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## The Effect of PGF2 $\alpha$ Injection on Post-Thaw Motility in Sperm of Nubian Goats

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### ABSTRACT

This study aims to determine the effect of PGF2 $\alpha$  injection on the post-thaw motility (PTM) in sperm of Nubian goats. Three male Nubian goats (3-4 years) with good reproductive ability were used. This study used a 3 x 3 Latin square design. The experimental animals received a physiological NaCl injection as a control (P1); 37.5  $\mu$ g of PGF2 $\alpha$  (P2), and 75  $\mu$ g of PGF2 $\alpha$  (P3). Semen was collected using an artificial vagina with one-week storage intervals between treatments. The collected semen was then diluted and frozen using a simple freezing method. Observation of semen quality before freezing included macroscopic and microscopic examinations. Macroscopic examination consisted of volume, pH, color, odor, and consistency, while microscopic examination consisted of motility, concentration, viability, and abnormality. PTM examination was done by mixing a drop of sperm suspension and one drop of physiological NaCl on an object glass and covered prior to observation under microscope. The results were analyzed using a Latin square pattern variant analysis, followed by Duncan's test. The PTM values of sperm (%) of Nubian goats in P1, P2, and P3 respectively are 28.71 $\pm$ 10.24, 50.03 $\pm$ 13.70, and 54.84 $\pm$ 12.04 (P<0.05). Injection of PGF2 $\alpha$  to Nubian goats by injection increased the PTM.

Keywords: Nubian goat, PGF2 $\alpha$ , PTM

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### Introduction

Goats are one type of ruminant livestock producing meat and milk that have a lot of potential and are attractive to the people of Indonesia. Some developing countries use goat as a commodity to alleviate poverty. The role of livestock is very strategic for the life of rural communities and has developed in almost all regions of Indonesia. Nubian goats are a superior type of goat originating from Africa, which are classified as dairy and meat goats (Husin *et al.*, 2007). Nubian goats is well adapted in Indonesia. Population of Nubian goats can be increased by implementing artificial insemination (AI) technology. One of the factors that determine the successful application of AI is the availability of quality frozen semen (Hoesni, 2013) which is closely related to sperm quality. Decreased sperm quality will reduce the number of conceptions (Dethan *et al.*, 2010).

Several studies reported that the sperm quality of Nubian goats is relatively lower compared to that of PE goats, but similar that of kacang goats. Nubian goats have a semen volume of 0.43 $\pm$ 0.05 mL, a sperm concentration of 2.77 $\pm$ 0.27 million/mL, sperm motility of ++, and sperm viability of 58.30 $\pm$ 27.30%; whereas PE goats have a semen volume of 0.86 $\pm$ 0.40 mL,

sperm concentration of 3.10 $\pm$ 0.57 million/mL, sperm motility +/+++, and sperm viability of 75.98 $\pm$ 4.61% (Hastono *et al.* (2013), In Kacang goats, the sperm concentration is 2.763 $\pm$ 395.0 million/mL with a motility of 3.7 (Armansyah *et al.*, 2018).

The administration of prostaglandin F2 alpha (PGF2 $\alpha$ ) to Mariz goats prior to semen collection increased sperm motility and reduce the number of abnormal sperm (Mwafaq *et al.*, 2013), although different results are reported by Armansyah *et al.* (2018). Armansyah *et al.* (2018) reported that the effect of PGF2 $\alpha$  did not have a significant effect on the sperm quality of Kacang goats, but still showed an increasing trend. Improvement of sperm quality after administration of PGF2 $\alpha$  happens through increased genital tract contractions in response to PGF2 $\alpha$  (Sen *et al.*, 2019). In addition, the effect of prostaglandins is to stimulate the production of cyclic adenosine monophosphate which then stimulates testosterone synthesis (Hess, 2002). The testosterone hormone plays an important role in the process of spermatogenesis.

Some reports regarding the use of PGF2 $\alpha$  to improve sperm quality are limited to the assessment of fresh semen (Mwafaq *et al.*, 2013; Armansyah *et al.*, 2018). Masoumi *et al.* (2011)

reported an insignificant increase in post-thaw sperm motility in Iranian Holstein cattle.

### Materials and Methods

Three male Nubian goats (3-4-year-old) with good reproductive ability were used. The goats were fed with elephant grass and concentrate at rate of 200 g/goats/day. Drinking water was given *ad libitum*. This study was conducted using a Latin square 3 x 3 pattern design so that the experimental animals received a physiological NaCl injection as a control (P1), 37.5  $\mu$ g of PGF2 $\alpha$  (P2), and 75  $\mu$ g of PGF2 $\alpha$  (P3). Schematically, the treatment given to each experimental animal is presented in Table 1.

Table 1. Treatment on experimental animals

Week	Treatment		
	I	II	III
1	P1	P2	P3
2	P2	P3	P1
3	P3	P1	P2

P1= Physiological NaCl (control); P2= 37.5  $\mu$ g of PGF2 $\alpha$ ; P3= 75  $\mu$ g of PGF2 $\alpha$ .

### Research procedure

Semen was collected at one-week intervals using an artificial vagina. Semen collection was carried out 30 minutes after the injection of PGF2 $\alpha$  by intra muscular according to the instructions Olfati *et al.* (2013). The sample injection pattern is presented in Table 1. Immediately after being collected, the semen quality was evaluated macroscopically for volume, pH, color, odor, and consistency.

### Sperm motility

Sperm motility can be observed by mixing a drop of semen with NaCl 0.9% on an object glass. The mixture is then observed under a microscope with a magnification of 40 x 10. The number of motile spermatozoa is calculated based on the movement of spermatozoa that is fast progressive (A), slow progressive (B), circular (C), and vibratory (D). Determination of the percentage of sperm motility is as follows:

$$\text{Percentage of motility} = \frac{A}{A + B + C + D} \times 100\%$$

### Sperm concentration

Sperm concentrations were calculated using Hemocytometer counting chambers with 200x dilution. The sample was filled into the haemocytometer counting chamber which had been covered using a glass cover and observed with a magnification of 40 x 10. The sperm concentration obtained was  $Y \times 10^6$  (Y = number of sperm in 5 chambers) (Azzahra *et al.*, 2016).

The number of sperm/mL =  $N \times 5 \times DF \times 10,000$

N: The average number of sperm in the chamber

DF: Dilution factor

5: A factor of 5 boxes of 25

10,000: The counting-chamber depth factor

### Sperm viability

Sperm viability was examined by placing one drop of spermatozoa on the glass object, to which one drop of 2% eosin was added. A thin blood cover was made and fixed on a methylated light. It was then examined using a microscope with 40 x 10 magnification. Dead sperm would absorb the red color, while living sperm did not absorb color or were white.

$$\text{Percentage of live sperm} = \frac{\text{Number of live sperm}}{\text{Number of dead and live sperm}} \times 100\%$$

### Sperm abnormality

Sperm abnormalities were observed by making a preparatory review in advance, namely by dripping one drop of semen on a glass object, to which one drop of eosin was added and then fixated on a methylated light. It was then observed using a microscope with 40x10 magnification. Morphological examination was done by observing sperm deformities or abnormalities, which include primary abnormalities (small/large head size, double head or double tail, and abnormal head shape) and secondary abnormalities (head rupture, tail breaking on the neck or middle, and folded tails). At least 200 spermatozoa were observed and the calculation according to WHO (1999):

$$\text{Percentage of abnormality} = \frac{\text{Abnormality}}{\text{Abnormality} + \text{Normal}} \times 100\%$$

### Semen extender

Andromed extender were made by mixing Andromed and aquades in a ratio of 1: 4 (5 mL of Andromed: 20 ml of aquades) until they were homogeneous; the mixture was then stored in an incubator at 36-37°C (Astuti, 2017). The amount of diluent needed to thin the semen was calculated using the following formula.

$$\text{Amount of diluent} = \frac{\text{Final volume} \times \text{motility} \times \text{concentration}}{\text{Sperm concentration per straw}} \times \text{straw volume}$$

### Semen dilution loading into straw and equilibration

Fresh semen that had been evaluated was then diluted with semen extender and equilibration process was carried out at 5°C for 4 hours, followed by filling and sealing.

### Semen freezing stage

The initial freezing stage involved pre-freezing the straws that had been filled with semen and arranging them on a 8-cm-high shelf above the surface of liquid nitrogen for 10 minutes, in the surface of liquid nitrogen (temperature around -130°C) in tightly closed

styrofoam. After the pre-freezing was finished, the next step was to put the semen into a goblet which was then put in liquid N $_2$  at -196°C. The straws were stored in liquid nitrogen (Waluyo, 2014).

#### Sperm post-thaw motility quality check

The quality of sperm's PTM was observed by dripping spermatozoa on an object glass, to which one drop of physiological NaCl was then added. The mixture was then covered with a glass cover and observed under a microscope with 40x10 magnification. The number of motile sperm was calculated based on the category of spermatozoa movements: fast progressive (A), slow progressive (B), circular (C), and vibratory (D). The percentage of sperm motility was determined using the following formula established by the WHO (1999):

$$\text{Percentage of motility} = \frac{A}{A + B + C + D} \times 100\%$$

#### Data analysis

Results of the semen quality examination were analyzed using a Latin Square Design (LSD) followed by Duncan's test.

### Results and Discussion

Examination of fresh ejaculate determines the feasibility of semen for further processing. Volume of Nubian goat semen obtained in all treatments in this study was 1.00-1.40 ml. Similar to Bretzlaff (1995) that the volume of goat semen ranges from 0.5 to 3.0 ml. The semen volume is the same as that of the Nubian goats reported by Husin *et al.* (2007), which is 0.50-1.5 ml, and by Ali and Mustafa (1986), which is 1.5 mL. The semen volume varies depending on the goat type, age, body size, season, level of food and frequency of ejaculation (Toelihere, 1981).

Macroscopic observation indicated the color was good and normal. Dethan *et al.* (2010) stated that the color of semen is related to the level of concentration and consistency of sperm. The thicker the semen, the higher the concentration of sperm, and the creamier and more yellow the semen, the thicker the sperm consistency (Hastono *et al.*, 2013).

According to Taming *et al.* (2003) normal semen has slightly thick to very thick. One of the factors that influence the consistency of semen is the frequency of ejaculation. The level of consistency with a higher viscosity indicates a higher number of spermatozoa. Purwasih *et al.*

(2013) state that the thickness or consistency along with the properties of semen is proportional to the sperm concentration. Suyadi *et al.* (2012) state that semen with very thick consistency levels would have higher levels of spermatozoa concentrations compared to semen with slightly thick.

Ejaculate's pH vary from 5.7-6.0. The difference in pH of fresh semen produced can be caused by the different composition in each of the ejaculated semen (Nahriyanti *et al.*, 2017). According to Elya *et al.* (2010) pH is determined by the balance of cations and anions in the accessory glands. Dethan *et al.* (2010) states that pH varies greatly, depending on livestock species, and the pH has a correlation with concentration, i.e. if the concentration is high, the pH will be slightly acidic. Based on the results of the examination, it can be concluded that the fresh semen of Nubian goats produced has a slightly acidic pH and a high concentration.

Microscopically, the average concentration of fresh semen of Nubian goats in the three groups was around 241.687-350.0 x 10 $^7$  mL. These results are still in accordance with the report of Bretzlaff (1995) that the sperm concentration in goats is around 250-500 x 10 $^7$  mL. Kamal *et al.* (2005) states that the sperm concentration of Nubian goats in the summer is 2080 million/mL. According to Ax *et al.* (2008) sperm concentrations are influenced by feed, livestock breed, age, temperature and ejaculation frequency. Viability is the life force of spermatozoa as an indicator of sperm quality (Sukmawati *et al.*, 2014). The average viability of Nubian goats obtained in this study was 59.40-81.04%.

The percentage of abnormal sperm obtained in this study was around 21.34 - 41.40%. Garner and Hafez (2000) report that sperm abnormality in goats ranges from 5-20%. According to Herdiawan (2004), sperm abnormality can be caused by physical changes in media, namely changes in osmotic pressure. These events can cause changes in the structure of spermatozoa, such as a bent tail or a detached head. Less than 20% of sperm abnormalities is suitable for AI (Garner and Hafez, 2000). According to Salisbury *et al.* (1985), sperm abnormalities are caused by the age, individual variations, and the physical condition of the animal itself.

Motility of Nubian goat motility in different periods are presented in Table 2. The PTM values (%) in P1, P2, and P3 were 28.71 $\pm$ 10.24, 50.03 $\pm$ 13.70, and 54.84 $\pm$ 12.04 (P<0.05), respectively. The PTM values in the treatment

Table 2. Sperm motility of Nubian goats at 3 examination periods after administration of PGF2 $\alpha$

Semen quality check	Treatment group		
	P1	P2	P3
Initial motility (%)	81.19 $\pm$ 6.26 <sup>a</sup>	55.77 $\pm$ 12.05 <sup>b</sup>	62.70 $\pm$ 9.87 <sup>c</sup>
Pre-freeze motility (%)	52.55 $\pm$ 2.54 <sup>a</sup>	50.50 $\pm$ 14.61 <sup>a</sup>	55.20 $\pm$ 7.13 <sup>a</sup>
Post-thaw motility (%)	28.71 $\pm$ 10.24 <sup>a</sup>	50.03 $\pm$ 13.70 <sup>b</sup>	54.84 $\pm$ 12.04 <sup>b</sup>

<sup>a,b,c</sup> Different superscripts on the same line show significant differences (P<0.05). P1 = Control (physiological NaCl); P2= 37.5 $\mu$ g of PGF2 $\alpha$ ; P3= 75  $\mu$ g of PGF2 $\alpha$ .

groups are included in the eligible to be AI. Garner and Hafez, 2000). In general, the PTM figures obtained from this study are relatively higher than the PTM figures reported by Masoumi *et al.* (2011). Masoumi *et al.* (2011) reported that the PTM values of groups given PGF2 $\alpha$  and controls were 36.1 and 35.6%, respectively.

The PGF2 $\alpha$  administration was able to maintain the spermatozoa motility during the storage in freezer, however this ability was not significantly influenced by the increasing of PGF2 $\alpha$  dosages. This can be proven from the findings that the value of initial motility (of fresh semen) in the control group tends to decrease in the pre-freeze and post-thaw phases. The values of initial motility; pre-freeze motility; and post-thaw motility on P1 vs. P2 vs. P3 (%) are 81.19 $\pm$ 6.26 vs 52.55 $\pm$ 2.54 vs 28.71 $\pm$ 10.24; 55.77 $\pm$ 12.05 vs. 50.50 $\pm$ 14.61 vs. 50.03 $\pm$ 13.70; and 62.70 $\pm$ 9.77 vs. 55.20 $\pm$ 7.13 vs 54.84 $\pm$ 12.04.

The results in line with Siregar *et al.* (2014) and Herawati and Widiarso (2003), that the administration of PGF increases sperm mass motility. Herawati and Widiarso (2003) reported that the addition of 2.0 mg of PGF2 $\alpha$  could increase the motility of goat sperm fresh ejaculate. The sperm motility increases because prostaglandins activates the contractile element of spermatozoa, the fibrous layer that surrounding the central acronema in the head spermatozoa. Gottlieb *et al.* (1988) reported that PGF2 $\alpha$  plays an important role in regulating sperm motility, by mediate the content of adenosine triphosphate (ATP) in spermatozoa.

According to Bygdeman *et al.* (1985), PGF2 $\alpha$  in seminal plasma plays a role in stimulating the kinetic activity and motility of sperm during ejaculation. However, the exact mechanism of increasing PTM after in vivo administration cannot be explained, because some of the arguments (Schlegel *et al.*, 1981; Gottlieb *et al.*, 1988) tend to support the findings when PGF2 $\alpha$  is given in vitro (into diluents), as has been proven by Prestiya *et al.* (2020).

### Conclusions

Based on the results of this study, it can be concluded that administration of PGF2 $\alpha$  by injection can increase the PTM value of Nubian goat sperm.

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