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Original Research

Strategies for Improving Fertility in Buffalo Bulls under Summer Condition in Upper Egypt

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Abstract

The purpose of this study was to determine the effect of i.m administration of (250 mg) GnRH on predicted testicular volume (PTV), scrotal circumference (SC), sperm assessment, and eventual fertility after ten weeks (period of the experiment), particularly in the summer (heat stress). Fifteen buffalo bulls (10 treatment and five control) with comparable age (1.5-2 years old) and body weight (300-400 kg) were used in this study. These animals must be housed in the Farm of Animals Production Department, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt, under identical conditions (administration, diet, etc.). PTV, SC, and blood samples were collected soon before GnRH administration. Blood samples were taken from all animals after GnRH injection (treated groups) and normal saline in the control groups. PTV and SC were assessed every 15 days for the first 75 days. All blood samples were centrifuged after being stored at 4°C for 6 hours. Serum was isolated and kept at - 20°C until the hormone meagered. The ejaculate volume, individual motility, living sperm percent, and sperm concentration were all measured in the collected semen from these animals. Five females in estrus were bred by each animal from the treatment and control groups. At 60-75 days after mating, the pregnancy was detected by rectal palpation technique. Testosterone concentrations, PTV, and SC values rose considerably ($P<0.01$) in treated bulls compared to controls after GnRH therapy. Serum testosterone levels climbed from 5.38 0.75 ng/ml to 14.86 1.51 ng/ml. Individual motility, the overall number of sperm/ejaculates, and the number of living sperm were significantly affected by GnRH administration. Furthermore, after receiving GnRH injections, the rate of pregnancy increased in females inseminated by treated bulls. It was 84.56.8% for treated buffalo bulls and 76.57.89% for control buffalo bulls. GnRH injection considerably enhanced PTV and SC levels, according to the findings. Furthermore, semen quality and fertility improved dramatically in treated bulls compared to controls, which may be affected by summer heat stress. GnRH also improves the reproductive performance of buffalo bulls in the summer.

KEYWORDS
GnRH, buffalo-bull, summer, Testosterone, Semen, Testicular volume

INTRODUCTION

In general, stress damage's reproductive function, but the mechanism of damage that causes it has not been well elucidated and may vary between species. Heat stress adversely affects the reproductive performance of males. It reduces spermatogenic activity (Takahashi, 2012). Gonadotropin-inhibitory hormone (GnIH) is a likely conduit for the effect of stress on the reproductive axis (Kirby *et al*. 2009). GnRH is a peptide hormone produced inside the neurons of the dorsomedial nucleus (DMN) of the hypothalamus and released in a synchronized, pulsatile manner from neurons at the medial basal hypothalamus-median eminence (D'Occhio *et al*., 2000; Yina and Gore, 2010). GnRH moves into the portal vessels and is delivered to gonadotroph cells in the anterior pituitary gland. Two distinguishing features of GnRH agonists, compared with natural sequence GnRH, are that agonists have a higher affinity for GnRH receptors and a longer half-life in circulation (Karten and Rivier, 1986). The acute phase of treatment, which can last for several days, is characterized by an immediate large increase in plasma LH and FSH, followed by a return to basal concentrations (Gong *et al*., 1995 and 1996). Continued exposure to GnRH agonists leads to a chronic phase in which pulsatile secretion of LH is blocked (D'Occhio and Aspden, 1996). The latter occurs because of the downregulation of GnRH receptors on gonadotrophs cells (Hazum and Conn, 1988).

In several studies, bulls receiving GnRH agonist had elevated basal concentrations of LH compared with control animals and maintained for long-term (D'Occhio and Aspden, 1996; Jime´nez-Severiano, *et al*., 2003). In the middle trimester of the gestation period, there are GnRH receptors that affect the fetal gonads, and there is also a store that can be released from the gonadotropins. Recently, Giriboni *et al*. (2019) declared that the GnRH agonists can be used to induce a rapid increase in the synthesis and secretion of gonadotropins, and thus in androgen secretion. This hormone may be injected to increase the reproductive capacity of males. Therefore, the present study aimed to evaluate the effect of GnRH injection on testosterone concentrations, testicular response (PTV and SC), and its action on the

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subsequent fertility of treated buffalo bulls.

MATERIALS AND METHODS

Ethical approval

The collection of samples and care of the animal used in this study followed the guidelines for experimental animals established by Research Ethics Committee, Faculty of Veterinary Medicine; Assiut University (Under the No.06/2023/0028).

Animals and their management

The present study was performed on 15 buffalo bulls of similar ages (1.5-2 years old) and body weights (300-400 kg). All animals have been put into animal production Experimental Farm of the Faculty of Agriculture, Al-Azhar University, Assiut, Egypt. The animals were housed in a free-stall barn. They fed 4 kg/head/day commercial concentrate ration and rice straw and Egyptian clover *ad-libitum* and had free access to drinking water. This study was achieved during the summer season from June to August. The animals had previously been trained to mate occasionally.

To investigation the effect of GnRH injection, the buffalo-bulls (n=15) were divided into treated group (n= 10) and control group (n= 5). The animals in treated groups received intramuscular administration of single dose of 250 mg GnRH (Fertagyl, Intervet Unterschleissheim, Germany) according to Abdel-Malak *et al*. (1992). However, the control group was injected with normal physiological saline. The injection of GnRH and saline started at a fixed time between 08:00 and 09:00 hr A.M.

Testicular measurements

The scrotal circumference (SC) and predicted testicular volume (PTV) measurements were performed every two weeks before and after injection of GnRH. The SC was estimated by using a flexible measuring tape after the testes had been gently forced into the scrotum by applying pressure with the hand above the head of the epididymis and ensuring that the scrotal skin was smooth and tense. The testicular length and width were estimated by using a caliper. The PTV was calculated according to Toelle and Robinson (1985).

Semen collection and evaluation

Before and after injection of GnRH, the buffalo-bulls were sexually prepared and the ejaculates (especially 2nd ejaculate) were collected at early morning hours once a week by using artificial vagina and restrained non-pregnant buffalo-cow. Within 2-3 minutes after collection, the ejaculates were transferred to the laboratory, then kept in water bath at 25-28°C for the subsequent evaluation. The semen parameters (volume, individual motility, alive sperm % and sperm concentration) were estimated according to Zemjanis (1970).

Blood samples collection and hormonal assay

The collected blood samples from the injected and controlled animals were kept at 4°C for six hours and then centrifuged. The serum was separated and stored at -20°C until the hormonal assay. The level of testosterone (Singer and Zumoff, 1992) was determined by using enzyme-linked immunosorbent assay (ELISA) commercial kits. (Catalogue Number: BC-1115, BioCheck, Inc S. San Francisco, CA 94080, USA).

Pregnancy rate

After 10 weeks post-injection of GnRH, treated (n=10) and control (n= 5) buffalo-bulls) could mount 75 healthy females in heat (5 females for each animal). The pregnancy rate was calculated per rectum at 60-75 days after mating for treated and control buffalo bulls.

Statistical analysis

Data were expressed as the means ± SE for all parameters, then analyzed by using analysis of variance (ANOVA). The means ± SE were tested at least significant difference (LSD). All tests were done by using PC-stat computer program. Results were considered significant at $P<0.05$ or less.

RESULTS

Tables 1-3 shows the obtained results in the present study. A single injection of GnRH led to a pronounced release of testosterone (Table 1). Serum testosterone (ng/ml) level (means ±SE) increased significantly after four weeks. After treatment, testosterone concentrations elevated (8.54±0.67 ng/ml) significantly ($P<0.05$) when compared with control (7.76±0.79 ng/ml). The mean maximum value of testosterone in treated animals after the end of the study was 14.86±1.51 ng/ml. These concentrations increased significantly ($P<0.01$) compared with the control.

The PTV and SC increased significantly ($P<0.01$) in the treated group as compared to control animals (Table 2). After ten weeks

Table 1. The effect of single injection of GnRH upon Testosterone concentration in buffalo-bulls.

Time after GnRH injection (weeks)	Buffalo-Bull	
	Control (n=5)	Treated (n=10)
0	5.38±0.75	5.47±0.96
1	6.43±0.76	6.98±0.67
2	6.43±0.53	7.76±0.55
3	7.53±0.78	8.14±0.62
4	7.76±0.79	8.54±0.67
5	8.03±0.29	9.53±0.55*
6	8.66±0.69	10.72±0.78*
7	8.69±0.76	11.56±0.61*
8	9.24±0.63	12.58±0.69*
9	9.78±0.53	13.67±1.52*
10	9.94±1.05	14.86±1.51

post-injection of GnRH, the values of PTV were 191.26±2.02 and 150.29±2.32 c.c in treated and control buffalo-bulls respectively. Moreover, the increasing mean values of SC of treated buffalo-bulls were significant (P<0.001) between pre-injection (30.19±1.23 cm) and post-injection (41.56±2.16 cm).

Regarding semen quality and pregnancy rate (Table 3), there were significant increases in the individual motility, alive sperm %, and the total number of sperm/ ejaculates after injection of GnRH. The pregnancy rate improved in buffalo cows mated by treated buffalo bulls. This rate was 84.56±8.27 % for injected buffalo bulls and 76.87±7.89 % for control buffalo bulls.

DISCUSSION

The obtained results revealed that the administration of a single injection of GnRH led to a significant increase in the release of testosterone (P<0.01) as well as, significant increases in PTV and SC values (P<0.01). These results agree with that reported by Devkota *et al.* (2011); Ali *et al.* (2012) and Mohammed *et al.* (2021) in ram. The basis for increased testosterone secretion in bulls during treatment with GnRH is not explained but is likely related to the elevated basal concentrations of LH (D'Occhio *et al.*, 2000), or may be attributed to increased testicular levels of steroid acute regulatory (StAR) protein and steroidogenic enzymes involved in testosterone biosynthesis (Aspden *et al.*, 1998). Furthermore, Leydig cell activity was reported to increase which led to increase testosterone concentrations (Byerley *et al.*, 1990 Thompson *et al.*, 1992).

The interaction of LH with receptors in the Leydig cells activates the adenyl cyclase system, including protein kinase activation and RNA synthesis, resulting in an increased pregnenolone production from cholesterol by the mitochondria in the Leydig cells that lead to the formation and release of Testosterone (Pineda, 1989). The interpretation of the difference in the value of testosterone in response to the administration of GnRH may be due to the possibility of its secretion by a separate compartment within the testes (Schanbacher and Echternkamp, 1978). Exogenous treatment of GnRH led to improve sexual behavior and the quality of spermatozoa in fertile stallions during the non-breeding season (Sieme *et al.*, 2004). They also reported the possibility of a direct gonadal or epididymal effect of exogenous GnRH in the stallion. Additionally, Giriboni *et al.* (2019), cited that the administration of two daily doses of GnRH for 7 weeks in rams resulted in an increase in testosterone concentration, scrotal circumference, and the percentage of sperm with progressive motility in the ejaculate. In addition, GnRH injection could induce an improvement of testicular blood flow. This improvement occurred significantly after the GnRH injection (Samir *et al.*, 2015). A possible explanation for this may be related to the effect of GnRH. Blood flow is important to testis function because any defect in the arterial blood flow to the testis could cause impaired spermatogenesis secondary to defective energy metabolism at the mitochondrial level (Hsu *et al.*, 1994). Moreover, Janett *et al.*, (2008) reported that administration of anti-GnRH in stallion sup-

Table 2. The effect of GnRH injection upon the predicted testicular volume (PTV) and scrotal circumference (SC) in buffalo- bulls.

Time after GnRH injection (weeks)	Predicated testicular volume (c.c)		Scrotal circumference (c.c)	
	Control (n=5)	Treated (n=5)	Control (n=5)	Treated (n=5)
-4	142.21±2.86^{1a}	142.41±2.57^{1a}	30.29±1.23^{1a}	30.19±1.49^{1a}
-2	142.75±1.77^{1a}	142.73±2.42^{1a}	30.31±1.59^{1a}	30.26±2.13^{1a}
0	143.55±1.33^{1a}	143.65±2.22^{1a}	30.42±2.19^{1a}	30.36±1.76^{1a}
2	144.35±2.59^{1a}	146.21±2.33^{1a}	30.66±3.24^{1a}	30.46±1.27^{1a}
4	145.23±2.49^{1a}	157.86±2.47^{2b}	31.25±1.46^{1a}	34.52±1.28^{2b}
6	146.45±2.27^{1a}	161.48±2.19^{2b}	32.18±2.27^{1a}	37.34±1.71^{2c}
8	148.18±2.17^{1a}	177.61±2.79^{2c}	33.32±1.62^{1a}	39.23±2.47^{2d}
10	150.29±2.32^{1a}	191.26±2.02^{2d}	34.68±2.23^{1a}	41.56±2.16^{2c}

Means with the same superscript letters within the same column are non-significant.
Means with the same superscript number within the same row are non-significant.

Table 3. The effect of single injection of GnRH upon buffalo-bull's semen parameters and pregnancy rate.

Time* (week)	Total No. of sperm/ ejaculate (10^9)		Individual motility (%)		Alive spermatozoa (%)	
	Control (n=5)	Treated (n=10)	Control (n= 5)	Treated (n=10)	Control (n= 5)	Treated (n=10)
-2	8.16±1.67	8.17±1.31	69.62±1.43	70.58±1.51	74.23±0.58	74.57±1.52
-1	8.17±1.51	8.18±1.25	69.69±1.25	70.87±1.16	74.57±1.16	75.35±1.73
0	8.22±1.27	8.15±0.87	70.43±1.32	70.55±0.59	75.23±1.27	75.75±0.57
1	8.33±0.83	8.38±1.36	70.48±1.15	70.65±1.18	75.84±1.13	76.36±1.25
2	8.34±0.95	8.47±1.08	70.65±1.06	71.19±1.63	76.27±1.18	76.63±0.93
3	8.39±1.15	8.76±1.23	71.25±1.22	72.65±1.49	76.39±0.73	76.88±1.08
4	8.42±1.21	9.24±1.17	71.43±0.85	72.39±0.79	76.67±1.14	77.04±0.56
5	8.63±0.87	9.33±0.83	71.72±1.06	72.77±1.13	77.36±1.26	78.24±1.28
6	8.68±0.56	9.46±0.35*	71.75±1.21	73.38±0.54*	77.59±0.76	78.31±1.17
7	8.79±1.05	10.33±0.18*	72.23±1.44	73.73±0.89*	77.73±0.45	79.85±1.23*
8	9.25±1.12	10.85±0.27*	72.63±1.41	74.23±1.44*	77.81±1.04	80.59±1.15*
9	9.37±1.52	11.72±0.65*	72.78±1.63	74.83±1.08*	77.96±1.26	83.13±1.09*
10**	9.41±0.59	12.48±0.23*	72.87±1.09	75.85±1.49*	78.35±0.73	85.74±0.57*

*Time before and after injection of GnRH.
Mean with the same superscript letters within the same column are non-significant.
Mean with the same superscript number within the same row are non-significant.
** Pregnancy rate in injected bulls was 84.56±8.27 %, while it was 76.57±7.89 % in control bulls.

pressed the secretion of testosterone and testicular functions as well as decreased the semen quality.

Concerning the effect of GnRH injection on PTV and SC, these studies also indicated that GnRH had a significantly increasing impact on PTV and SC. This result agree with Ali *et al*. (2012) and Devkota *et al*. (2011) who reported that administration of GnRH weekly significantly increased the testosterone levels and scrotal circumference when compared to control bulls. Moreover, D'Occhio and Aspden (1996) and D'Occhio *et al*. (2000) reported that the bull injected with GnRH showed an increasing rate of testicular growth. However, Chandolia *et al*. (1997) detected retardation in testicular growth in bulls injected with GnRH, while Ronayne *et al*. (1993) found that GnRH had no effect on testicular growth. Furthermore, Safarineiad *et al*., (2009) found that decreased GnRH levels due to intensive, long-term treadmill running reduced all testicular functions and semen quality.

The obtained result concerning the significantly improved semen agreed with El-Khawaga *et al*. (2011) and Romanello, *et al*., (2018). Moreover, the GnRH analog enhanced testosterone secretion and improved semen quality followed by improving the reproductive capacity of males (Giriboni *et al*., 2019). Also, they added that the administration of GnRH analog (buserelin) probably led to an increase in FSH and LH in the seminal plasma, which might explain the improvement in sperm quality. The improvement in semen quality may be attributed to the administration of GnRH analog (buserelin) probably led to an increase in FSH and LH in the seminal plasma, which might explain the improvement in sperm quality (Giriboni *et al*., 2019).

Regarding the effect of GnRH on the pregnancy rate of cows mounted by treated and control bulls, the present study revealed that cows mounted by treated bulls had a higher pregnancy rate. This agrees with that reported by Andersson (1992). This finding is attributed to the improved semen quality of the treated bulls (Braun *et al*., 1988) and the increasing values in the total number and the percentage of motile spermatozoa (Gabor *et al*., 1998). Abd-Malak *et al*. (1992) reported that the administration of GnRH increased the production of estradiol -17ß, which plays a role in epithelial functions associated with the maintenance of fertile spermatozoa in the cauda epididymidis (Tekpetey and Amann, 1988). Also, Devkota *et al*. (2011) demonstrated that the reaction time and libido of the bull greatly improved after treatment with GnRH.

During the summer, bulls experience the action of heat stress leads to a decrease in sexual performance, and temporary sterility may occur (Kamel, 1996). The same author also reported that infertility in Egyptian buffalo bulls is a problem of high ambient temperature and the insufficiency of the heat-regulating mechanism in these animals. Heat stress leads to a decrease in the plasma concentration of LH and a lowering in steroidal capacity and spermatogenesis (Goswami *et al*., 1991, Wolfenson *et al*., 2000). These researchers recommend the use of hormonal injections to improve sexual activity during the summer.

CONCLUSION

The present study indicated that using a single injection of GnRH in buffalo bulls subjected to heat stress (during the summer season) induce a beneficial effect on the predicted testicular volume, scrotal circumference, and semen quality. GnRH can be used in these months of heat stress (during summer) to improve sexual activity and fertility in buffalo bulls.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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