



EFFECT OF GnRH ANALOGUE ON LIBIDO AND SEMEN CHARACTERISTICS OF PUBERAL BUFFALO BULLS

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ABSTRACT

Egyptian puberal buffalo bulls (n=9); aged 15-18 months; allocated into three groups (n= 3/group) to verify the effect of different doses (8 µg, 12 µg or 16 µg) of GnRH analogue (Buserelin acetate) on the sexual desire and semen characteristics. Bulls treated with 12 µg of Buserelin were superior to those injected with 8 µg or 16 µg where it shortened the reaction time (6.51 ± 0.38 min. vs. 7.48 ± 0.41 min. and 7.46 ± 0.40 min., respectively) and improved the semen quantity: increased the ejaculate volume (2.22 ± 0.13 ml vs. 1.89 ± 0.13 ml and 1.54 ± 0.11 ml, respectively) and sperm cell concentration ($957 \pm 86.60 \times 10^6$ sperm/ml vs. $928 \pm 86.76 \times 10^6$ sperm/ml and $779 \pm 53.62 \times 10^6$ sperm/ml, respectively), improved the sperm motility ($57.13 \pm 1.36\%$ vs. $63.29 \pm 1.55\%$ and $56.11 \pm 1.51\%$, respectively) and sperm livability ($66.80 \pm 1.23\%$ vs. $69.12 \pm 1.45\%$ and $67.85 \pm 1.87\%$, respectively), and lower sperm abnormalities ($5.08 \pm 0.33\%$ vs. $11.30 \pm 0.95\%$ and $9.16 \pm 0.49\%$, respectively). Instead, Buserelin injection increased testosterone levels in a dose-dependent-manner (1.73 ± 0.57 ng/ml, 4.61 ± 1.28 ng/ml and 4.79 ± 1.21 ng/ml in 8 µg, 12 µg and 16 µg groups, respectively). These results demonstrated that GnRH injection at a dose of 12 µg is optimal to maximize the libido and semen quality of pubertal buffalo bulls.

KEY WORDS: Buffalo bulls, GnRH, Semen, Testosterone

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1. INTRODUCTION

Puberty is defined as the age at which the germinative organs of the male become functional and the reproduction becomes possible [28]. Age of puberty can be enhanced; to a certain extent; by genetic improvement, nutritional and managemental methods [5] or by hormonal application [18]. In cow bull; an early increase in LH secretion is important for early sexual development [15], while its suppression leads to delay in the testicular development [8].

Gonadotropin releasing hormone (GnRH); the key hormone of reproduction; is synthesized in the hypothalamus, and stimulates the secretion of luteinizing

hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary. These gonadotropins are actively involved in male reproductive functions and stimulate the secretion of testosterone hormone required for spermatogenesis and sperm transport.

Madgwick et al. [22] found that the treatment of beef bull calves with GnRH, twice daily, from 4 to 8 weeks of age, might increase the plasma LH concentrations and advance testicular development and reduce age at puberty. Rao [26] demonstrated that the administration of GnRH improved libido

in bulls suffering from low or lack of sex drive.

Chandolia *et al.* [9] indicated that supplementation of yearling bulls with GnRH enhanced testicular growth and increased spermatogenesis and Sertoli cell number in testicular tissue. Moreover, Holstein-Friesian bulls injected with GnRH showed a significant correlation between testis volume and serum testosterone concentration [17]. Although some reports showed that the GnRH administration improved sperm output in Murrah bulls [26], Ayrshire bulls [3], Friesian bulls [11, 20] and buffalo bulls [2], other studies did not reveal any effect to GnRH treatment on the sperm output in Montbeliarde post-pubertal bulls [23] and Nili-Ravi buffalo bulls [30]. Moreover, earlier work [16, 20, 23, 30] indicated that an exogenous treatment with GnRH has no significant effect on the ejaculate volume of bull semen. Abdine *et al.* [2] noticed that the treatment of Egyptian buffalo bulls with GnRH increased the sperm motility (75% vs. 70 % in control group). However, Sajjad *et al.* [30] found that the GnRH treatment of buffalo bulls did not affect the sperm motility. Abdine *et al.* [2] reported that the percentage of sperm abnormalities decreased in Egyptian buffalo bulls treated with GnRH than that in the control bulls.

Bulls treated with GnRH analogues *e.g.* Buserelin, Nafarelin, Leuprolide, were shown to have a characteristic normal plasma LH concentrations but elevated testosterone [24, 27, 29]. It has been found that bull's testosterone secretion is actually enhanced during treatment with GnRH agonist [10, 21, 24, 29].

Although several researches have been reported about the effect of GnRH on the sexual desire and semen of bulls using the same doses [1, 8, 22, 30], till now, no reports was recorded about the effect of using different doses of GnRH on the sexual behavior and the semen producing potential of puberal buffalo bulls. The present study is an attempt to display the effect of several different doses of GnRH

analogue (Buserelin) on sexual desire, serum testosterone and semen characteristics of puberal buffalo bulls.

2. MATERIAL AND METHODS

2.1. Animals

The present study was carried out on nine apparently healthy Egyptian buffalo bulls and free from any genital disorders belonged to the Educational Farm of Faculty of Veterinary Medicine, Benha University; aged 15-18 months; with 5-6 body condition score (1 = extremely emaciated, 9 = excessively fat) was determined according to Campanile *et al.* [7] at the beginning of the experiment. All animals were housed in free stall barn, fed 4 kg/head/day concentrate ration as well as Barseem in winter and darawa in summer *ad libitum* and had a free access to drinking water.

2.2. Experimental design

The present experiment (18 weeks) was divided into 3 sub-periods; pre-treatment, treatment and post-treatment periods; each of which was six weeks in duration. All bulls underwent semen collection and evaluation, sexual desire assessment and blood sampling once weekly. At the end of the pre-experimental period, bulls were assigned randomly into three groups according to treatment dose of Buserelin acetate, a GnRH analogue (Receptal, Intervet Schering Plough; 1ml = 4µg) as follow:

Group I (n=3): Each Bull was injected with 8µg Buserelin acetate *i.m.* once weekly.

Group II (n=3): Each Bull was injected with 12 µg Buserelin acetate *i.m.* once weekly.

Group III (n=3): Each Bull was injected with 16 µg Buserelin acetate *i.m.* once weekly.

2.2.1. Sexual desire assessment

The buffalo bulls libido' was assessed by measuring the reaction time which elapsed

from approaching the bull to the teaser until the ejaculatory thrust was completed [12].

2.2.2. Semen collection and evaluation

Semen samples were collected from each animal by means of an artificial vagina [6] and a non-estrous female teaser. Semen samples were evaluated for volume, sperm motility, livability, abnormalities and concentration [13, 14].

2.2.3. Blood sampling and hormonal assay

The collected blood samples (10 ml) by using plain vacutainer tubes were centrifuged at 3000 rpm for 15 minutes and the separated sera were stored at -20°C until assayed for testosterone concentration by using ELISA kits according to Sarker et al. [31].

2.3. Statistical analysis

The obtained data were tabulated and statistically analyzed, where appropriate, according to the SPSS program ver. 14. Out of the total expected 162 trials of semen collection, an actually 150 trials were used in the statistical analysis.

3. RESULTS

As shown from table (1), the reaction time showed a non-significant difference between buffalo bulls treated by different doses of GnRH analogue. However, the shortest reaction time (6.51 ± 0.38 min.) appeared with buffalo bulls treated by $12\mu\text{g}$ in comparing to those treated with $8\mu\text{g}$ (7.48 ± 0.41 min.) and $16\mu\text{g}$ (7.46 ± 0.40 min.) Buserelin acetate. With the dose of $12\mu\text{g}$, the improvement that occur in the reaction time occur mainly during the treatment (4.30 ± 0.34 min.) compared to those before (8.75 ± 0.37 min.) and after (6.38 ± 1.02 min.) treatment (Table, 2).

A significant ($P < 0.05$) difference on the ejaculate volume was found between buffalo bulls treated by different doses of GnRH analogue (Table, 1). It was $1.89 \pm$

0.13 ml, 2.22 ± 0.13 ml and 1.54 ± 0.11 ml with $8\mu\text{g}$, $12\mu\text{g}$ and $16\mu\text{g}$ respectively. Table (1). With the dose of $12\mu\text{g}$, the ejaculate volume was 2.19 ± 0.19 ml during treatment compared to those before (1.97 ± 0.15 ml) and after (3.32 ± 0.42 ml) treatment (Table, 2).

A significant ($P < 0.05$) difference on the sperm individual motility was shown between buffalo bulls treated by different doses of GnRH analogue (Table, 1). It was $63.29 \pm 1.55\%$, $57.13 \pm 1.36\%$ and $56.11 \pm 1.51\%$ with $8\mu\text{g}$, $12\mu\text{g}$ and $16\mu\text{g}$ respectively (Table, 1). With the dose of $12\mu\text{g}$, the sperm individual motility was $60.42 \pm 1.70\%$ during treatment compared to those before ($54.58 \pm 2.27\%$) and after ($54.17 \pm 3.75\%$) treatment (Table, 2).

A non-significant difference on the sperm livability was shown between buffalo bulls treated by different doses of GnRH analogue (Table, 1). It was $69.12 \pm 1.45\%$, $66.80 \pm 1.23\%$ and $67.85 \pm 1.87\%$ with $8\mu\text{g}$, $12\mu\text{g}$ and $16\mu\text{g}$ respectively (Table, 1). With the dose of $12\mu\text{g}$, the sperm livability was $68.83 \pm 1.52\%$ during treatment compared to those before ($65.17 \pm 2.10\%$) and after ($65.17 \pm 3.75\%$) treatment (Table, 2).

A highly significant ($P < 0.01$) difference on the total sperm abnormalities was shown between buffalo bulls treated by different doses of GnRH analogue (Table, 1). It was $11.30 \pm 0.95\%$, $5.08 \pm 0.33\%$ and $9.16 \pm 0.49\%$ with $8\mu\text{g}$, $12\mu\text{g}$ and $16\mu\text{g}$ respectively (Table, 1).

A highly significant ($P < 0.01$) difference on the sperm cell concentration was shown between buffalo bulls treated by different doses of GnRH analogue (Table, 1). It was 928 ± 86.76 , 957 ± 86.60 and $779 \pm 53.62 \times 10^6$ sperm /ml with $8\mu\text{g}$, $12\mu\text{g}$ and $16\mu\text{g}$ respectively (Table, 1). With the dose of $12\mu\text{g}$, the sperm cell concentration was $1490 \pm 98.97 \times 10^6$ sperm /ml during treatment compared to those before ($397 \pm 47.29 \times 10^6$ sperm /ml) and after ($1050 \pm 108.55 \times 10^6$ sperm /ml) treatment (Table, 2).

With the dose of 12 μ g, the total sperm abnormalities was $4.37 \pm 0.56\%$ during treatment compared to those before ($5.32 \pm 0.41\%$) and after ($7.00 \pm 0.45\%$) treatment (Table, 2).

A significant ($P < 0.05$) difference on the serum testosterone concentration was shown between buffalo bulls treated by different doses of GnRH analogue (Table,

1). It was 1.73 ± 0.57 , 4.61 ± 1.28 and 4.79 ± 1.21 ng/ml with 8 μ g, 12 μ g and 16 μ g respectively (Table, 1). With the dose of 12 μ g, the serum testosterone concentration was 9.18 ± 2.53 ng/ml during treatment compared to those before (0.72 ± 0.14 ng/ml) and after (3.59 ± 1.93 ng/ml) treatment (Table, 2).

Table 1 Reaction time, semen characteristics and serum testosterone concentration of puberal buffalo bulls treated with different doses of GnRH analogue (Buserelin acetate).

Parameters	Treatment group			Overall mean
	8 μ g	12 μ g	16 μ g	
Reaction time (min.)	7.48 ± 0.41^a	6.51 ± 0.38^a	7.46 ± 0.40^a	7.13 ± 0.23
Ejaculate volume (ml)	1.89 ± 0.13^a	2.22 ± 0.13^a	1.54 ± 0.11^{ab}	1.88 ± 0.07
Individual motility (%)	63.29 ± 1.55^a	57.13 ± 1.36^b	56.11 ± 1.51^b	58.49 ± 0.88
Sperm livability (%)	69.12 ± 1.45^a	66.80 ± 1.23^a	67.85 ± 1.87^a	67.83 ± 0.90
Sperm total abnormalities (%)	11.30 ± 0.95^a	5.08 ± 0.33^c	9.16 ± 0.49^b	8.29 ± 0.39
Sperm cell conc. ($\times 10^6$)	928 ± 86.76^a	957 ± 86.60^a	779 ± 53.62^a	885 ± 44.17
Serum testosterone conc.(ng/ml)	1.73 ± 0.57^b	4.61 ± 1.28^a	4.79 ± 1.21^a	3.79 ± 0.65

Values presented in the table are Mean \pm S.E. Values with different superscript letters within the same row differed significantly at least at $P < 0.05$.

Table 2 Reaction time, semen characteristics and serum testosterone concentration of puberal buffalo bulls treated with 12 μ g GnRH analogue (Buserelin acetate).

Parameters	12 μ g GnRH analogue treated group			Overall mean
	Before	During	After	
Reaction time (min.)	8.75 ± 0.37	4.30 ± 0.34	6.38 ± 1.02	6.51 ± 0.38
Ejaculate volume (ml)	1.97 ± 0.15	2.19 ± 0.19	3.32 ± 0.42	2.22 ± 0.13
Individual motility (%)	54.58 ± 2.27	60.42 ± 1.70	54.17 ± 3.75	57.13 ± 1.36
Sperm livability (%)	65.17 ± 2.10	68.83 ± 1.52	65.17 ± 3.75	66.80 ± 1.23
Sperm total abnormalities (%)	5.32 ± 0.41	4.37 ± 0.56	7.00 ± 0.45	5.08 ± 0.33
Sperm cell conc. ($\times 10^6$)	397 ± 47.29	1490 ± 98.97	1050 ± 108.55	957 ± 86.60
Serum testosterone conc.(ng/ml)	0.72 ± 0.14	9.18 ± 2.53	3.59 ± 1.93	4.61 ± 1.28

Values presented in the table are Mean \pm S.E.

4. DISCUSSION

It is well known that a positive correlation present between the androgenic activity of buffalo bulls and the secretory functions of the anterior pituitary gland regulated by

the GnRH [19, 25, 30]. The present study revealed that injection of puberal buffalo bulls with GnRH analogue seemed to have a significant positive effect on the sexual behavior and semen producing potentials,

findings which come in agreement with some previous studies [2, 9, 20, 23, 30].

The improvement in the sexual behavior was measured by the reaction time that was declined during treatment compared to that before and after treatment. These obtained results came in parallel to the results recorded on estimating the serum testosterone for the different treated groups under investigation (Tables 1, 2). Referring to the semen results (Tables 1, 2), it has been emphasized that the quantity of buffalo semen, including the semen volume and the sperm cell concentration was improved by the exogenous injection of GnRH, a finding which was anticipated regarding the mutual effects of GnRH, gonadotropines and testosterone on the testicular and accessory functions [4, 19, 25, 30].

Parallel to the improvement in the semen quantity of buffalo bulls, an improvement was also observed on the semen quality of buffalo bulls injected with GnRH analogue including sperm motility, sperm livability and sperm abnormalities (Table, 1) that might be anticipated in corporation with the increased ejaculate volume and sperm cell concentration, a finding which came in agreement with some previous studies [2, 13, 23, 30]. The present study revealed that the improvement in sexual desire and both; semen quantity and quality was more prominent in buffalo bulls injected by GnRH analogue at a dose of 12 µg rather than those injected either at 8 or 16µg (Table, 1). That mean, the small dose of GnRH (8 µg) might induce a little stimulant effect on the pituitary gland to release more FSH/ ICSH and consequently a little improvement in the secretory functions of the testes and accessory sex glands. On the other hand, the large dose of GnRH (16 µg) might be too high to block even a little quantity of the released gonadotropines from the pituitary gland.

In conclusion, GnRH analogue (Buserelin acetate) injection seems to improve the semen quality and quantity of puberal buffalo bulls. A dose of 12 µg Buserelin

acetate is the optimal dose to maximize the libido, semen quality and quantity of puberal buffalo bulls. The synergistic effect of the exogenous injection of GnRH on the semen producing potentials of buffalo bulls occasionally occur only during the period of GnRH administration, a point of research interest which need to be confirmed.

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