The Effect of Gonadotropin-Releasing Hormone (Gnrh) on Semen Quality and Testosterone Level of Nubian Goats

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Abstract. *This research aimed to determine the effect of gonadotropin-releasing hormone (GnRH) in improving semen quality and hormone testosterone in Nubian goats. The experimental design was a 3x3 Latin square with the experimental animals that received a physiological NaCl injection as a control (T0), 50 μg GnRH (T1), and 100 μg GnRH (T2). The semen samples were collected using an artificial vagina at 24 hours after treatment, and then semen characteristics were evaluated both macroscopically and microscopically. Subsequently, the blood samples were collected at 60 minutes after the injection of GnRH for the analysis of testosterone concentration using the enzyme-linked immunosorbent assay (ELISA) method. The observations showed a cream coloration with thick consistency in the semen collected from all treatment groups. In addition, the average (±SD) of semen volume (mL), concentration (10^6), motility (%), viability (%), abnormality (%) of spermatozoa and testosterone levels (ng/mL) in the T0 vs T1 vs T2 groups were 1.8 ± 0.52 vs 1.5 ± 0.70 vs 2.6 ± 1.63 ($P > 0.05$), 807 ± 409.98 vs 895 ± 509.73 vs 1,215 ± 270.14 ($P > 0.05$), 37.00 ± 0.333 vs 34.00 ± 0.309 vs 65.00 ± 0.110 ($P > 0.05$), 63.00 ± 0.144 vs 59.00 ± 0.121 vs 57.00 ± 0.145 ($P < 0.05$), 33.00 ± 0.382 vs 15.00 ± 0.199 vs 7.00 ± 0.040 ($P > 0.05$), 13.16 ± 9.37 vs 28.13 ± 1.21 vs 33.13 ± 2.30 ($P < 0.05$), respectively. It was concluded that GnRH treatment is capable of reducing spermatozoa abnormalities and increasing the testosterone concentrations of Nubian goats.*

Introduction

Semen and hormonal concentrations are important indicators of the quality of male reproduction (Novita et al. 2006). Several studies have shown a lower quality of spermatozoa in Nubian goats, compared with the Peranakan Etawah (PE), and similar to the Kacang goat. Hastono et al. (2013) reported that spermatozoa of Anglo-Nubian goats show the creamy coloration, thick consistency, semen volume of 0.43 ± 0.05 mL, concentration of 2.77 ± 0.27 million/mL, motility ++, and viability of 58.30 ± 27.30%, while the PE spermatozoa present with a creamy-yellow color, watery consistency, semen volume of 0.86 ± 0.40 mL, concentration of 3.10 ± 0.57 million/mL, motility +/+++++, and viability of 75.98 ± 4.61. In Kacang goats, the number of spermatozoa was 2.763 ± 395.0 million/mL with motility of 3.7 (Armansyah et al. 2018). The testosterone levels of Nubian goats at puberty range around 5.4 ng/mL (Souza et al. 2011), which is relatively lower than recorded for Kacang goats. Armansyah et al. (2018) reported an average testosterone level of Kacang goats in the control, prostaglandin F2 alpha, and seminal vesicle extracts treatment groups to be 10.27 ± 5.42, 18.51 ± 19.46, and 29.57 ± 12.96 ng/mL, respectively.

Improving the quality of Nubian semen involves processes that increase the volume and concentration of spermatozoa, increasing the gonadotropin-releasing hormone (GnRH) circulation in blood. This compound has a decapeptide in the structure and is synthesized in the arcuate nucleus of the hypothalamus (Suparman and Suparman 2016) with a basic function of regulating adenohipofisa activity, subsequently stimulating the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Isnaini and Wahjuningsih 2014). FSH is the main gonadotropin hormone affiliated with the process of spermatogenesis (Akmal et al. 2015), while LH, which is secreted by the anterior pituitary, controls the development of germ cells. Collectively, they have been associated with the release of androgens by interstitial cells, necessary in the production of mature sperm, and also in the stimulation of Leydig cells to secrete testosterone (Andalusia et al. 2008), which assist in the formation of spermatozoa (Hasbi and Gustina 2018).

The GnRH hormone is often used to increase the reproductive capacity of male animals, including buffalo (Sajjad et al. 2007), PE goats (Hamdan 1999),

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and sheep (Azawi et al. 2012; Kiyma et al. 2000). However, there has been no research on its application for similar a purpose on Nubian goats. Azawi et al. (2012) stated that the breed and area of origin influence the reproductive activity of sheep. Therefore, research is needed to determine the effect of GnRH in improving the quality of semen and the hormone testosterone in Nubian goats.

Materials and Methods

This research required the use of three male Nubian goats aged 2–3 years, with a 3 × 3 latin square design. The male goats were raised in individual cages, were fed 3–4 kg of king grass and 0.5–0.7 kg of concentrate daily, and had water consumption *ad libitum*. The experimental animals received physiological NaCl injections as controls (T0), 50 µg gonadorelin (Fertagyl, BV Boxmer, Holland, T1), and 100 µg gonadorelin (T2), and each treatment was performed three times and on alternate weeks. This procedure was conducted according to the instructions of Hamdan (1999), as schematically presented in Table 1.

Research Procedures

Semen collection

Semen was collected using an artificial vagina, previously dried and cleaned alongside the rubber and reservoir tubes, in order to prevent contamination. The sample was collected in one ejaculation/week for three weeks, while the time interval between treatment and collection was in accordance with Azawi et al. (2012).

Semen quality examination

Semen macroscopic examination

After collection, the quality was macroscopically evaluated based on volume, color, consistency, and pH.

Semen microscopic examination

Spermatozoa motility

Spermatozoa motility was evaluated by dripping the sample on a glassslide, then one drop of physiological NaCl was added, followed by observation through a microscope with 40 x 10 magnification. The number of motile sperm was calculated based on the movement, categorized as fast progressive (A), slow progressive (B), circular (C) and fibration (D). The percentage was determined using the following formula:

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

Spermatozoa concentration

Spermatozoa concentrations were calculated using a haemocytometer pipette and a Neubauer counting chamber, and the semen was sucked up to a scale of 0.5, followed by the addition of a 3% NaCl solution, until 101 was reached. Subsequently, the solution was gently homogenized using an "8" pattern for 2–3 minutes, then a few drops were discarded, followed by another round of homogenization. The sample was filled into the Neubauer counting chamber, and closed with a glass cover. Spermatozoa counting was performed in five chambers, and observed under a microscope with 40 x 10 magnification. The concentration obtained was Y x 5 x 10^6 (Y = the number of spermatozoa in 5 boxes) (Indriani et al. 2013).

Spermatozoa Viability

The examination of viability was performed by introducing one drop of spermatozoa on a glass slide, followed by the addition of one staining eosin drop. A smear preparation was made and fixed on a spritus lamp, and then was evaluated using a microscope of 40 x 10 magnification. The dead cells absorb red pigmentation, while the live spermatozoa tend to not absorb any color, leading to a white appearance. The spermatozoa were then counted and divided by the total visible, and presented as a percentage value (Masir et al. 2017; Padrik et al. 2010).

$$\%\ \text{Live} = \frac{\text{Total of live spermatozoa}}{\text{Total live and dead spermatozoa}} \times 100\%$$

Spermatozoa Abnormalities

This observation was performed by dripping spermatozoa and eosin on the object glass, fixed on a spritus lamp, and observed in a microscope with 40 x 10 magnification (Klimas et al. 2012; Rahmiati et al. 2015). The morphological examination identified deformities that are categorized as primary (small/large head size, double head or double tail, and abnormal head shape) and secondary abnormalities (head rupture, tail breaking at the neck or middle, and folded tail). The minimum spermatozoa observed were 200 cells, and the calculations was conducted using the following formula:

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

Blood sampling

A total of 3 mL blood samples without anticoagulants were collected through the jugular vein using a syringe, 60 minutes after administering the GnRH injection (Hamdan 1999). The blood was then placed into a centrifuge tube and left for 60 minutes to freeze, and the serum was subsequently separated from blood objects (clots), followed by centrifugation at 1200 rpm for 10 minutes. The collected serum was transferred into a microtube and stored in the freezer at –20°C (Gholib et al. 2016).

Hormone testosterone analysis

Hormone analysis was performed according to the procedure from the testosterone catalog from DRG

diagnostic (EIA-1559, DRG Instruments GmbH, Germany). Briefly, a standard solution of 0.2 ng/mL to 16 ng/mL was prepared. A total of 25 µL of each standard solution and samples were transferred into the micro-plate well. Subsequently, 200 µL of conjugate enzyme was added and the mixture was incubated for 60 minutes at room temperature, and then the micro-plate was washed three times using a 300 µL washing solution in each well. Furthermore, 200 µL of substrate solution was added to each well and incubated for 15–20 minutes at room temperature, followed by the termination of enzyme reaction with a stop solution of 100 µL 0.5 M H_2SO_4. The absorbance was read using an ELISA reader (Pratomo and Yudi 2016).

Data Analysis

Data resulting from the quality examination of semen and testosterone were analysed using ANOVA followed by the Duncan multiple range test.

Results

The results of volume, concentration, motility, viability and abnormalities examination on Nubian goat spermatozoa in the three treatment groups, including the physiological NaCl group (A), 50 µg GnRH (B), and 100 µg GnRH (C), are presented in Table 2.

Discussion

The color of semen collected from Nubian goats in all treatments was cream with thick consistency. This was in accordance with Ax et al. (2000), who recorded milky white or cream in color of goat semen, while Tambing et al. (2003) reported on the interrelatedness of both characteristics, indicating an association between a thinner semen and paler coloration.

The average semen volume obtained was 1.50–2.60 mL, while the spermatozoa volume at T0, T1, and T2 were 1.80 ± 0.52, 1.50 ± 0.70, and 2.63 ± 1.63 mL (P>0.05), respectively. The semen volume tends to increase in goats treated with 100 µg, although no statistically significant effect was recognized. In addition, the semen volume in the 100 µg treatment group was higher than the value obtained with PE goat as reported by Hamdan (1999), who capped at 1.30 mL, using a similar GnRH dose level.

The semen volume was included in the normal category, although Garner and Hafez (2000) reported that a normal range was from 0.80 to 1.20 mL. Hafizuddin et al. (2020) report that semen volume in Anglo-Nubian x PE (Anpera) crossbred goats in the age groups of 24 months, 30 months, 36 months, and more than 48 months were 0.60 ± 0.08 mL, 0.78 ± 0.05 mL, 0.84 ± 0.18 mL, and 0.75 ± 0.03 mL, respectively. Meanwhile, the occurrence of an increase per ejaculation is often associated with the optimal working capacity of the testes and the accessory glands, resulting from the influence of GnRH (Hamdan 1999). However, the variation between high and low volume is affiliated with the frequency of ejaculation, species, age, season, nutrition, libido, and animal condition (Tambing et al. 2003). According to Pamungkas et al. (2008), besides the differences in goat species, the collection method and frequency as well as the age have also been identified as influencing factors.

The result of microscopic semen examination indicated that a spermatozoa concentrations in T0, T1, and T2 were 807 ± 409.98, 895 ± 509.73, and 1.215 ± 270.14 (10^6/mL) (P > 0.05), respectively. Numerically, the average concentration tends to increase in the treatment group, with no statistically significant effect. This was possibly due to an elevation in the levels of FSH and testosterone, which have been implicated in spermatogenesis activities of goat (Hamdan 1999). However, the spermatozoa concentration observed in this study (range 807–1.215 (10^6/mL)) was lower than the normal value observed in goat, which is 3.50 x 10^9 to 6.00 x 10^9/mL (Ax et al. 2000). These were lower than the values recorded for PE, as reported by Hamdan (1999), using similar dosages. Husin et al. (2007) reported on the influence of differences in species, as the concentration of spermatozoa was assumed to be due to its genetic quality (Situmorang 2002). This has also been affiliated with variations in the age of the male, which increases up to the 22nd month (Heriyanta et al. 2014).

Spermatozoa motility levels in T0, T1, and T2 were 37.00 ± 0.33, 34.00 ± 0.30, and 65.00 ± 0.11%, respectively, with a quality ranging from 34% to 65% (P > 0.05). Meanwhile, the average value tends to increase more in those treated with GnRH at a dose of 100 µg, although no statistically significant effect was observed. The minimal elevation in motility was possibly related to the increased concentration of spermatozoa per mL of semen (Hamdan 1999), which is known to accelerate the depletion of food. This increases the metabolic waste products in semen liquid, subsequently reducing the durability and capability of spermatozoa present (Garner and Hafez 2000).

The results in this study were lower than those reported by Hamdan (1999) on PE goats at the same treatment dose of 0, 50, and 100 µg, which were 69.00 ± 7.76, 75.00 ± 2.64, and 73.66 ± 3.51%, respectively, although the normal motility according to Garner and Hafez (2000) is 60–80%. These values are used as the simplest benchmark in assessing the quality of semen (Pamungkas et al. 2008), and Suyadi et al. (2012) affiliates a high motility with a greater occurrence of fertilization.

The average spermatozoa viability ranges from 57% to 63%, with the specific values at T0, T1, and T2 being 63.00 ± 0.14, 59.00 ± 0.12, and 57.00 ± 0.14% (P > 0.05), respectively. Furthermore, the result for Nubian goats in this study was relatively lower compared with the PE species as reported by Hamdan (1999), designating 81.33 ± 3.05, 85.67 ± 1.53, and 86.33 ± 0.58%, at the similar treatment dose. Accord-

ing to Hastono et al. (2013), the live spermatozoa in Anglo-Nubian goats was 58.30 ± 27.30%, with less than 15% sperm (Bintara 2011). Moreover, the occurrence of living and dead forms is often influenced by nutritional and environmental factors. This is in line with the study by Wahyuningsih et al. (2014), who indicated age, genetic, temperature, season, and feed as the factors influencing the quality of fresh semen obtained from superior males. According to Hamdan (1999), the high and low percentage recorded as alive on examination is dependent on the time interval between the ejaculation and completing the object.

Spermatozoa abnormalities in T0, T1, and T2 were 33.00 ± 0.382, 15.00 ± 0.199, and 7.00 ± 0.040% ($P < 0.05$), respectively, characterized by an average decline in those treated with GnRH. The nature of abnormality evaluated in this research include folded tail, broken head, and broken tail. Sajjad et al. (2007) reported the possibility for treating buffaloes to decrease the incidence of spermatozoa abnormalities, directly or indirectly influenced by the increase in the testosterone level (Ronayne et al. 1993; Sajjad et al. 2007). This is in accordance with the results obtained in the current study, with the blood serum testosterone levels T0, T1, and T2 reaching 13.16 ± 9.37, 28.13 ± 1.21, and 33.13 ± 2.30 ng/mL, respectively.

The provision of GnRH in Nubian goats conferred no significant effect ($P > 0.05$) on the semen quality, except for abnormalities, possibly due to variation in species, age, season and nutrition; hence, its effect is categorized as sub-optimal. This is in line with Pamungkas et al. (2008), who recorded the influence of the collecting method and frequency, as well as the age, besides by the diversity in species. According to Monaco et al. (2015), a GnRH injection does not significantly affect volume, motility, and viability, although an increase in sperm concentration was expressed in treated camels.

In this research, it was observed that GnRH possesses the capacity to enhance testosterone concentration, and the statistical analysis result on the effect of treatment in all groups after 60 minutes showed significant differences ($P < 0.05$). Furthermore, the groups administered with 50 µg and 100 µg had relatively higher values compared with the control. This is associated with the high level of GnRH in the plasma and is possibly due to the capacity of hormones to increase its content released by the hypothalamus, which subsequently influences the pituitary to release *gonadotropin hormone* (GtH) (Dewantoro et al. 2017).

Sanford et al. (1977) reported the increase of testosterone concentrations in sheep aged 2–3 years after injecting 50 *µg* GnRH compared with controls. This occurs for 3–4 hours after administration, with the peak value recorded at 1 hour after administration, with 4.08 ± 0.54 and 7.97 ± 1.25 ng/mL, respectively. Ronayne et al. (1993) observed that the administration of 250 *µg* GnRH for cows in India increased testosterone levels 10–15 times compared with the controls at 60 minutes post-administration. Hamdan (1999) also reported elevation of the testosterone level in PE goats injected with 50 *µg* and 100 *µg* GnRH (8.28 ± 0.99 and 9.84 ± 1.97 ng/mL, respectively), using blood samples that were collected after 60 minutes. Moreover, Elkhawagah et al. (2011) reported an upsurge in the blood serum testosterone concentrations of 1.73 ± 0.57, 4.61 ± 1.28, and 4.79 ± 1.21 ng/mL on buffaloes aged 15–18 months, using three different GnRH doses of 8, 12, and 16 µg, respectively. In contratry, Kumar et al. (2016) reported the inability of a 10 µg GnRH injection in bulls aged 6–16 months to increase overall testosterone concentration, although this value increased in cows aged 14–16 months (from 0.97 ± 0.08 to 11.4 ± 2.22 ng/mL) after two hours of administration. This was due to the fact that cows under 14 weeks of age are not yet undergoing puberty, resulting from the imperfect hormones present in the body.

Based on aforementioned facts, the highest concentration of the hormone testosterone was generally observed at 60 minutes or 120 minutes after administering GnRH. Also, an increased blood serum level is affiliated with the elevated amount of LH, resulting from the stimulation of the anterior pituitary. This upsurge subsequently promotes the production of testosterone through interstitial cells (McLachlan et al. 1995).

The results show the tendency for an increase in the dose of GnRH to elevate testosterone concentration. This is evidenced by the values recorded in groups of 50 µg and 100 µg, being 28.13 ± 1.2 and 33.13 ± 2.30 ng/mL, respectively. Dewantoro et al. (2017) have stated that the hormone mechanism is highly dose-dependent, as it works normally (optimal) at a certain level; otherwise, the biological potential toward its target is diminished. Meanwhile, there is a possibility that the injected treatment is unable to efficiently stimulate the target release at lower (suboptimal) doses.

The testosterone concentration in Nubian goats evaluated in the control group was 13.16 ± 9.37 ng/mL, indicating a higher value, compared with the Anglo-Nubian species (5.4 ng/mL), as reported by Souza et al. (2011). Also, the record on other types of goats, including the two-year-old white variety, showed a concentration of 4.30 ± 0.47 ng/mL (Polat et al. 2011), while PE showed 6.82 ± 4.18 ng/mL, Kejobong showed 12.00 ± 6.56 ng/mL, and Bligon showed 9.23 ± 4.73 ng/mL (Rachmawati et al. 2014). In addition, the differences were assumed to have occurred as a result of genetic and breed factors.

The mechanism of testosterone elevation due to the higher dose of a GnRH injection is associated with its ability to stimulate the pituitary neurons to release gonadotropin hormone (GtH-I and GtH-II). These are subsequently released systemically, causing an increase in the serum concentration of both hormones (Kusuma et al. 2012), known to play a role

Table 1. Treatment patterns in experimental animals

Subject	Period		
	I	II	III
1	A	B	C
2	B	C	A
3	C	A	B

A – physiological NaCl (control, T0);
B – 50 μg gonadorelin (T1); C – 100 μg gonadorelin (T2)

Table 2. The average (± SD) volume, concentration, motility, viability, abnormality of spermatozoa, and testosterone levels of Nubian goats with GnRH treatment

Treatment	Group		
	Control (T0)	50 **μg** (T1)	100 **μg** (T2)
Volume (mL)	1.8 ± 0.52 [a]	1.5 ± 0.70 [a]	2.6 ± 1.63 [a]
Concentration (10^6/mL)	807 ± 409.98 [a]	895 ± 509.73 [a]	1,215 ± 270.14 [a]
Motility (%)	37.00 ± 0.33 [a]	34.00 ± 0.30 [a]	65.00 ± 0.11 [a]
Viability (%)	63.00 ± 0.14 [a]	59.00 ± 0.12 [a]	57.00 ± 0.14 [a]
Abnormality (%)	33.00 ± 0.38[a]	15.00 ± 0.19 [b]	7.00 ± 0.040 [b]
Testosterone (ng/mL)	13.16 ± 9.37[a]	28.13 ± 1.21[b]	33.13 ± 2.30[b]

[ab]Different superscripts in the same row showed significant difference ($P < 0.05$)

in spermatogenesis. Furthermore, they have been affiliated with the stimulation of gonads in the production of steroid hormones, including testosterone and 17ß-estradiol. Specifically, GtH-I encourages the development and proliferation of Sertoli cells responsible for the production of androgen binding protein (ABP), needed for arousing the spermatogonium to initiate the process of spermatogenesis. In addition, GtH-II stimulates interstitial or Leydig cells to secrete the hormone testosterone, while the interstitial cell stimulating hormone (ICSH) influences the development of seminiferous tubules and Sertoli cells needed in the production of ABP. This conjoins with testosterone to incite spermatogenesis, alongside the collective function of stimulating sperma development.

Conclusion

Based on the result and discussion, it was concluded that GnRH treatments possess the capacity to reduce spermatozoa abnormalities and also increase testosterone concentrations in Nubian goats.

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Conflict of Interests

The authors declare that they have no conflict of interests.

Author's Contribution

Syafruddin Syafruddin, Faris Iryandi, Riska Asria Sa'adatur Rahmi, Husnurrizal Husnurrizal wrote the manuscript and conducted the research, Hafizuddin Hafizuddin and Tongku Nizwan Siregar conceptualized the research, and Teuku Armansyah TR., Budianto Panjaitan, Arman Sayuti, Amalia Sutriana, Dwinna Aliza revised the final form of the manuscript.

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