DRG Instruments GmbH, Germany). Testosterone analyses were conducted following the manufacturer's instructions as previously described by Hafizuddin *et al*. (2023).

### *Data Analysis*

Data related to libido, macroscopic and microscopic sperm qualities, as well as testosterone concentration in the *in vivo* treatment, were analyzed using T-tests, while sperm motility post-dilution and pre-freezing after *in vitro* treatment were analyzed using one-way Analysis of Variance (ANOVA). The correlation between testosterone concentration and libido performance was analyzed using Pearson's bivariate correlation analysis.

## Results

The testosterone concentration in Gembrong goats between the two treatments showed no significant difference ($p>0.05$), although there was an increased tendency in group G2 (11.72±1.90 ng/mL) as compared to G2 (13.30±3.90 ng/mL), as presented in Table 1. This non-significant difference in testosterone concentration did not align with the libido of Gembrong goats. The PGF2α administration had a significant effect ($p<0.05$) on the libido of Gembrong goats between G1 and G2.

The concentration of testosterone in blood plasma showed a weak correlation with the libido of Gembrong goats ($R^2 = 0.0611$). The correlation between testosterone concentration and libido is presented in Fig. 1. The quality of spermatozoa was not affected by the *in vivo* administration of PGF2α ($p>0.05$), although almost all the variables tested showed an increased trend, with the exception of sperm viability and abnormality variables. Although the *in vivo* administration of PGF2α did not improve sperm quality, the *in vitro* administration of PGF2α in the phase II trial was able to escalate pre-freezing sperm motility in Gembrong goats ($p<0.05$), in Table 2.

**Table 1:** Fresh semen quality of Gembrong goats after administration of 75 µg of PGF2α (G1) and physiological NaCl (G2) *in vivo*

| | Mean ± SD | |
|---|---|---|
| Variable | G1 (n = 3) | G2 (n = 3) |
| Testosterone (ng/mL) | 11.072±001.90 | 13.30±003.90 |
| Libido score | 5.000±001.00[b] | 8.67±001.15[a] |
| Volume (mL) | 0.005±000.00 | 0.87±000.64 |
| Sperm concentration ($\times 10^6$ cells/mL) | 1.185±431.34 | 2.30±950.00 |
| Motility (%) | 79.031±003.72 | 77.45±003.01 |
| Viability (%) | 87.059±003.35 | 86.78±002.75 |
| Abnormalities (%) | 2.016±000.95 | 1.00±001.73 |

[a] [b]Different superscripts in the same row indicate significant differences ($p<0.05$)

**Table 2:** The sperm quality of Gembrong goats subjected to *in vitro* PGF2α administration

| | Administration | | | |
|---|---|---|---|---|
| Sperm motility | G1a (n = 3) | G1b (n = 3) | G2a (n = 3) | G2b (n = 3) |
| Post-dilution motility (%) | 79.3±9.240 | 62.9±6.360 | 75.23±7.39 | 66.58±20.92 |
| Motility pre-freezing (%) | 75.04±5.43[c] | 55.98±0.74[a] | 70.21±0.04[b] | 52.19±18.53[a] |

[a,b,c] Different superscripts in the same row indicate significant differences ($p<0.05$). G1a: PGF2α *(in vivo)* and Andromed + PGF2α *(in vitro)*, G1b: PGF2α *(in vivo)* and Andromed without PGF2α *(in vitro)*, G2a: Physiological NaCl *(in vivo)* and Andromed + PGF2α *(in vitro)*, G2b: Physiological NaCl *(in vivo)* and Andromed without PGF2α *(in vitro)*

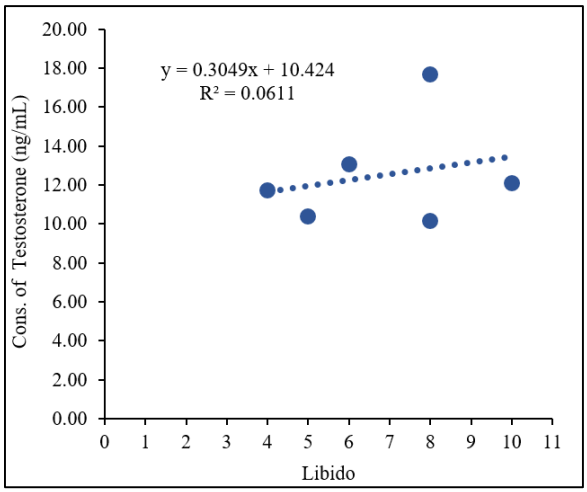


**Fig. 1:** Correlation between testosterone hormone concentration and libido of Gembrong goats after *in vivo* PGF2α treatment

## Discussion

Gembrong goats injected with PGF2α showed similar serum testosterone concentrations with the control group (p>0.05). Da *et al*. (2013) reported that there was no difference in testosterone concentrations after *in vivo* administration of PGF2α in male Barki sheep. Siregar *et al*. (2014) also reported similar results, stating that PGF2α administered to male white rats did not increase testosterone levels.

However, these findings differed from those reported in some other studies (Armansyah *et al*., 2018; Sari *et al*., 2019). Armansyah *et al*. (2018) observed that PGF2α treatment in Kacang goats increased testosterone level to 18.51±19.46 ng/mL, compared to the control group which was administered with physiological NaCl (10.27±5.42 ng/mL). Sari *et al*. (2019) reported that injecting PGF2α in Bali cattle increased testosterone concentrations 30 min following injection.

Variations in testosterone levels after PGF2α administration may be due to the difference in the time of testosterone measurement and frequency of PGF2α administration. In this study, testosterone concentration was measured 30 min after PGF2α injection, whereas, in Kacang goats, testosterone increased two days after administration (Armansyah *et al*., 2018). An increase in testosterone levels in local sheep after PGF2α administration at different frequencies, one week before sample collection. In addition to variations in testosterone measurement times and PGF2α administration frequency, the breed may also affect the response to PGF2α administration. In Bali and Aceh cattle, there was a difference in response after 30 min of PGF2α administration to testosterone concentration. Testosterone concentration in Bali cattle increased, while in Aceh cattle did not show the increment, although both breeds showed an improvement in sperm quality (Sari *et al*., 2021; 2019).

Although there was no significant difference, the *in vivo* administration of PGF2α in Gembrong goats tended to increase testosterone concentration in blood plasma. The mean plasma testosterone concentration of Gembrong goats in G1 and G2 was 11.72±1.90 and 13.30±3.90 ng/mL (p>0.05). One possible explanation for the non-significant difference result observed among groups could be owing to the small sample size. It is well known that the PGF2α hormone stimulates the hypothalamus to produce GnRH. GnRH then stimulates the pituitary gland to produce ICSH or LH (De Moraes *et al*., 2021). Furthermore, LH stimulates Leydig cells to increase the production of the testosterone hormone (Nedresky and Singh, 2019). An increase in testosterone concentration after PGF2α administration is also related to an increase in spermatozoa concentration (Estienne, 2014). Prostaglandin stimulates the production of cAMP, which in turn stimulates testosterone synthesis (Shah *et al*., 2021). Additionally, testosterone, together with FSH, plays a role in increasing the process of spermatogenesis in the seminiferous tubules (Estienne, 2014). CAMP plays a role in regulating the glycolysis pathway, which affects the energy formation required for spermatozoa movement (Dahan and Breitbart, 2022).

The PGF2α hormone acts directly on the process of testosterone production in the Leydig cells. The PGF2α stimulates the formation of cAMP (Pereira *et al*., 2017), which is a cyclic, ring-shaped molecule that is produced from ATP, a second messenger in the endocrine cells of vertebrates. Protein kinase a synthesis is catalyzed by cAMP, which is necessary for the transport of cholesterol from the cytoplasm to the mitochondria (Postler, 2021). Steroidogenic Acute Regulatory protein (StAR) and the Peripheral Benzodiazepine Receptor (PBR) transport cholesterol from the outer membrane of the mitochondria to the inner membrane (Patra *et al*., 2023). Cholesterol is a precursor to the synthesis of testosterone.

Data on the concentration of testosterone in Gembrong goats have not been reported to date. In this study, each individual Gembrong goat was relatively similar in all aspects other than treatment that could affect testosterone levels. Therefore, the testosterone concentration in the control Group (G1) can be used as a reference for the normal testosterone concentration in Gembrong goats. The concentration of testosterone in the Gembrong goats at G1 was lower than in the other breeds of goats. A concentration of 4.30±0.47 ng/mL of testosterone was observed in white goats (Polat *et al*., 2011), 6.82±4.18 ng/mL for the Etawah crosses, 12.00±6.56 ng/mL in the Kejobong goats and 9.23±4.73 ng/mL in Bligon goats (Rachmawati *et al*., 2014). Factors such as age, gender and season are likely

to influence these differences (Gholib *et al*., 2016), breed (Hafizuddin *et al*., 2021) and environment (García-Cruz *et al*., 2022). Testosterone levels in Boer and Barbari goats increased after receiving selenium with concentrations of 9.76 ng/mL and 5.21 ng/mL, respectively (Mariani *et al*., 2022).

*In vivo* administration of PGF2α enhanced the libido of Gembrong goats, however, the increase in libido level was not escorted by the increase in testosterone level. The present data revealed that the concentration of testosterone in the blood plasma correlates weakly with the libido of Gembrong goats ($R^2$ = 0.0611). The weak correlation between testosterone concentration and libido might be due to the non-significant increase in testosterone concentration in the study. Syafruddin *et al*. (2020) reported that libido in male animals was influenced by testosterone concentration. Although PGF2α administration significantly increased libido scores ($p<0.05$), libido scores cannot be used as an indicator of fertility in Gembrong goats because neither testosterone levels nor sperm quality increased significantly. Ungerfeld *et al*. (2020) reported different results, stating that PGF2α administration did not have a positive effect on libido in Male Saint Croix sheep.

A positive correlation between testosterone and libido has been reported in Bligon, Kejobong and Etawah crossbreed goats (Rachmawati *et al*., 2014), Simmental cattle (Baharun *et al*., 2022) and Zebu cattle (Mondal *et al*., 2019). Libido is an expression of sexual behavior under endogenous control, primarily LH or testosterone in the blood, as well as exogenous stimulation through physiological processes and reproductive experience (Mondal *et al*., 2019). The expression of libido is mediated by hormonal mechanisms, primarily LH and testosterone (Baharun *et al*., 2022).

The incapability of PGF2α to improve sperm quality in this study might be related to the fact that PGF2α's action was not optimal since it only acted directly. The mechanism for improving sperm quality occurs through both direct (Ruan *et al*., 2011) and indirect actions (Capitan *et al*., 1990). The direct actions of PGF2α result from smooth muscle contraction around the epididymis and stimulate testis capsule contractions (Şen and Akcay, 2015). Testis capsule contraction as a response to PGF2α is crucial for increasing spermatozoa numbers (Masoumi *et al*., 2011). The indirect effects of PGF2α on sperm quality occur through the increase in testosterone concentration (Capitan *et al*., 1990).

The inability of PGF2α to increase the sperm quality of Gembrong goats may also be related to the short interval between blood and semen sample collection after PGF2α injection, which was 30 min. This duration might not have been sufficient for the full effects of PGF2α to be realized and there could also have been differences in individual responses. Hess (2002) reported that PGF2α injection would lead to an increase in testosterone within 40-50 min and would last for 8 h. Titirungreung *et al*. (2012) also reported no significant difference in testosterone concentration after 30 min of PGF2α injection in Friesian Holstein cows, but testosterone level was higher in the group of cows injected with PGF2α compared to the control group. The increase in testosterone concentration indicates that the testes are functioning normally, have a certain consistent pattern and are an indication of testis activity and spermatogenesis.

The results of the *in vitro* study show that adding PGF2α to the extender can preserve the motility of Gembrong goat sperm (G1a and G2a). This result is in line with previous reports (Prestiya *et al*., 2020). According to Schlegel *et al*. (1981), PGF2α has a role in the elevation of sperm motility. Incubation of spermatozoa with 15-hydroxydehydrogenase prostaglandin causes the inactivation of PGF2α and resulted in a decrease in the motility of the sperm. The escalation in sperm motility following the administration of PGF2α is due to the fact that PGF2α can activate the contractile elements of sperm, which is the fibrous layer surrounding the central axoneme of the main part of the sperm (Susilawati, 2011). Gottlieb *et al*. (1988) reported that PGF2α possesses a crucial role in regulating sperm motility by mediating the content of ATP inside the sperm. The improvement in sperm quality after *in vitro* administration is likely also related to the concentration of MDA. The addition of PGF2α in semen extender can reduce the concentration of MDA (Al-Dahan *et al*., 2020). MDA is the final product of lipid peroxidation (Situmorang and Zulham, 2020), which enhances the free radicals formation and oxidative stress leading to damage to the structure and function of the plasma membrane of bull spermatozoa (Hosen *et al*., 2015).

## Conclusion

*In vivo* administration of PGF2α can increase libido, while *in vitro* administration can enhance the pre-freezing motility of Gembrong goat sperm.

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## Author's Contributions

**Husnurrizal:** Responsible for designed, conducted data collection, laboratory analyses, data analysis and initial manuscript preparation.

**Sri Wahyuni:** Contributed to laboratory analyses and reviewed the final manuscript.

**Hafizuddin:** Contributed to the designed, data collection, data analysis and reviewed of the manuscript.

**Cut Nila Thasmi:** Contributed to the visualization.

**Muhammad Rizky Ramadhan:** Contributed to performed, selecting samples, sample collection and writing the initial manuscript.

**Maulana Ahsan:** Contributed to the data collection, laboratory analysis and visualization.

**Tongku Nizwan Siregar:** Responsible for the designed of the study, conducted the project as a leader, data collection, data analysis and manuscript preparation and reviewed the manuscript.

## Ethics

This research has obtained ethical certification from the veterinary ethics committee of the faculty of veterinary medicine, Universitas Syiah Kuala with No. 169/KEPH/IX/2022.

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