Journal of Reproduction and Infertility 3 (1): 08-16, 2012
ISSN 2079-2166

DOI: 10.5829/idosi.jri.2012.3.1.63186

Ultrasound Imaging of the Testes and Accessory Sex Glands in Buffalo Bulls Treated with Gonadotrophic Releasing Hormone

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Abstract: The current study was designed to find out the effect of different doses (8, 12 or 16 µg) of GnRH analogue (Buserelin acetate, BA) on the ultrasonographic picture and biometry of buffalo bulls' reproductive organs. Animals (n=9, aged 15-18 months) were allocated into three groups (n= 3/group) according to the hormonal dose that was administered once weekly for six weeks. Results revealed that the testicular diameter and epididymal tail width did not differ significantly before and after treatment. The epididymal tail length did not differ significantly before and after treatment with the 8µg, while it differed significantly ($P<0.05$) with doses of 12 and the 16 µg GnRH. The ampulla ductus deference and seminal vesicle heights differed significantly ($P<0.05$) before and after treatments. The prostatic body differed significantly ($P<0.05$) after treatments with 8 and 16 µg. The pars disseminate of prostate gland and the bulbourethral gland height did not differ significantly regardless the dose of treatment. The bulbourethral gland length differed significantly after treatment with the 8 µg, while it did not show significant differences with doses of 12 and the 16 µg of GnRH. In conclusion, the present results demonstrated that GnRH injection at a dose of 12 µg efficiently affect buffalo bulls reproductive organs. Moreover, ultrasonography is an affirmative indicator to the response of male reproductive system to exogenous GnRH analogue treatment.

Key words: Buffalo bulls % GnRH % Prepubertal % Testosterone % Ultrasonography

INTRODUCTION

Gonadotrophic releasing hormone (GnRH) agonists and antagonists are increasingly used to modulate the fertility, behavior and productivity of male animals. The integrity of the hypothalamic-pituitary connection is essential for testicular maturation and function [1]. Administration of estrogenic compounds (estradiol or zeranol), suppressing the episodic release of GnRH via the hypothalamus, delayed the onset of puberty in bull calves [2,3]. Increased episodic secretion of GnRH heralds the onset of testicular maturation at puberty. A significant correlation between testis volume and serum testosterone concentration has been indicated after injection of GnRH in Holstein- Friesian bulls [4].

The present investigation aimed to studying changes in the reproductive organs (testes, epididymis and accessory sex glands) after treatment of Egyptian buffalo bulls with GnRH before age of puberty by means of ultrasonographic and hormonal evaluation.

MATERIALS AND METHODS

Animals: The present study was carried out on a total number of nine puberal Egyptian buffalo bulls belonged to the Educational Farm - Faculty of Veterinary Medicine - Benha University. Bulls aged 15-18 months with 3-4 body condition score [5] and 400-450 Kg body weight during the period from April to October, 2009. All animals 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 (clover in winter and corn crops in summer).

Experimental Design: Animals (n=9) were allocated into three groups (n= 3/group) according to the dose of Buserelin acetate (GnRH analogue; Receptal®, Intervet International B.V. European Union (EU)). GnRH was injected i.m. at a dose of 8, 12 or 16 µg once weekly for six weeks. Blood sampling and an ultrasound examination of

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all animals were done every two weeks for 6 times (3 times before and 3 times after treatment with GnRH analogue).

Ultrasonographic Examination of Male Reproductive Organs: An ultrasound examination of bulls was done by means of 6-8 MHz linear probe of Scanner 240 Vet (Pie medical company®, Netherlands) per cutaneous to investigate the testis and epididymis and per rectum to evaluate the accessory sex glands (ampulla ductus deference (ADD), vesicular glands, prostate gland (body and pars disseminate) and the bulbo-urethral glands) according to the different methods previously described [6, 7]. Briefly; the testes were located extra-abdominally within a pendulous scrotum in the inguinal region. Testes and the epididymal tail were visualized in longitudinal plane scrotal wall, with the later present at the distal extremity of testes. The accessory sex glands were observed per rectum. The urinary bladder-urethral junction was located a few centimeters cranial to the pelvic brim. ADD was scanned from point dorsal to the bladder with excretory ducts on dorsal wall of the urethral lumen. The paired seminal vesicles were located lateral to the neck of the urinary bladder and cranial to the prostate gland. The pars disseminate of prostate gland was visible within the pelvic urethra between the anechoic urethral lumen and urethral muscle. The prostatic body was located dorsal to the neck of the bladder. The bulbourethral glands appeared embedded within the bulbospongiosus muscle at the dorso-lateral aspect of the pelvic urethra when it begins to curve posteriorly around the ischial arch.

Blood Sampling and Hormonal Assay: The collected blood samples (10 ml) by using plain vacutainer tubes wer centrifuged at 3000 rpm for 15 minutes and the separated sera were stored at -20°C until assayed for testosterone concentration by using EIA-1559 ELISA kit (DRG Instruments GmbH, Marburg, Germany) and the OD absorbance has been determined at 450 ±10 nm by means of by Stat Fax 2100 ELIZA Reader (Awareness Technology, Inc. USA) according to manufacturer instructions. Briefly, 25 µl of samples were incubated with 200 µl of enzyme conjugate for 60 min. rinsed 3 times with diluted wash solution (400 µl/ well), then re-incubated with substrate solution (200 µl) for 15 min. and finally the enzymatic reaction was stopped by adding of stop solution (100 µl). The OD absorbance of each well was determined at 450±10 nm with a microtiter plate reader thereafter. The testosterone assay ranged between 0.2 - 16 ng/ml and the sensitivity was 0.17 pg/ml.

Statistical Analysis: The obtained data during each period (before and after treatment) were pooled, tabulated and statistically analyzed by using student t test (to compare the changes in measurements after treatment) and ANOVA (to compare between different groups) where appropriate, according to the SPSS program (Ver. 14). P-Values <0.05 were regarded as significant.

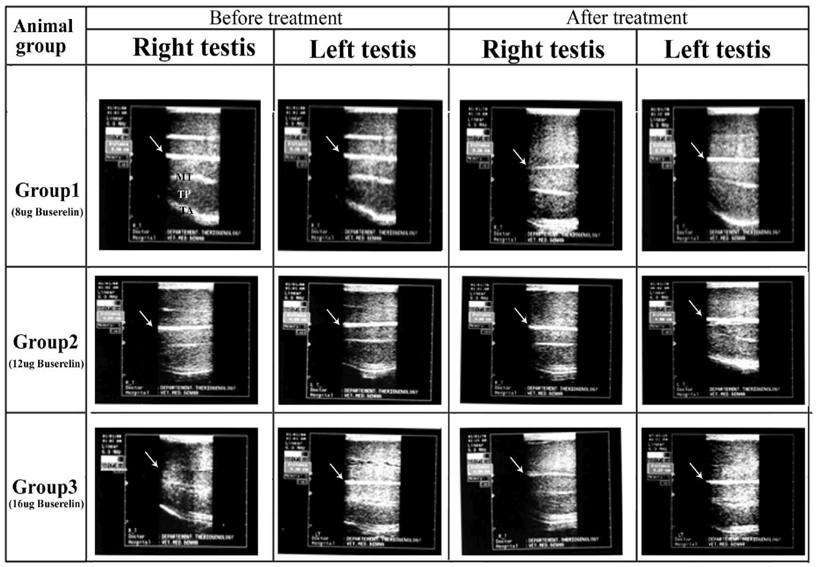
RESULTS

Tsticular Diameter: Ultrasonographic imaging of puberal Egyptian buffalo bulls' testes appeared as homogeneous hypoechoic parenchyma with centrally located hyperechoic mediastinum testis and surrounded by a distinct hyperechoic tunics (Ultrasonogram 1). The diameter of buffalo testes examined by means of an ultrasound after GnRH treatment showed that there was no significant effect of hormonal dose on the mean medio-lateral diameter. Also, within the different treatments, the testicular diameter did not significantly differ before and after treatment (Table 1).

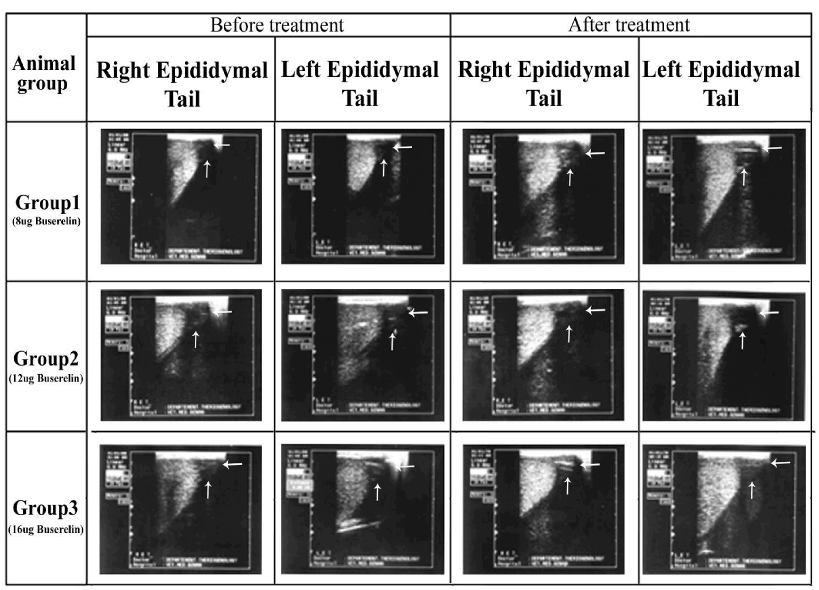
Epididymis: The epididymal tail in the ultrasonographic image appeared as heterogeneous and less echogenic than testis (Utrasonogram 1). After treatment, the epididymal tail length differed significantly among the different doses of GnRH analogue, higher values were recorded after 12 µg injection. Within the different treatments, the epididymal tail length did not differ significantly before and after treatment with the 8 µg, while it differed significantly ($P<0.05$) with the 12 and 16 µg GnRH analogue. On the other hand, the epididymal tail width did not show any significant differences neither between nor within treatment (before and after) groups (Table 1).

Ampulla Ductus Deference: The ADD by means of ultrasound appeared as slightly anechoic irregularly shaped lumen terminated in small hyperechoic excretory ducts on dorsal wall of the urethral lumen (Ultrasonogram 2). After treatment, the ADD differed significantly among the different doses of GnRH analogue, higher values were recorded after 12 µg injection. Moreover, within the different treatments, ADD did vary significantly before and after treatment in all groups under experimentation.

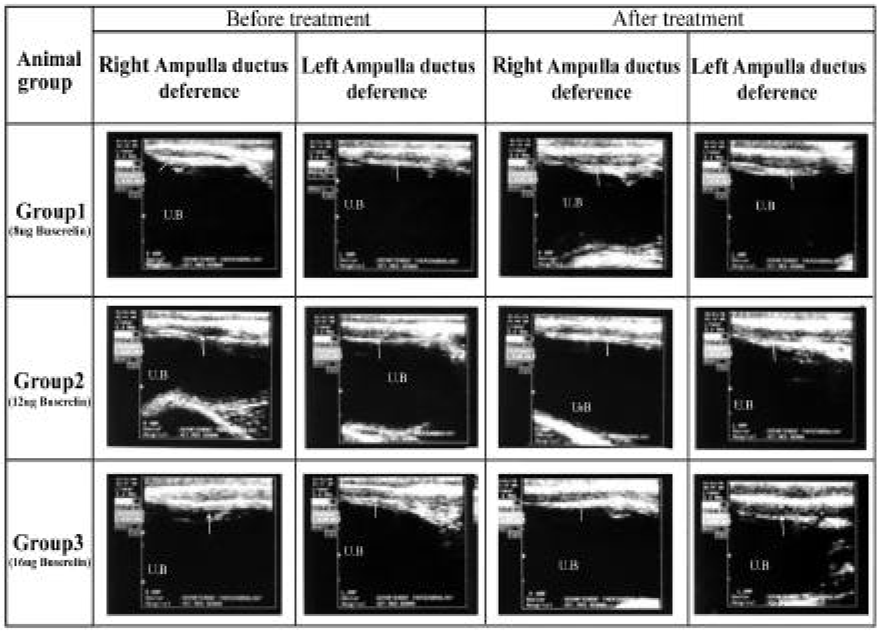
Seminal Vesicles: Ultrasonographic image of puberal Egyptian buffalo bulls' Seminal vesicles showed that the gland was irregular in shape, with isoechoic lobes of



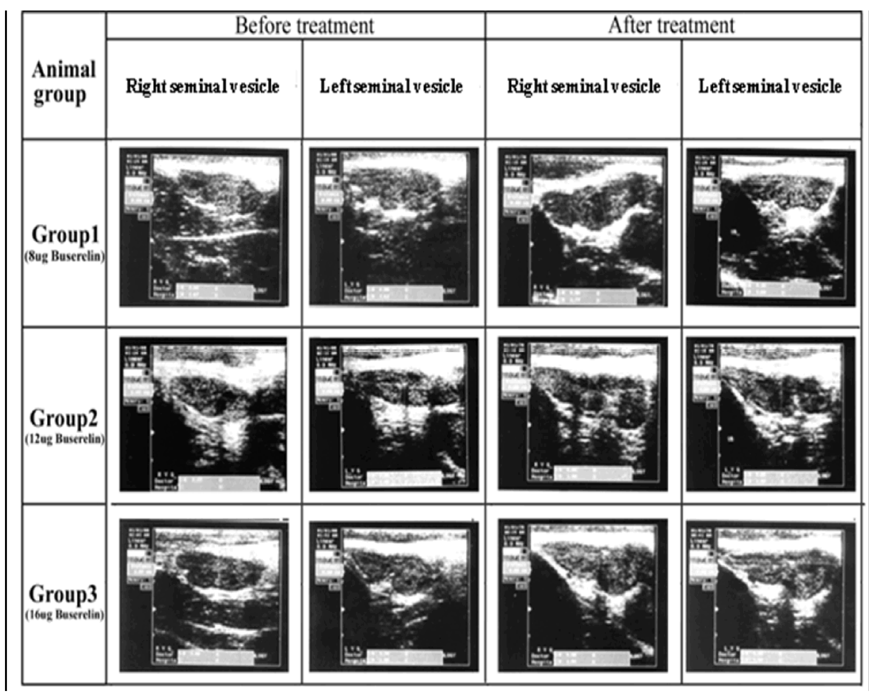
Ultrasonogram 1: Ultrasonographic image of puberal Egyptian buffalo bulls' testes treated with GnRH analogue (Buserelin). The testes appeared as homogeneous hypoechoic parenchyma with centrally located hyperechoic mediastinum testis and surrounded by a distinct Hyperechoic tunics. No significant difference was found in the testicular morphometry between the different treated groups after GnRH injection. MT: Mediastinum testis. TP: Testicular parenchyma. TA: Tunica albuginea.



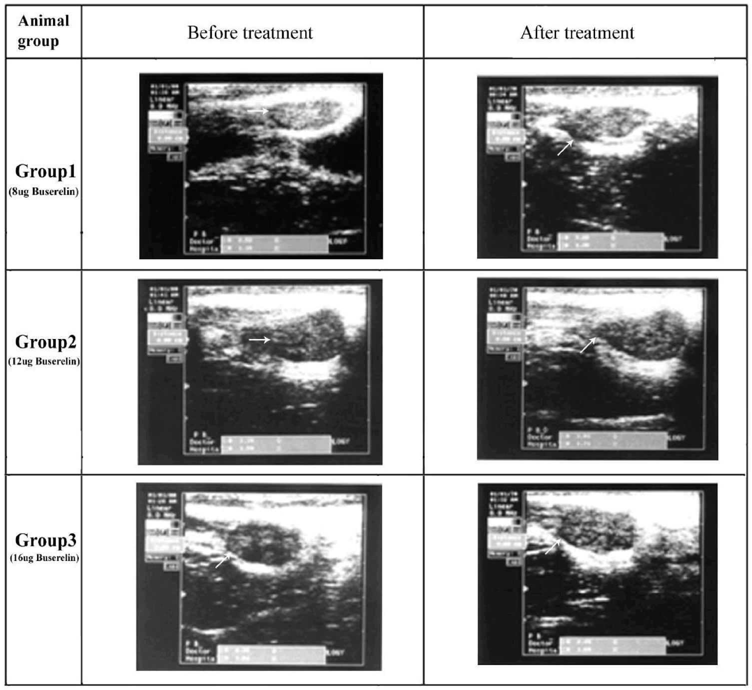
Ultrasonogram 2: Ultrasonographic image of puberal Egyptian buffalo bulls' epididymal tail treated with GnRH analogue (Buserelin). The epididymal tail appeared as heterogeneous and less echogenic than testis. Note the significant increase in the epididymal tail length after treatment with 12 µg and 16 µg GnRH analogue.Ultrasonogram 3 Ultrasonographic image of puberal Egyptian buffalo bulls' Ampulla ductus deference (ADD) treated with GnRH analogue (Buserelin). The ADD appeared as slightly anechoic irregularly shaped lumen terminated in small hyperechoic excretory ducts on dorsal wall of the urethral lumen. Note the significant increase in the ADD height after treatment with 8 µg, 12 µg and 16 µg GnRH analogue.



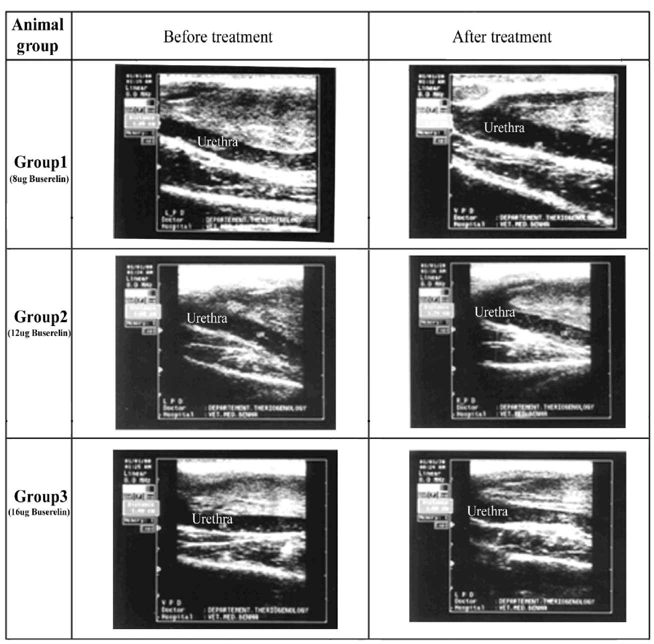
Ultrasonogram 3: Ultrasonographic image of puberal Egyptian buffalo bulls' Seminal Vesicular glands treated with GnRH analogue (Buserelin). The Seminal Vesicular glands appeared irregular in shape, with isoechoic lobes of glandular tissue separated by more hypoechoic regions with variable number of small anechoic fluid filled vesicles. Note the significant increase in the seminal Vesicularglands height after treatment with 8 µg, 12 µg and 16 µg GnRH analogue.



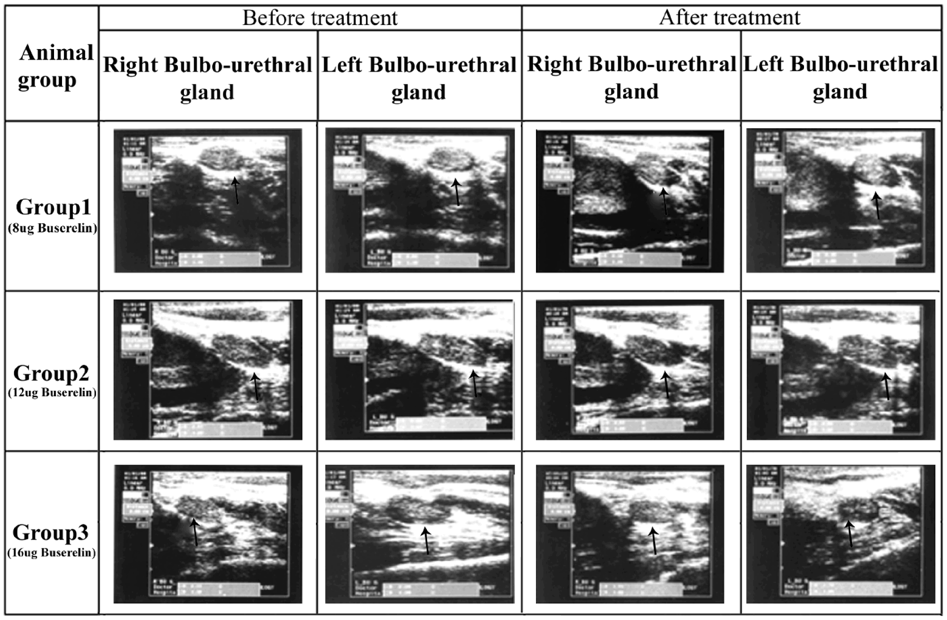
Ultrasonogram 4: ltrasonographic image of puberal Egyptian buffalo bulls' Prostatic body treated with GnRH analogue (Buserelin). The Prostatic body appeared as a uniformly hyperechoic structure located dorsal to the neck of the urinary bladder. Note the significant increase in the Prostatic body height after treatment with 8 µg and 16 µg GnRH analogue.



Ultrasonogram 5: Ultrasonographic image of puberal Egyptian buffalo bulls' Disseminate prostate treated with GnRH analogue (Buserelin). The Disseminate prostate appeared as number of hyperechoic streaks radiating dorso-medially between the anechoic urethral lumen and urethralis muscle. No significant difference was found in the Disseminate prostate morphometry between the different treated groups after GnRH injection.



Ultrasonogram 6: Ultrasonographic image of puberal Egyptian buffalo bulls' Bulbo-urethral glands treated with GnRH analogue (Buserelin). The Bulbourethral glands appeared as ovoid uniformly hyper echoic structure embedded within the bulbospongiosus muscle on the dorso- lateral aspect of the pelvic urethra. Note the significant increase in the Bulbourethral glands length after treatment with 8µg GnRH analogue.



Ultrasonogram 7: Ultrasonographic image of puberal Egyptian buffalo bulls' Bulbo-urethral glands treated with GnRH analogue (Buserelin). The Bulbourethral glands appeared as ovoid uniformly hyper echoic structure embedded within the bulbospongiosus muscle on the dorso- lateral aspect of the pelvic urethra. Note the significant increase in the Bulbourethral glands length after treatment with 8µg GnRH analogue.

Table 1: Changes in the ultrasound dimensions (cm) of the bulls' reproductive organs subjected to different doses of gonadotrophic releasing hormone during the prepuberal period.

	8 µg		12 µg		16 µg	
	Before	After	Before	After	Before	After
Testes diameter	5.15 ± 0.06	5.09 ± 0.11	4.88 ± 0.06	4.86 ± 0.08	4.80 ± 0.18	5.00 ± 0.19
Epididymal tail (length)	2.32 ± 0.05	2.32 ± 0.03^{B}	$2.29\pm0.05^{\dagger}$	$2.48\pm0.05^{\ddagger,A}$	$2.10\pm0.06^{*}$	$2.33\pm0.07^{**,B}$
Epididymal tail (width)	2.18 ± 0.05	2.13 ± 0.04	2.09 ± 0.04	2.06 ± 0.04	1.86 ± 0.04	1.96 ± 0.05
Ampulla ductus deference	0.49 ± 0.01^{b}	$0.62\pm0.01^{a,B}$	$0.60\pm0.02^{\dagger}$	$0.68\pm0.02^{\ddagger,A}$	$0.51\pm0.01^{*}$	$0.56\pm0.02^{**C}$
Seminal vesicles	1.50 ± 0.04^{b}	$1.63\pm0.05^{a,B}$	$1.58\pm0.07^{\dagger}$	$1.81\pm0.05^{\ddagger,A}$	$1.39\pm0.06^{*}$	$1.74\pm0.05^{**,AB}$
Prostate gland (body)	1.29 ± 0.09^{b}	1.47 ± 0.03^{a}	1.59 ± 0.03	1.58 ± 0.04	$1.37\pm0.05^{*}$	$1.53\pm0.05^{**}$
Prostate gland (Pars disseminata)	1.26 ± 0.06	1.38 ± 0.07	1.45 ± 0.10	1.56 ± 0.10	1.33 ± 0.09	1.32 ± 0.10
Bulbourethral gland (width)	1.17 ± 0.03	1.16 ± 0.02	1.08 ± 0.04	1.12 ± 0.02	1.09 ± 0.02	1.15 ± 0.04
Bulbourethral gland (length)	2.16 ± 0.05^{b}	$2.36\pm0.05^{a,A}$	2.37 ± 0.07	2.40 ± 0.12^{A}	2.04 ± 0.05	1.99 ± 0.10^{B}

Values (Mean ±SEM) with different superscript letters (a, b, †, ‡, *, **) within the same raw were significant differences before and after treatments in groups treated with 8, 12 and 16 µg of GnRH analogue. On the other hand, Values with different superscript letters (A, B, C) within the same raw indicated significant differences between groups after treatments. P-value was set at <0.05.

glandular tissue separated by more hypoechoic regions with variable number of small anechoic fluid filled vesicles (Ultrasonogram 3). Regarding the effect of GnRH treatment, the present results showed a significant (P<0.05) difference in the mean value of seminal vesicle (SV) height among treated groups, with the higher response was observed in 12 µg treated bulls. Within the different treatments, SV differed significantly (P<0.05) after treatment with GnRH analogue as compared with before treatment (Table 1).

Prostatic Gland: The prostatic body in ultrasound image appeared as a uniformly hyperechoic structure located dorsal to the neck of the urinary bladder

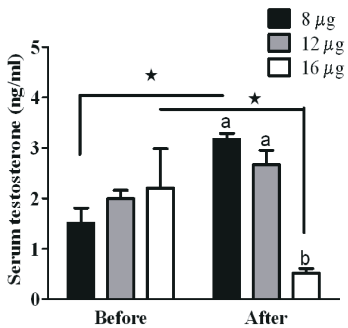


Fig. 1: Changes in serum concentration of testosterone in puberal Egyptian buffalo bulls' before and after treated with GnRH analogue (Buserelin).

(Ultrasonogram 4). The Disseminated part of prostate gland appeared as number of hyperechoic streaks radiating dorso-medially between the anechoic urethral lumen and urethralis muscle (Ultrasonogram 5). Regarding the effect of GnRH treatment, the present results showed a significant ($P<0.05$) difference in the mean value of the prostatic body height between different GnRH analogue treated groups. Within the different treatments, the prostatic body height differed significantly ($P<0.05$) before and after treatment with the 8 and 16 µg, while it did not show any significant difference with the 12 µg GnRH analogue. Nevertheless, the height of the disseminated part of prostate gland did not show any significant difference neither between nor within the treated groups (Table 1).

Bulbourethral Gland: Ultrasonographic image of bulbourethral glands of Egyptian buffalo appeared as ovoid uniformly hyper echoic structure embedded within the bulbospongiosus muscle on the dorso- lateral aspect of the pelvic urethra (Ultrasonogram 6). Regarding the effect of GnRH treatment, the current results in table 1 illustrated a significant ($P<0.05$) difference in the mean value of bulbourethral gland length between GnRH analogue treated groups, with higher values in 12 µg treated group. Within the different treatments, the bulbourethral gland length differed significantly ($P<0.05$) before and after treatment with the 8µg, while it did not vary with the 12 µg or the 16 µg GnRH analogue. Even so, the mean value of bulbourethral gland height was not significantly different between or within the different treated groups.

Serum Concentration of Testosterone: Investigation of testosterone in animals exposed to different doses of GnRH analogue revealed a highly significant ($P<0.005$) variation between treated buffalo bulls (Fig. 1). The lowest serum testosterone concentration (0.52±0.09 ng/ml.) appeared with buffalo bulls treated with 16 µg while it was 3.17±0.12 and 2.67±0.28 ng/ml after injection of 8 and 12 µg GnRH, respectively (Fig. 1).

DISCUSSION

Ultrasonography is one of the most widely used diagnostic tools in modern medicine used to visualize many internal organs, their size, structure and any pathological lesions with real time tomographic images, besides, evaluating the response to and changes in treatment. Gonadotropin releasing hormone (GnRH) controls the activity of the gonadotrope cells of the pituitary gland and, as a consequence, is a critical component of the endocrine cascade that determines the growth, development and functional activity of testicular tissue [8]. In prepubertal bull calves, the early transient rise in gonadotrophin secretion between 10 and 20 wk of age has been found to play a role in the attainment of sexual maturation [9].

The current study indicated that GnRH administered before the age of puberty was associated with changes in the epididymal tail length and accessory sex glands (ADD and seminal vesicles height and bulbourethral gland length) and lacking to affect testicular or epididymal diameter, such effect was very much evident in 12 µg treated bulls.

The ultrasound findings of the testes and epididymal tail diameter of prepuberal buffalo bull in the current work indicated the presence of a non significant change in the testicular diameter and sperm storage capacity of epididymal tail before and after treatment by different doses of GnRH analogue, a finding which might be due to the short course of treatment. However, an increase in the epididymal tail length in buffalo bulls treated by 12 and 16 µg when compared to that observed during treatment by 8 µg GnRH analogue could be anticipated to the stimulatory role of testosterone produced from Leydig cells in response to pituitary LH release following the treatment. Administration of a single injection of GnRH to dairy bulls at 12-13 month of age led to a pronounced release of LH and testosterone [10]. The continuous administration of GnRH analogue to 5-month old bulls for 28 or 56 d resulted in a chronically decrease in the LH pulse frequency, a non significant effect on basal LH, but there was an increase in the testosterone concentration [11]. This indicated that, treatment with GnRH for a prolonged period [12] or with a highly potent GnRH analogue [13] resulted in the depression of spermatogenesis and reduced the size and volume of the tests.

Accessory glands viewed in buffalo bulls after GnRH analogue in the current study revealed a significant increase in the height of ADD and SV and the length of bulbourethral glands which might indicate the increase in serum androgens following treatment. Growth of the prostate and vesicular glands, based on dimensions, is in linear manner with age, but vesicular gland length increases more rapidly after 32 wk of age prior to puberty (46 weeks) [14]. Chronic administration of GnRH at lower doses had a stimulatory effect on the pituitary gland and indirectly affects gonadal function, while in the very high dose range; it has dose-response suppressive effects on prostate, testis and seminal vesicle weights [15].

Regarding effect of GnRH on testosterone secretion, the present results showed a negative effect of high dose on testicular Lydig cells activity and consequently hormonal secretion. On the other hand, 8 and 12 µg doses had a significant positive feedback on testicular hormonal secretory function. Chronic treatment with the GnRH agonist was found to increase blood concentrations of LH and FSH and testosterone concentration in prepubertal bulls [16]. The absence of differences between the later mentioned groups even though the different in hormonal dose, might be attributed to the timing of sampling. Concentrations of testosterone in jugular and spermatic vein blood reached maximum concentrations 3 hr after GnRH (approximately 1 hr after the LH and FSH peak) then returned to pre-injection levels [17].

The age at which a bull is mature for the purpose of breeding has attained greater significance since it necessarily affects the time elapsing before his progeny can be evaluated.

From the present study it can be concluded that GnRH injection at a dose of 12 µg was the most powerful dose affect on bulls' reproductive organs. The change in the biometrics of the genital organs might be assumed in association with the change in the semen quality and quantity of buffalo bulls injected by GnRH analogue. Moreover, the ultrasonography was an affirmative indicator to the semen producing potentials of male reproductive system.

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