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Research Article

Effects of Oral Administration of Gonadotrophin Stimulant (Theriogon®) on Sexual Behavior and Semen Characteristics in Bulls

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Abstract

Background and Objectives: Application of artificial insemination necessitates proper selection and evaluation of sires for libido and fertility. Deficiencies in either one of these characters can seriously compromise herd production. The objective of the current study was to investigate the effects of oral administration of GnRH stimulant (Theriogon®) on sexual behavior and semen characteristics of bulls. **Materials and Methods:** The study was performed on 16 native Egyptian bulls that were randomly assigned in 2 groups, control group (n = 8 bulls) received amino acid cocktail and treatment group (n = 8 bulls) received GnRH stimulant orally (100 mg kg^{-1} b.wt.,) once/week for 4 weeks. Serum and semen samples were collected twice per week starting 1 week before treatment and extended for 2 weeks after the last treatment. On semen collection site, libido index and reaction time were assessed. Collected semen samples were evaluated for ejaculate volume, pH, sperm motility, percentage of live sperm, sperm cell concentration, total sperm number per ejaculate and sperm cell abnormalities. Testosterone concentration was assessed in serum samples using radioimmunoassay (RIA). **Results:** Oral administration of GnRH stimulant resulted in significant gradual increase in testosterone levels from 2.36 ng mL^{-1} before treatment to 7.33ng mL^{-1} at week 5 after treatment, then declined to level similar to controls at week 7. The increase of testosterone levels was associated with increase in sexual activity expressed by increased libido index and decreased reaction time. Some parameters of semen quality represented by ejaculate volume, sperm cell concentration and total sperm count per ejaculate were also improved after GnRH stimulant treatment. **Conclusion:** Results of the current study suggest that treatment with GnRH stimulant (Theriogon®) could be used to improve sexual activity and some aspects of semen quality in bulls. Further studies are required to investigate the effect of Theriogon treatment on fertilizing ability.

Key words: Bull, GnRH stimulant, libido, semen quality, sperm motility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the last decades, wide application of artificial insemination (AI) necessitated proper selection and evaluation of sires with good libido and highly fertile semen. Libido in bulls has been defined as the willingness and eagerness to mount and attempt service, with mating ability described as complete service. Deficiencies in either one or more of these characters can seriously compromise herd production[1]. High libido bulls are required for maximizing herd fertility and for its probable beneficial effect on the fertility of subsequent female progeny. Several attempts to improve bull libido and semen quality using different treatments were reported previously. Libido and semen quality were significantly improved after treatment with GnRH in bulls[2-4], buffalo-bulls[5,6], dromedary camel bulls[7] and dogs[8]. Injection of PGF2α also increased the number of spermatozoa and testosterone level in bulls[9,10]. α-amino-para-hydroxy-hydrocinnamic acid (synthetic L-Tyrosine, Theriogon®) is an aromatic amino acid derived from phenylalanine by the action of phenylalanine hydroxylase, it is also ingested directly from dietary protein. L-Tyrosine is necessary for the synthesis of catecholamine (dopamine, norepinephrine and epinephrine), thyroxin and melanin[11]. L-Tyrosine mediates its action on gonadal function indirectly through the stimulant effect of catecholamine on both GnRH (GnRH secreting neurons are directly innervated by catecholamine terminals) and growth hormone secretion[12,13]. To our knowledge, there is no published studies on the effect of Theriogon® on sexual behavior and semen characteristics in bulls. Therefore, the present study aimed at studying the effect of α-amino-p-hydroxy-hydrocinnamic acid (Theriogon®) administration on serum testosterone level, sexual behavior and semen quality parameters of Egyptian bulls.

MATERIALS AND METHODS

Animal treatment protocols were approved by Ethics Committee of Alexandria University, Egypt in 2015.

Animals and treatments: The present study was performed on 16 native Egyptian bulls, aged 7-8 years. Animals were kept under the same nutritional and managemental conditions. All bulls were exercised for half an hour every other day and were apparently healthy and free from any genital or reproductive disorders. Animals were randomly assigned in 2 groups; control group (n = 8) received amino acid cocktail (100 mg kg^{-1} b.wt.,) and treatment group (n = 8), bulls were administered oral doses equal to 100 mg kg^{-1} b.wt., of α-amino-p-hydroxy-hydrocinnamic acid (Theriogon®, ADWEA pharmaceuticals, Egypt) weekly for 4 weeks. The drug or amino acid cocktail was dissolved in 200 mL water and administered orally using drenching guns.

Semen collection and evaluation: Semen was collected from all animals twice a week starting one week before any treatment to ensure the soundness of the animals and extended 2 weeks after last treatment. Briefly, semen was collected by artificial vagina and a non-estrous female teaser. Ejaculate volume was estimated visually using graduated collection tubes. Semen pH was measured immediately after collection using pH meter. Sperm cell concentration (10^6/mL) was estimated using hemocytometer, total sperm count per ejaculate was calculated by multiplying the ejaculate volume in sperm cell concentration/mL. Progressive motility was evaluated by light microscopy (x400) with a thermal stage (37°C). The percentage of live spermatozoa and sperm morphology were determined by the supervital eosin/nigrosine staining technique, at least 200 spermatozoa were counted in each sample[14].

Assessment of sexual activity: Indices of sexual activity were assessed during semen collection. Libido index, the degree of sexual interest within 10 min and reaction time, the time that elapses between male recognition of the female and the completion of copulation[1]. In this case the time elapsed required for the bull to complete the ejaculation in the artificial vagina.

Radioimmunoassay (RIA): Blood samples were collected at same times of semen collection using plain vacutainer tubes. Serum was separated by centrifugation at 3000 rpm for 15 min. Serum testosterone concentration was assessed using Radioimmunoassay (RIA) technique[15]. Kits were purchased from Beckman Coulter Diagnostics (Active© DSL-400 RIA), that was verified before for bovine species[15]. Detection range (0.08- 25 ng mL^{-1}), intra-assay coefficient (4.4%).

Statistical analysis: Statistical analyses were performed using R software (version 3.3.1), package 'stats' (R Core Team., 2013). Descriptive statistics were calculated using the psych package and data presented as mean±standard error of the mean (SEM). Semen characteristics, serum testosterone concentration and sexual activity were compared between both control and treatment groups and throughout a 7 weeks observational period (1 week before treatment, 4 weeks of

treatment and 2 weeks after). To describe the influence of oral administration of GnRH stimulant on sperm characteristics (Sperm cell concentration, total sperm count per ejaculate, progressive motility, percentage of live spermatozoa and sperm morphology), sexual activity (Libido index and reaction time) and serum testosterone concentration, during each of the 7 week observational period and all possible interactions, generalized linear mixed models (GLMM) were developed with family set to "Poisson", with the "log" link function, using the lme4 package[16].

Individual animal and the day of data collection were included as random effects for all models and $p \leq 0.05$ was considered significant.

The models included treatment groups (Treated and control), the week of observational period (week 1 through week 7) and their interactions as fixed effects. Statistically significant effects were further analyzed using Tukey's honestly significant difference (HSD) multiple comparison procedures using the multcomp package[17], $p \leq 0.05$ was considered significant.

RESULTS

GnRH stimulant increase serum testosterone and improve the libido of bulls: GnRH stimulant treatment depicted in Fig. 1 resulted in a significant increase in serum testosterone levels starting at week 2 (5.98 vs 2.33 ng mL^{-1}; $p = 0.03$) and peaking at week 5 (7.33 vs. 2.4 ng mL^{-1}; $p = 0.01$) in Theriogon treated and control group, respectively. Then serum testosterone was declined starting 6 to reach to level similar to controls at week 7. No changes in serum testosterones levels were observed in the control group over the treatment period. Indices of sexual activity, libido index and reaction time were significantly improved in response to GnRH stimulant treatment as compared to control group. Libido index (Fig. 2a) was increased from 3.8 before treatment to 6.09 at week 5 ($p = 0.021$), whereas reaction time (Fig. 2b) was reduced from 108.8-43.25 sec ($p = 0.042$). Effects of GnRH on sexual activity declined after cessation of treatment.

Effect of GnRH stimulant treatment on ejaculate characteristics: To investigate the effect of GnRH stimulant treatment on ejaculate characteristics, collected ejaculates were evaluated for pH, ejaculate volume, sperm cell concentration per mL, total sperm number per ejaculate. Semen pH was not impacted by Theriogon® treatment, whereas, ejaculate volume, sperm cell concentration and total sperm per ejaculate were significantly increased post

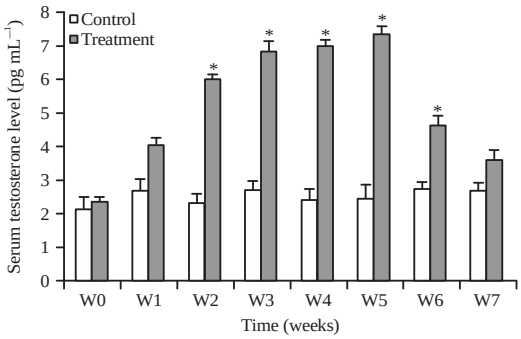

Fig. 1: Effect of GnRH stimulant treatment on serum testosterone levels in bull, treatment group bulls (n = 8) were received oral doses of 100 mg kg^{-1} b.wt., of GnRH stimulant/week for 4 weeks, control group (n = 8) were received same doses of amino acid cocktail. Blood samples were collected twice/week starting one week before treatment (W0) and extended 2 weeks after GnRH stimulant treatment for measurement of serum testosterone by RIA
All parameters are expressed as Mean±SEM. * indicates differences ($p<0.05$) between treatment and control group or the same week of treatment

Theriogon® treatment. Ejaculate volume (Fig. 3a) was increased from 3.9 mL before treatment to 6.8 mL at week 5 post treatment ($p = 0.03$). Sperm cell concentration (Fig. 3b) started to increase significantly from week 2 ($p = 0.032$) after treatment, reaching maximum value at week 6 (1668.1×10^6 sperm/mL. $p = 0.038$), then started to decline at week 6. Likewise, total sperm count per ejaculate showed similar pattern (Fig. 3c) $p = 0.041$).

Effects of GnRH stimulant treatment on sperm motility and viability: Individual sperm motility was assessed using diluted semen and light microscopy (x400), only progressively motile spermatozoa were counted. The superavital stain, eosin nigrosine was utilized to investigate sperm viability. Individual sperm motility and sperm viability showed slight but non-significant increase in response to Theriogon® treatment. Sperm viability showed upward trend compared to control group starting at week 2 (82.6 vs. 75.3%) through week 5 (85.4 Vs. 74.9%), then it declines at week 6 (79.1 vs. 76.1) post treatment as depicted in Fig. 4. Individual motility tended to increase in response to Theriogon® treatment starting week 3 through week 5 before declining at week 6 post treatment.

Effects of GnRH stimulant treatment on sperm cell abnormalities: Sperm cell abnormalities (Fig. 5) were

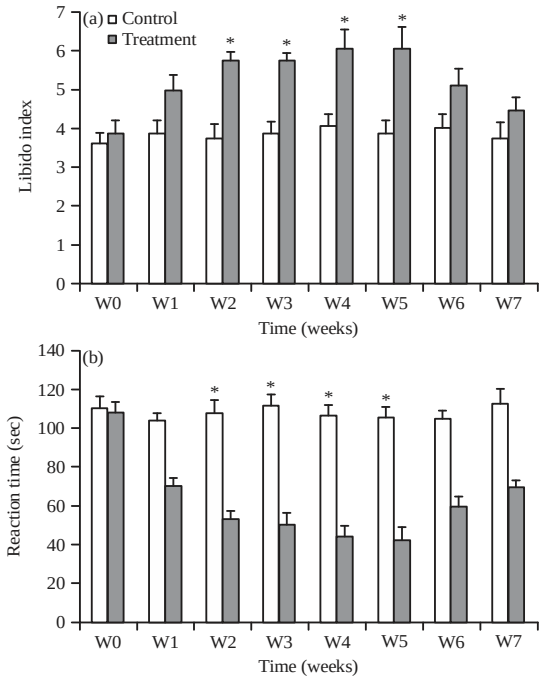

Fig. 2(a-b): Effect of GnRH stimulant treatment on sexual activity of bulls, treatment group bulls (n = 8) were received oral doses of 100 mg kg^{-1} b.w., of GnRH stimulant/week for 4 weeks, control group (n = 8) were received same doses of amino acid cocktail. Sexual behavior was observed twice/week starting one week before treatment (week 0) and extended 2 weeks after GnRH stimulant treatment for assessing (a) Libido index and (b) Reaction time

All parameters are expressed as Mean±SEM. * indicates differences (p<0.05) between treatment and control group for the same week of treatment

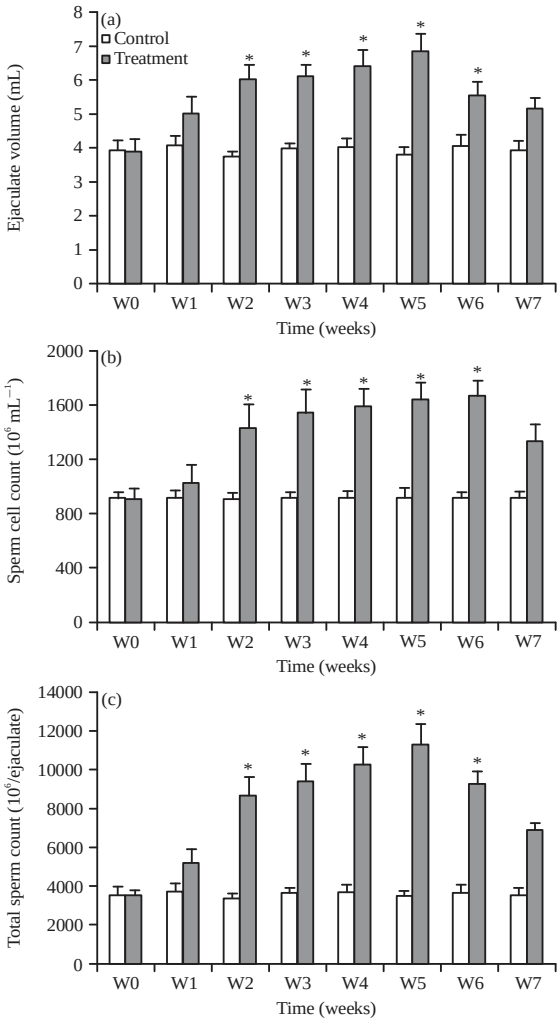

Fig. 3(a-c): Effect of GnRH stimulant treatment on semen characteristics in bulls, treatment group bulls (n = 8) were received oral doses of 100 mg kg^{-1} b.wt., of GnRH stimulant/week for 4 weeks, control group (n = 8) were received same doses of amino acid cocktail. Semen samples were collected twice/week starting one week before treatment (week 0) and extended 2 weeks after GnRH stimulant treatment for measurement of (a) Ejaculate volume, (b) Sperm cell concentration and (c) Total sperm count/ejaculate

All parameters are expressed as Mean±SEM. * indicates differences (p<0.05) between treatment and control group for the same week of treatment

significantly increased in response to GnRH stimulant treatment (p = 0.021). Primary abnormalities were increased from 2.2-5.3%. Secondary abnormalities were increased from 5.8-9.45%.

DISCUSSION

The results of current study demonstrated that oral administration of GnRH stimulant (Theriogon®) increases the levels of testosterone in bull's serum and subsequently increased sexual activity assessed by libido index and reaction time. Moreover, several parameters of semen quality were improved in response to GnRH stimulant administration. Previous studies demonstrated positive effect of tyrosine on female reproductive performance. For example, rats supplemented with 100 mg of L-tyrosine/kg BW at late diestrus had 3.4 more pups per litter than untreated controls[18]. Similar work demonstrated that sows received the same dose one day after weaning increased the litter size[19]. We previously demonstrated that the same dose of Theriogon® induced

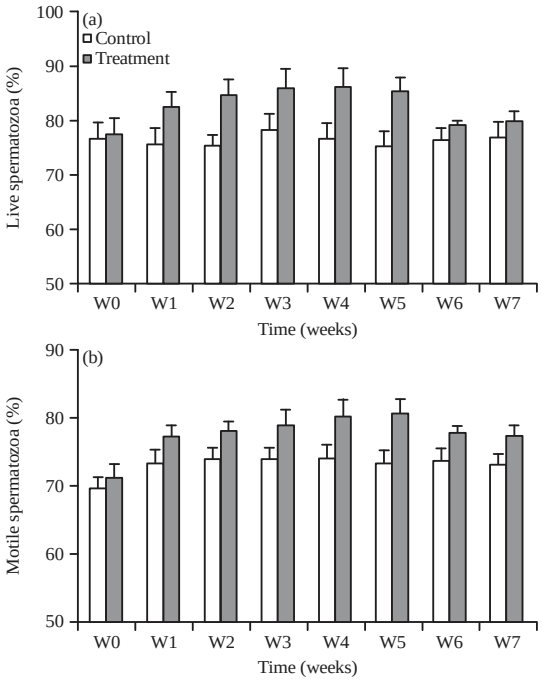

Fig. 4(a-b): Effect of GnRH stimulant treatment on sperm and viability and motility, treatment group bulls (n = 8) were received oral doses of 100 mg kg^{-1} b.wt., of GnRH stimulant/week for 4 weeks, control group (n = 8) were received same doses of amino acid cocktail. Semen samples were collected twice/week starting one week before treatment (week 0) and extended 2 weeks after GnRH stimulant treatment for measurement of (a) Sperm viability and (b) Motility

All parameters are expressed as Mean±SEM

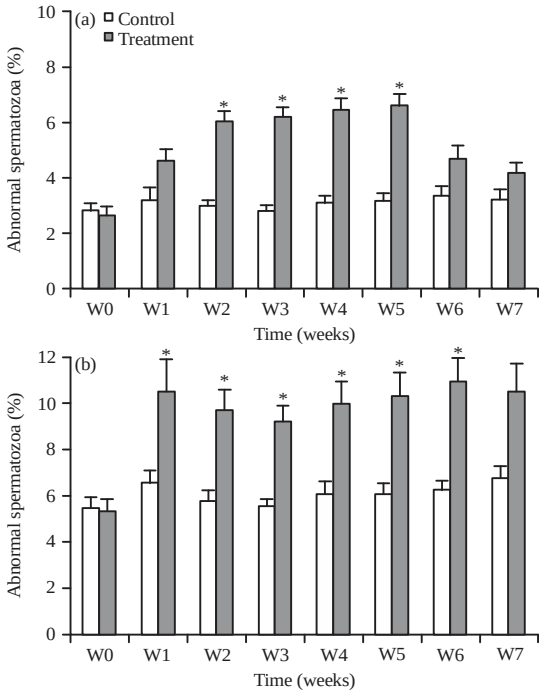

Fig. 5(a-b): Effect of GnRH stimulant treatment on sperm abnormalities, treatment group bulls (n = 8) were received oral doses of 100 mg kg^{-1} b.wt., of GnRH stimulant/week for 4 weeks, control group (n = 8) were received same doses of amino acid cocktail. Semen samples were collected twice/week starting 1 week before treatment (week 0) and extended 2 weeks after GnRH stimulant treatment for measurement of sperm (a) Primary and (b) Secondary abnormalities

All parameters are expressed as Mean±SEM. * indicates differences ($p<0.05$) between treatment and control group for the same week of treatment

puberty in delayed pubertal buffalo-heifers, which continued normal cyclicity afterward[20], also single oral dose of Theriogon® induced cyclicity in anestrous buffaloes[21]. Available data showed that when Theriogon® was administered to prepubertal rabbits, testicular descent occurred 16 days earlier in Theriogon® treated group (100 mg kg^{-1} b.wt.,) compared to control group. First attempt of mounting females was recorded at 90-97 days of age and 131-133 days of age for the Theriogon® treated and control group, respectively. Likewise, when pubertal rams treated with Theriogon®, an increase in circulating testosterone concentration and improved semen parameters (volume, motility, count) were observed, which was superior to the use of 10 µg-GnRH analogue (Receptal©) (El-Amrawi *et al.* unpublished).

Gonadotrophin stimulant, α-amino-p-hydroxy-hydrocinnamic acid, is an aromatic amino acid necessary for the synthesis of catecholamine (adrenaline, noradrenaline and dopamine). Catecholamine stimulates the hypothalamus to produce Gonadotropin releasing hormone (GnRH) via adrenergic receptor mechanism[22]. GnRH secreted from the hypothalamus stimulates the anterior pituitary to produce gonadotropin hormones (FSH and LH), which control folliculogenesis and spermatogenesis[23-26]. Spermatogenesis is induced and controlled directly by follicle stimulating hormone (FSH) and indirectly by luteinizing hormone (LH), the latter act via stimulating Leydig cells to secrete testosterone thus improves libido[23,27]. It has been reported that treatment with GnRH increased testosterone concentration in prepubertal bulls[28] and dromedary camel bulls[7]. The current study demonstrated that treatment with GnRH stimulant resulted in significant increase in serum testosterone levels

over time in treated bull. Increased testosterone is associated with improved sexual activity[29]. Libido or sex drive is defined as the willingness and eagerness of the bull to mount and serve[30]. Libido is a genetic competent of the male sexual behavior, many tests were developed to measure such trait including but are not limited to, libido index which is defined as the degree of sexual interest including service within 10 min[31] and reaction time which measured by the time that elapses between male recognition of an appropriate sexual stimulus and the completion of copulation[32]. Previous studies demonstrated that the reaction time and libido of the bull greatly improved after treatment with GnRH or testosterone[2,4,5,33,34]. The present study indicated that administration of GnRH stimulant improved the sexual activity of the animal, determined by increased libido index and decreased reaction time. A negative association between testosterone level and reaction time and positive association with libido index was evident in bulls. This could be attributed to the stimulatory effect of GnRH on pituitary gonadotropin secretion rate and in turn a higher production of serum testosterone that resulted in improvement of libido.

In the present study, a significant increase in the ejaculate volume, sperm concentration and total sperm count per ejaculate was demonstrated following Theriogon® treatment. Previous studies showed that treatment with testosterone[35] or GnRH[2,36] increases the ejaculate volume and sperm density. Increased testosterone stimulates the accessory genital glands mainly seminal vesicles, subsequently increasing the ejaculate volume by increasing the major component of the ejaculate; seminal plasma. Stimulation of seminal vesicles leads to increased fructose concentration in seminal plasma which in turn contribute to the increased sperm motility[2]. It was demonstrated that L-DOPA and its precursor, tyrosine, has antioxidant and antiradical properties in an *in vitro* model[37]. Also, it was shown that tyrosine contributes to the anti-oxidant capacity of seminal plasma[38], these findings explains improved motility and viability of spermatozoa.

Sperm abnormalities where classified according to the site origin to primary, originating from the testis and secondary originating from epididymis[39]. The overall percentage of sperm abnormalities is 20% in bulls[40]. The results of the present study revealed a significant increase in the sperm abnormalities (primary and secondary) after treatment with GnRH stimulant. However, the percentage of abnormalities did not exceed the permissible limit reported before[40]. This increase may be resulted from the squeezing effect of catecholamine on seminiferous tubules and epididymis.

CONCLUSION

From the results of the current study it was concluded that, oral administration of GnRH stimulant (100 mg kg^{-1} b.wt.) resulted in an improvement in libido and sperm count. Therefore, GRH stimulant could be used to improve sexual behavior in these species. Further studies are required to elucidate the molecular mechanisms of Theriogon® in male reproduction.

SIGNIFICANCE STATEMENT

The current study discovers the beneficial effects of oral administration of α-amino-p-hydroxy-hydrocinnamic acid (Theriogon®) on libido and semen characteristics in bulls. It provides new insights in improving sexual behavior and semen quality in farm animals. This study help researchers to uncover the critical area of using non-hormonal therapy to improve sexual behavior and semen quality in livestock species.

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