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Ultrasound Imaging of the Testes and Accessory Sex Glands in Male Goat Treated with GnRH During Non-Breeding Season

[1]Hamed T. Elbaz, [1]Emad M. Abdel Razek and [2]Ahmed Dawod

[1]Theriogenology Department, Faculty of Veterinary Medicine, University of Sadat City, Menofia, Egypt
[2]Husbandry and Animal Wealth Development Department, Faculty of Veterinary Medicine, University of Sadat City, Menofia, Egypt

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Corresponding Author:
Ahmed Dawod
Husbandry and Animal Wealth Development Department,
Faculty of Veterinary Medicine,
University of Sadat City,
Menofia, Egypt
Email: adawod1980@gmail.com

Abstract: The aim of the present study was to determine the effect of GnRH on the measurements of testes, accessory sex glands and testosterone hormonal concentrations during the non-breeding season. Eight mature healthy Egyptian Baladi male goat injected with Buserelin acetate (GnRH analogue) 8µg once weekly for one month. Ultrasonographic measurements of the scrotum and its contents and all accessory sex gland and blood samples collection were done one week before experiment and one week after the end of experiment. Results revealed that scrotal circumference, length and breadth of both testes did not differ significantly ($p>0.05$). While, the length and breadth of the tail of epididymis was increased significantly after treatment ($p<0.05$). The length and breadth of vesicular gland and bulbourethral gland differed significantly ($p<0.05$) after treatments. The ampulla and pars disseminata of prostate breadth was increased significantly ($p<0.05$). Treated bucks showed higher values for serum testosterone after treatment. In conclusion, the present results demonstrated that GnRH injection ameliorates male goat reproductive efficiency. Moreover, ultrasonography is an affirmative indicator to the response of male reproductive system to exogenous GnRH analogue treatment during the non-breeding season.

Keywords: Bucks, GnRH, Testosterone, Ultrasonography

Introduction

Male reproductive efficiency may be enhanced by melatonin treatment (Rosa *et al*., 2000), or by applying artificial lighting regimes (Delgadillo *et al*., 2004).The eCG injections stimulate testosterone secretion and testicular activity in rams (Hochereau-De *et al*., 1990; Price *et al*., 1991). There was a strong relation between testis volume and serum testosterone concentration after injection of GnRH in male animals (Schanbacher and Lunstra, 1977; Anderson, 1992; Gabor *et al*., 1995). On the contrary, Schneider *et al*. (1998) stated that prolonged administration GnRH resulted in decreasing of spermatogenesis, reduced the size and testicular volume. FSH can stimulate the testicular activity through its receptors on leydig cells, sertoli cells and peri-tubular myoid cells (Walker and Cheng, 2015). Rams administrated with two doses of eCG before joining with anestrous ewes, stimulate their ability to induce ewes reproductive activity and enhance the ram effect by increasing androgen hormone (Ungerfeld *et al*., 2014). Long-term treatment of GnRH at lower doses had a stimulatory effect on the pituitary gland and indirectly affects gonadal function but in large doses have a depressive effect on prostate, testis and vesicular glands (Warner *et al*., 1983). The non-breeding season can affect the reproductive pattern of rams desire, sexual activity, androgen concentration, testicular volume and semen parameters (Avdi *et al*., 2004; Dufour *et al*., 1984; Gündogan, 2007). Reproductive efficiency in bucks considered as seasonal breeding and reliant on breed and geographical distribution (Fatet *et al*., 2011). Seasonal variations in testicular measuraments have been described in many ram breeds (Dickson and Sanford, 2005). Testicular measuraments can be changed by photoperiod, Season, temperature and relative humidity

(Pelletier *et al*., 1982; Rosa *et al*., 2000). When the day light was decreased the testes and spermatogenesis reach their peak activity during the autumn season (Lincoln, 1998).on the other hand, season can ameliorates androgen secretion (Kafi *et al*., 2004), improve the testicular hemodynamics with changes in circulating androgen and estrogen in Shiba goats (Samir *et al*., 2018). Testicular echogenicity, FSH concentration and thyroid hormone can be used for puberty judgement (Bartlewski *et al*., 2017). Ultrasound can be used in the visualization the scrotal contents (Ragheb and Higgins, 2002; Ahmadi *et al*., 2012) and seminiferous tubules lumination (Giffin *et al*., 2014). Assessment of actual testicular dimensions in male goat can be done by ultrasonography (Samir *et al*., 2015). Therefore, the aim of the present study is to evaluate the effect of the Buserelin acetate (GnRH analogue) injections on the measurements of testes, epididymis, accessory sex glands and some hormonal parameters during the non-breeding season of Baladi male goat with the help of ultrasound.

Materials and Methods

Animals

The present study was carried out on a total number of eight adult bucks (Egyptian Baladi male goat) belonged to the educational farm, Faculty of Veterinary Medicine, Sadat City University, Egypt. Bucks aged between 2 and 2.5 years and averaging (40-50) kg body weight during the period from (May to June, 2017). All bucks were apparently normal, housed in free stall barn and fed a balanced ration (14% crude protein, 15% crude fiber, 9% ash and 2% fat), as well as free access of the drinking water and green fodders (Alfalfa).

Experimental Design

Animals (n=8) was injected with Buserelin acetate (GnRH analogue) (2 ml IM from Receptal®, Intervet, International B.V. European Union (EU)), each buck injected with a dose of 8 µg once weekly for one month. Ultrasound examination and blood sampling of all animals were done one week before experiment and one week after the end of experiment.

Testis Measurement

Scrotal circumference was measured by measuring steel tape (Ahmed and Noakes, 1995). Testis length was measured from top of the tail to the head of the epididymis for each testis using caliper (Islam and Land, 1977). All testes length was done for both right and left one.

Ultrasonographic Measurements of Reproductive Organs

Ultrasonographic imaging of the testes and epididymis were done in the standing position. An ultrasound examination of bucks was done by means of 5-7.5 MHz linear probe of Scanner (Sonoscape-A5V, Shenzhen, China) per scrotal cutaneous to investigate the testis, epididymis and spermatic cord according to method previously described (Gouletsou *et al*., 2003) one week before and one week after the end of experiment and the measurements were recorded for each side. Ultrasonographic imaging of the accessory sex glands including ampulla, vesicular, prostate and bulbourethral glands were scanned per rectum using ultrasound scanner with 5-7.5 MHz linear array trans-rectal probe. The transducer was fitted in a self-manufactured connector to favor its manipulation per rectum according to method previously done by (Mahmoud *et al*., 2013). All examinations were done by the same operator. The measurements of all accessory genital glands were recorded.

Blood Sampling and Hormonal Assay

The collected blood samples (10 ml) from jugular vein were allowed to clot at 4°C for 10 h in the refrigerator then centrifuged at 3000 rpm for 15 minutes and the separated sera were stored at -20°C until subsequent analysis. Serum (testosterone, FSH, LH) concentration was determined using ELISA kits (Calbiotech, Austin, Springer valley, CA, 91978, USA) using the micro-well method and the OD absorbance has been determined at 450±10 nm.

Statistical Analysis

Statistical analysis was performed using (GraphPad prism 5 software Inc., La Jolla, CA). Comparison between groups was made by self-paring t-test. Results are presented as means±standard errors. Values of $p<0.05$ were considered significant.

Results

The morphometric measures of both testes including length and scrotal circumference increased numerically not statistically after hormonal treatment ($p>0.05$). The ultrasonographic measurement of scrotum and its content as presented in (Table 1) revealed that the length, breadth of both testes increased numerically not statistically after hormonal treatment ($p>0.05$) (Fig. 1). While, the length and breadth of epididymal tail was increased significantly after treatment ($p<0.05$) (Fig. 2). Also, there was no significant difference in measurements of the spermatic cord ($p>0.05$). The ultrasonographic measurement of accessory sex gland and hormones concentrations as presented in (Table 2) revealed that the breadth of ampullae was increased

significantly after hormonal treatment ($p<0.05$) (Fig. 3). There was a significant difference in length and breadth measurements of vesicular glands after treatment ($p<0.05$) (Fig. 4). In addition, the breadth of pars disseminata of prostate gland was increased significantly ($p<0.05$) (Fig. 5). There was a significant difference in length and breadth measurements of bulbourethral glands after treatment ($p<0.05$) (Fig. 6). Furthermore, treated bucks showed higher values for serum testosterone ($p<0.05$) (increase from 1.8±0.18 to 3.25±0.23 ng/ml after treatment). While, FSH and LH were slightly decreased after treatment ($p>0.05$) as shown in (Fig. 7).

Table 1: Scrotal circumference (cm) and ultrasonographic measurements (mm) of tests, epididymal tail and spermatic cord before and after treatment (mean±SEM)

Item	Before treatment	After treatment
Scrotum		
Scrotal circumference (cm)	25.75±0.55	26.25±0.61
Ultrasonographic measurements		
Testes		
Length right	35.52±1.17	36.2±1.27
Breadth right	41.17±1.13	42.10±1.17
Length left	39.50±1.2	40.32±0.69
Breadth left	38.90±1.0	39.71±1.12
Epididymal tail		
Length right	22.10±1.29^{b}	23.36±0.76^{a}
Breadth right	17.20±0.64^{b}	20.05±1.3^{a}
Length left	22.96±1.64^{b}	24.0±1.5^{a}
Breadth left	16.40±0.39^{b}	17.85±0.34^{a}
Spermatic cord		
Breadth right	18.35±0.95	18.5±0.96
Breadth left	18.55±0.78	18.61±0.55

The values carrying different letters in the same row were statistically different ($p < 0.05$).

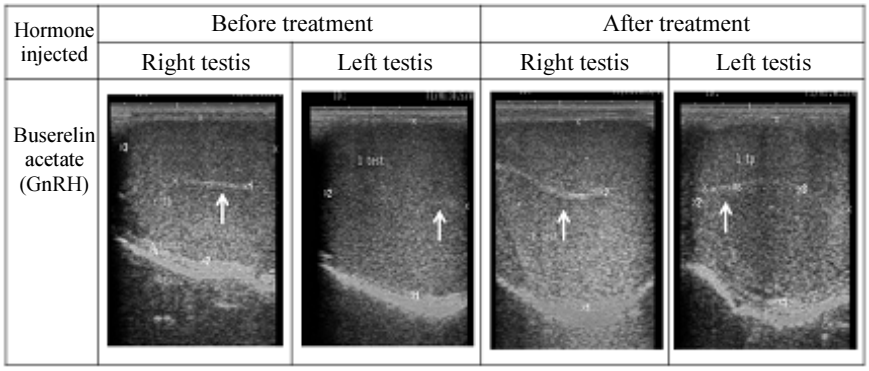


Fig. 1: Ultrasonographic image of buck's testes treated with GnRH analogue (Buserelin). No significant difference was found in length and breadth of testes and increase in echogenicity of mediastinum testes after GnRH injection (arrow)

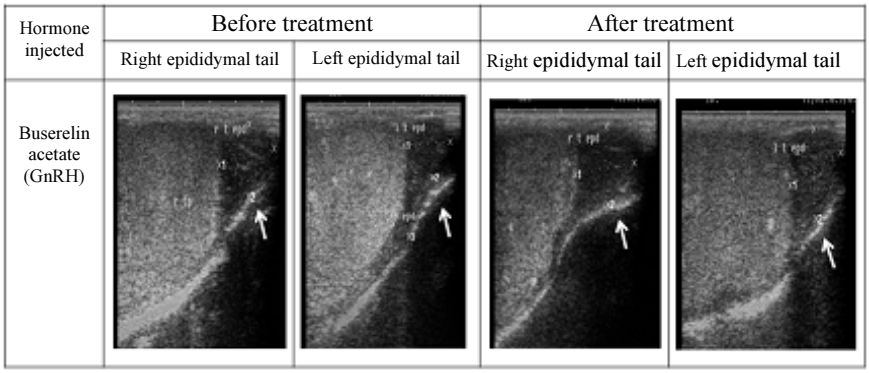


Fig. 2: Ultrasonographic image of buck's epididymal tail treated with GnRH analogue (Buserelin). Note the hypoechogenic texture and a significant increase in the epididymal tail length and breadth after GnRH injection

Table 2: Ultrasonographic measurements of accessory sex gland (mm) (ampulla, vesicular, p.disseminata of prostate and bulbourethral glands) and blood hormone concentrations (testosterone, FSH, LH) before and after treatment (mean ±SEM)

Item	Before treatment	After treatment
Accessory genital glands		
Ampulla		
Diameter right	5.1±0.32[b]	5.7±0.30[a]
Diameter left	5.05±0.33[b]	5.7±0.50[a]
Vesicular glands		
Length right	25.07±1.15[b]	29.5±1.72[a]
Diameter right	10.40±0.30[b]	11.75±0.59[a]
Length left	22.62±1.28[b]	25.47±0.91[a]
Diameter left	10.10±0.64[b]	12.47±0.77[a]
P.disseminata Prostate		
Diameter	14.10±0.39[b]	14.62±0.39[a]
Bulbourethral glands		
Length right	13.30±0.25[b]	15.2±0.57[a]
Diameter right	12.07±0.56[b]	14.1±0.69[a]
Length left	14.02±0.61[b]	14.7±0.57[a]
Diameter left	11.92±0.47[b]	12.9±40[a]
Hormone concentrations		
Testosterone (ng/ml)		
FSH (mIU/ml)	1.84±0.18[b]	3.25±0.23[a]
LH (mIU/ml)	3.86±0.27	3.65±0.21
	1.72±0.02	1.68±0.03

The values carrying different letters in the same row were statistically different ($p<0.05$).

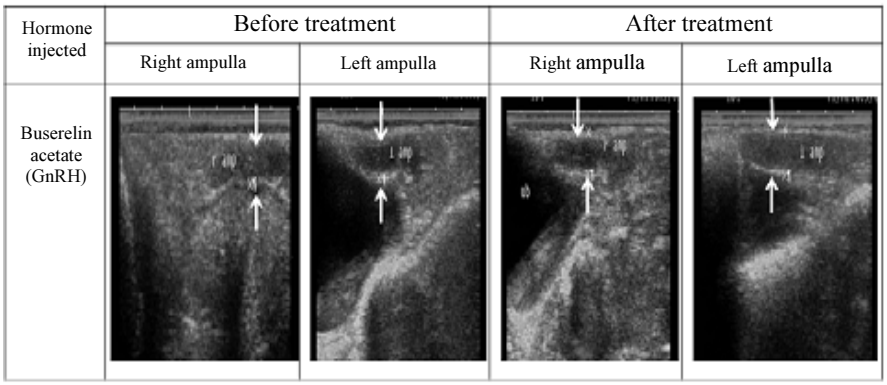


Fig. 3: Ultrasonographic image of buck's ampulla treated with GnRH analogue (Buserelin). Note the moderate non-echogenic texture and a significant increase in the breadth of ampulla after GnRH injection

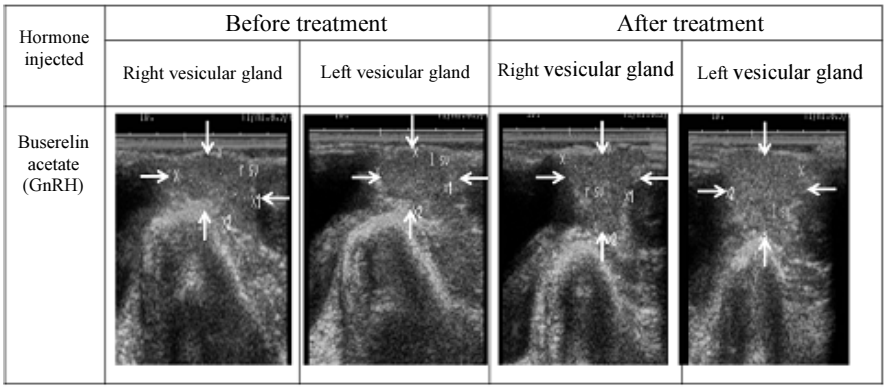


Fig. 4: Ultrasonographic image of buck's vesicular glands treated with GnRH analogue (Buserelin). Note the moderate hypoechogenic texture and a significant increase in length and breadth of vesicular glands after GnRH injection

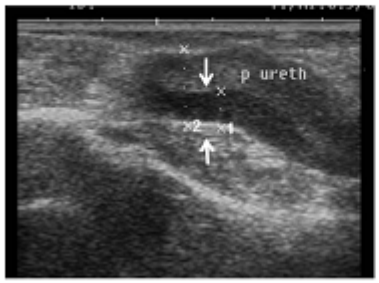
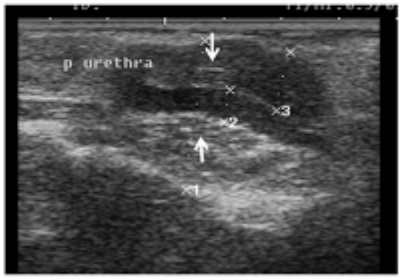
Hormone injected	Before treatment	After treatment
	Pelvic urethra and disseminate prostate	Pelvic urethra and disseminate prostate
Buserelin acetate (GnRH)		

Fig. 5: Ultrasonographic image of buck's prostate gland (p. disseminata) treated with GnRH analogue (Buserelin). Note the echogenic texture and a significant increase in the breadth and echogenicity of p. disseminata enclosing the lumen of pelvic urethra after GnRH injection (arrow)

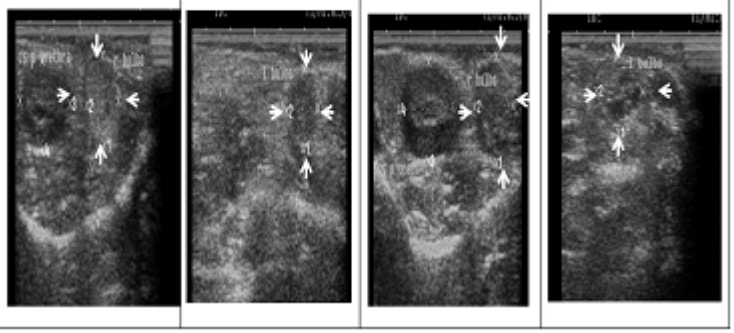
Hormone injected	Before treatment		After treatment	
	Right bulbourethral gland	Left bulbourethral gland	Right bulbourethral gland	Left bulbourethral gland
Buserelin acetate (GnRH)				

Fig. 6: Ultrasonographic image of buck's bulbourethral glands treated with GnRH analogue (Buserelin). Note the hypoechogenic texture and a significant increase in length and breadth of bulbourethral glands after GnRH injection

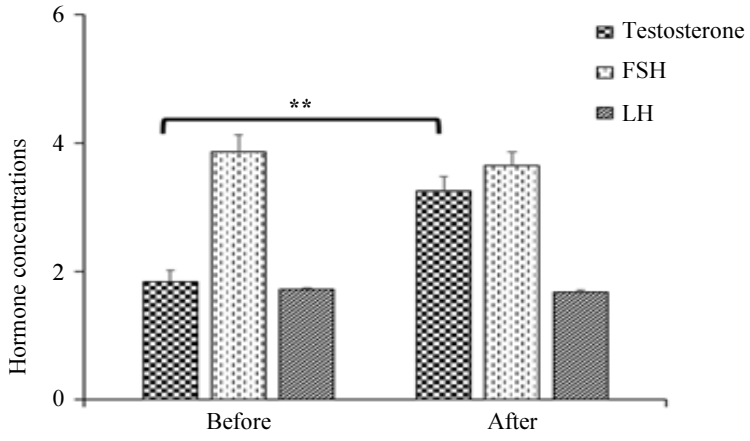


Fig. 7: Changes in serum concentration of testosterone, FSH, LH in bucks before and after treated with GnRH analogue (Buserelin).*Indicates significant difference before and after treatment ($p<0.05$)

Discussion

Ultrasonography was a modern diagnostic device used for examination of many internal organs including its size, structure and any pathological lesions with real time images and can evaluating the response to different treatments (El-Khawaga *et al*., 2012). Gonadotropin releasing hormone (GnRH) controls the activity of the gonadotrope cells of the pituitary gland which is a essential component of the endocrine cascade that determines the growth, development and functional activity of testicular tissue (Adams, 2005). In the present study the morphometric measures of both testes including length and scrotal circumference increased numerically not statistically. In accordance with the results obtained by (El-Khawaga *et al*., 2012) the testicular diameter did not significantly differ before and after treatment with GnRH. In the present study the ultrasonographic measurement of scrotum and its content revealed that the length and breadth of both testes increased numerically not statistically. Other results from a previous study (Medan *et al*., 2006) indicate that there are no variations in scrotal circumference and sperm cell concentrations between the summer and autumn in Shiba goats. Also, there was no effect on scrotal circumference and testes dimensions after GnRH injections (Ronayne *et al*., 1993). A finding which might be due to the short course of treatment, species difference and photoperiod.

In the present study the length and breadth of epididymal tail was increased significantly after treatment. Results from this study are in accordance with those obtained by (El-Khawaga *et al*., 2012) who mentioned that the stimulatory role of androgen produced from interstitial leydig cells in response to pituitary LH release following GnRH treatment. Matos and Thomas (1991) mentioned that a strong relation between testicular size with plasma FSH and testosterone concentrations. Aspden *et al*. (1998) Long term administration to supplemental GnRH or a potent GnRH agonist accelerate puberty in calves, increasing steroidogenic and spermatogenic activity of developing bulls.

Accessory sex glands viewed in Egyptian Baladi bucks after GnRH analogue in the current study revealed a significant difference in length and breadth measurements of (vesicular gland and bulbourethral gland) after treatment. In addition, the breadth of ampullae and pars disseminata of prostate gland was increased significantly after hormonal treatment which may be due to the higher level of serum androgen following treatment. In agreement with results obtained by (El-Khawaga *et al*., 2012). The growth and differentiation of accessory genital glands were mainly controlled by testosterone hormone (Risbridger and Taylor, 2006). A single injection of GnRH to yearling dairy bulls led to a distinct release of LH and androgen (Malak and Thibier, 1982). Recently, androgen and cyclooxygenase-2 as essential regulators for the growth and secretory activity of epithelial cells in the seminal gland of bucks (Emam, 2016). While, androgen concentrations did not differ between the peri- and post-pubertal rams, proposing that the size and functional activity of the accessory genital glands during that change were controlled by other factors (Camela *et al*., 2017). This discrepancy may be due to species difference, hormonal dose and timing of sampling. Regarding effect of GnRH on testosterone secretion, the present results showed a higher value for serum testosterone increase from (1.8±0.18 to 3.25±0.23 ng/ml) after treatment. In accordance with the results obtained by (El-Khawaga *et al*., 2012) who reported that doses 8 and 12 µg had a significant positive feedback on testicular hormonal secretory function of buffalo bull. In addition prolonged treatment with the GnRH agonist increase LH, FSH and testosterone concentration in prepubertal bulls (Jiménez-Severiano *et al*., 2003). However, the lowest testosterone value was recorded in the winter period (2.31±0.14 ng/ml) while the highest in autumn (17.81±1.07 ng/ml) (Sarlos *et al*., 2013). Even though, active immunization against GnRH depress synthesis of gonadotropins (LH and FSH), gonadal atrophy,affect gametogenesis, suppresses reproductive activity and form infertility of both male and female animals (Fagerstone *et al*., 2010). The concentrations of LH and testosterone were augmented four to eight fold 12–24 h after beginning continuous treatment of a potent GnRH agonist to mature rams (Lincoln *et al*., 1986). Karaca *et al*. (2015) stated that there is a strong relationship between reduction in sexual reaction time together with the increase in androgen concentration in rams. However, other studies reported that no correlation between circulating levels of testosterone and sexual desire in rams (Moghaddam *et al*., 2012). FSH and LH in the current study were slightly decreased after GnRH treatment. Xu *et al*. (1993) reported that there was a difference between groups in the pattern of seasonal variation in the total LH response to GnRH.Also, Bhasin and Swerdloff (1986) revealed that treatment of males with GnRH agonists causes a down-regulation of anterior pituitary gland which was associated with absence of pulsatile secretion of LH and FSH. Also, Ronayne *et al*. (1993) the continuous treatment of GnRH analogue to 5-month old bulls for 28 or 56 day forming increase in testosterone secretion and reduction in the LH. we proposed that elevated values of testosterone might generate a negative feedback mechanism on hypothalamic-pituitary-gonadal axis. The bucks used in the current study were physically mature and they were effectively monitored not to fluctuate in their body weight during this study. Therefore, the

changes in testes, epididymis and accessory sex glands can be attributed to GnRH injection.

Conclusion

The current study indicated that GnRH administration was associated with changes in dimensions of testes, tail of epididymis and accessory sex glands (vesicular gland, bulbourethral gland) and breadth of (pars disseminata of prostate and ampulla). The initial rise in testosterone concentrations may be necessary for initiation and maintenance of spermatogenesis during non-breeding season. These results could be an important step to improve the reproductive performance in bucks during non-breeding season in Egypt.

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Author's Contributions

Hamed T. Elbaz, Emad M. Abdel Razek and Ahmed Dawod: Conception, design and conduction of the study.

Emad M. Abdel Razek: Acquisition of data.

Hamed T. Elbaz and Ahmed Dawod: Analysis and interpretation of data.

Ahmed Dawod: Drafting the manuscript.

Hamed T. Elbaz, Emad M. Abdel Razek: Critical revision. All authors have read and approved the manuscript.

Ethics

All of the authors confirm that this article is original and no ethical issues are concerned with the present article.

Disclosure Statement

The authors warrant that there are no conflicts of interests among authors.

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