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Response of Alexandria Cockerels Reproductive Status to GnRH (Receptal) Injection

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Abstract: Fourty, 40 weeks old Alexandrian cockerels were distributed among 4 treatments to study the effect of GnRH analogue (Receptal) administration, on their reproductive performance. Birds of the second, third and fourth group were individually intramuscularly injected weekly with 0.1, 0.2 and 0.4 ml of Receptal, respectively for 2 months. Birds of the first group served as control. Receptal had significantly increased testosterone. Birds injected with the 0.1 and 0.2 ml of Receptal had significantly higher ejaculated volume as it increased by 49 and 38% respectively. On the other hand, the highest dose of Receptal decreased the ejaculate volume to 52 and 56% of the untreated males' volume on the first and second month, respectively. Birds injected with the 0.1 and 0.2 ml of Receptal had significantly higher sperm concentration as it increased by 28 and 18% respectively, meanwhile, raising the Receptal dose to 0.4 ml did not show any significant difference. The 0.1 and 0.2 ml doses of Receptal had significantly higher motility as it increased to reach 133 and 129% of control respectively, meanwhile, birds treated with 0.4 ml of Receptal had similar sperm motility as the controls. Cholesterol has increased significantly in a dose dependent manner. Total protein did not show significant differences except with the medium dose of Receptal as it increased to reach 112% of controls level. Seminal plasma constitutes showed a reflection of the blood status. It was concluded that the synthetic GnRH was capable of improving 40 week old cockerels' reproductive status.

Key words: Cockerels, GnRH, testosterone, ejaculated volume, sperm motility and seminal plasma

INTRODUCTION

Testicular function both in birds and mammals is controlled by information from the outer and inner environments, which are integrated in the brain to modulate Gonadotrophin Releasing Hormones (GnRH) secretion which is responsible of stimulating the gonadotropes of the pituitary to secrete Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) (Sharp and Gow, 1983). Although little information available concerning the effect of GnRH treatment on local chicken strains, it was well investigated in different mammalian species. Marai *et al*. (1998) reported a significant improvement in semen quality and reproductive performance of male rabbits injected with GnRH and an increase in testosterone in bulls was reported by (Kozdera *et al*., 1993).

Little is known about the effect of GnRH treatment on galliform species, as Muske *et al*. (1994) reported that at least eight GnRH have been characterized among vertebrates where domestic chicken shown to have two distinct forms, however, the genetic improvements of the modern strains of chickens led to impaired fertility, late sexual maturity and some reproductive problems associated with the related neuroendocrine axis (Weil *et al*., 1999). Therefore, the present study was conducted to investigate effects of different levels of GnRH analogue injection on 40 week old Alexandrian cockerels.

MATERIALS AND METHODS

The present study was carried out at the poultry research center, Faculty of Agriculture, Alexandria University, using 40 weeks old Alexandrian (native strain) cockerels.

Receptal solution: Receptal® solution used in this study is a veterinary therapeutic product as a ready-to-use injection solution of a synthetic releasing hormone that releases Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) from the pituitary and was in the form of 10 ml liquid injectable, with a chemical entity of Buserelin acetate (0.0042 mg/ml). Purchased from Intervet Ltd. P.O. Box 4079 Auckland, New Zealand.

Experimental design: A total of forty, 40 weeks old Alexandrian cockerels were randomly and equally distributed among 4 treatment groups each of 2 sub groups (5 birds each). Birds of the second, third and fourth group were individually intramuscularly injected weekly with 0.1, 0.2 and 0.4 ml of Receptal under the neck, respectively for 2 months. Birds of the first group served as untreated control. Feed and water were provided *ad libitum* throughout the 2 months experimental period.

Data collected: Semen specimens were artificially collected biweekly free of transparent fluid by abdominal massage technique. Ejaculated volume was measured to the nearest 0.01 ml. A weak eosin-formalin (10%

formalin) solution was used for evaluation of sperm concentration by the improved Newbuer hemocytometer slide as described by Smith and Mayer (1955). Sperm motility was estimated at 400 X magnification on stage warmer adjusted at 38°C (Kamar, 1960). Seminal plasma was obtained by centrifugation of semen samples at 3500 rpm for 20 min at 4°C and was stored at -20°C until later analysis. Seminal plasma samples were analyzed for total protein, albumin, total lipids and cholesterol, levels, colorimetrcally using available commercial kits.

Blood samples were collected biweekly from the wing vain for biochemical analysis. Serum total protein, albumin, total lipids and cholesterol, levels were determined colorimetrcally using available commercial kits. Serum testosterone (ng/ml) was determined by enzyme immunoassay using commercial kits purchased from Biosource.

Statistical analysis: Means and standard errors were estimated for each studied trait. Data were analyzed using SAS (1996) program, using general linear model. Significant differences among treatments means were separated using Duncan's multiple range procedure (Duncan, 1955).

RESULTS AND DISCUSSION

Testosterone: Data concerning testosterone concentration as influenced by Receptal administration are presented in (Fig. 1 and 2). Overall, birds injected with different doses of Receptal from 40-48 weeks of age had significantly higher testosterone compared to the untreated males ($p \leq 0.0001$). Testosterone increase was in a dose dependent manner, as it increased by 104, 163 and 218% compared to control with 0.1, 0.2 and 0.4 doses of Receptal, respectively (Fig. 1). Regarding interaction of treatments with months ($p \leq 0.0001$), the increase in testosterone concentrations reached 145, 213 and 226% by the end of the first month and 303, 349 and 474% by the end of the second month compared to control with 0.1, 0.2 and 0.4 doses of Receptal, respectively (Fig. 2).

The increase in testosterone concentration as a result of GnRH analogue treatments comes in good agreement with the finding of Hirschenhauser *et al.* (2000) who reported that injection of White geese with GnRH analogue (Ovurelin) resulted in an increased plasma testosterone concentration compared to the untreated birds. Also, Bollwein *et al.* (2008) reported elevation in testosterone of stallion after administration of human chorionic gonadotropin.

Ejaculated volume: Effect of GnRH analogue treatments on semen ejaculated volume is illustrated on (Fig. 1 and 3). Overall, birds injected with the 0.1 and 0.2 ml of Receptal from 40-48 weeks of age had significantly higher ejaculated volume as it increased by 49 and 38% respectively, compared to the untreated males ($p \leq 0.0001$) meanwhile, birds treated with 0.4 ml of Receptal had significantly lower ejaculated volume as it reached 47% of the untreated birds volume (Fig. 1). Regarding interaction of treatments with months ($p \leq 0.0006$), it can be noted that the lowest dose of

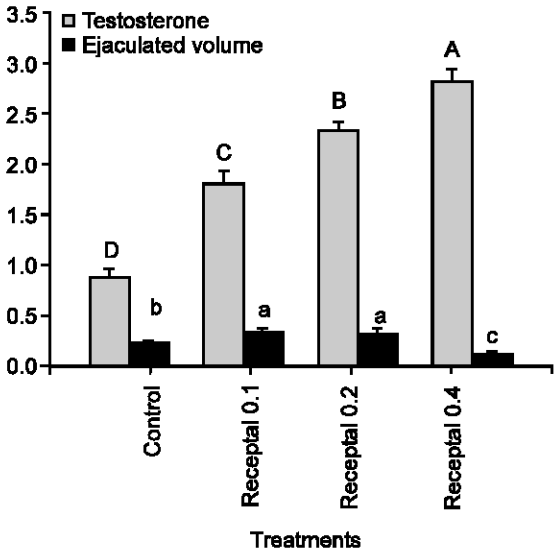

Fig. 1: Testosterone concentration (ng/ml) and Ejaculated volume (ml) of Alexandria cockerels injected weekly for 2 months with 3 doses of GnRH analogue (Receptal). A,B,C denote significant differences in testosterone concentrations between treatments. a,b,c denote significant differences in ejaculated volume between treatments

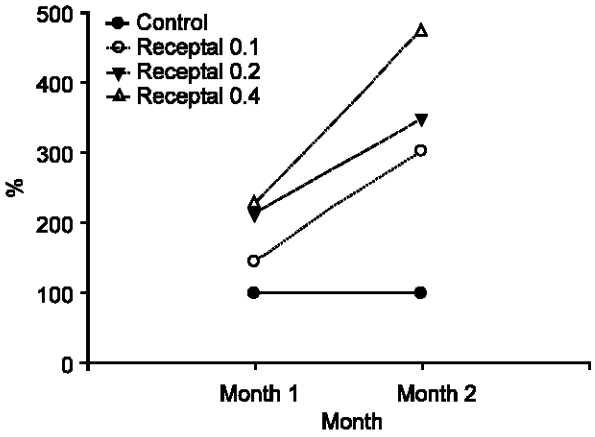

Fig. 2: Effect of different levels of GnRH analogue (Receptal) weekly injection on Alexandria cockerels Testosterone concentration, relative to control (where control concentrations represent 100%)

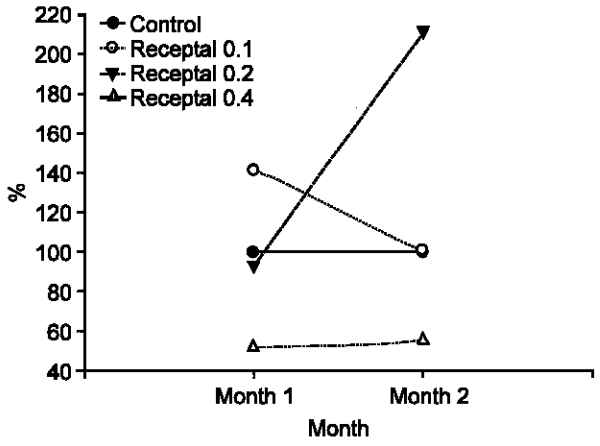

Fig. 3: Effect of different levels of GnRH analogue (Receptal) weekly injection on Alexandria cockerels semen ejaculated volume, relative to control (where control concentrations represent 100%)

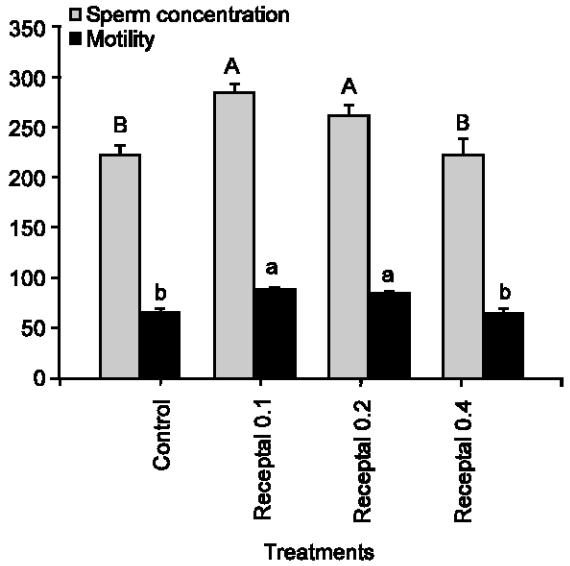

Fig. 4: Sperm concentration (n*10^7/ml semen) and motility (%) of Alexandria cockerels injected weekly for 2 months with 3 doses of GnRH analogue (Receptal). A,B,C denote significant differences in sperm concentrations between treatments. a,b,c denote significant differences in motility between treatments

Receptal resulted in a significant increase in the ejaculated volume in the first month which was lost by the second month, whereas the 0.2 ml dose of Receptal did not have a significant effect on the semen ejaculated volume till the second month as it increased dramatically to reach 211% of the untreated males volume. On the other hand, the highest dose of Receptal had an adverse effect, as it decreased the ejaculate volume to 52 and 56% of the untreated males volume on the first and second month, respectively (Fig. 3).

The increase in semen volume has been also shown in Holestein Friesian and Jersey bulls treated with GnRH (Bhaskaran and Dubey, 2004 and 2005).

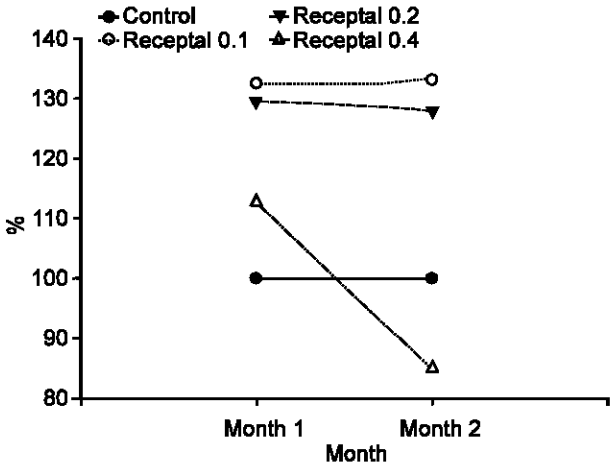

Fig. 5: Effect of different levels of GnRH analogue (Receptal) weekly injection on Alexandria cockerels sperm concentration, relative to control (where control concentrations represent 100%).

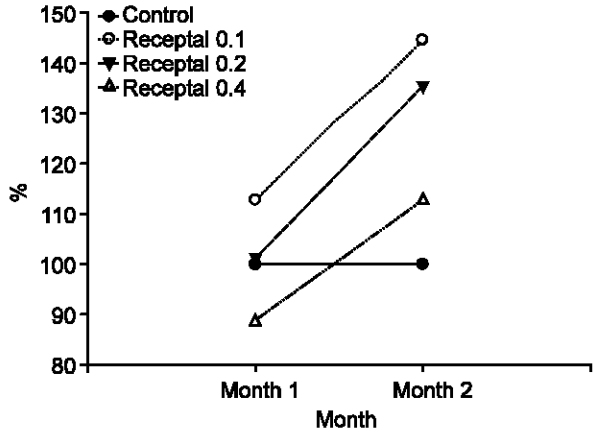

Fig. 6: Effect of different levels of GnRH analogue (Receptal) weekly injection on Alexandria cockerels sperm motility, relative to control (where control concentrations represent 100%)

Sperm concentration: Data concerning sperm concentration as influenced by Receptal administration are presented in (Fig. 4 and 5). Overall, birds injected with the 0.1 and 0.2 ml of Receptal from 40-48 weeks of age had significantly higher sperm concentration as it increased by 28 and 18% respectively, compared to the untreated males ($p \leq 0.0001$) meanwhile, raising the Receptal dose to 0.4 ml did not show any significant

Table 1: Effect of different levels of GnRH analogue (Receptal) weekly injection on Alexandria cockerels blood and seminal plasma analysis (Mean±SE)

	Blood Analysis			
Items	Total lipids (mg/dl)	Cholesterol (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)
Control	385.02±13.52	85.35±3.07 B	3.90±0.09 B	1.36±0.03 C
0.1 ml Receptal	415.45±12.09	98.50± 5.23 A	4.05±0.12 B	1.52± 0.04 B
0.2 ml Receptal	411.85±14.92	100.21±2.86 A	4.37±0.10 A	1.50± 0.04 B
0.4 ml Receptal	401.03± 8.65	101.46± 2.41 A	4.11± 0.15 B	1.67± 0.06 A
P value	NS	***	**	***
	Seminal Plasma			
Items	Total lipids (mg/dl)	Cholesterol (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)
Control	170.65±8.30 D	25.18±2.68 C	0.838±0.06 B	0.459±0.03 D
0.1 ml Receptal	209.13±10.4 C	30.35±1.37 B	0.740±0.01 C	0.647±0.01 A
0.2 ml Receptal	237.98±3.83 B	33.08±1.96 A	1.014±0.03 A	0.605±0.02 B
0.4 ml Receptal	274.05±6.93 A	28.28±0.26 B	0.762±0.01 C	0.528±0.02 C
P value	***	***	***	***

A, B, C Different letters within a column denote significant differences between treatments. *, **, *** Probability levels $p \leq 0.05$, 0.001 and 0.0001, respectively.

difference compared to the untreated males (Fig. 4). Regarding interaction of treatments with months ($p \leq 0.0423$), the increase in sperm concentrations reached 113, 101 and 89% by the end of the first month and 145, 136 and 113% by the end of the second month compared to control with 0.1, 0.2 and 0.4 doses of Receptal, respectively (Fig. 5).

The increase in sperm concentration was also shown in Holestein Friesian and Jersey bulls treated with GnRH (Bhaskaran and Dubey, 2004). Also, Fathi *et al.* (2000) reported that GnRH injection improved sperm cell concentration of the naked neck cockerels especially after long-term treatment.

Sperm motility: Effect of GnRH analogue treatments on sperm motility is illustrated on Fig. 4 and 6. Overall, birds injected with the 0.1 and 0.2 ml of Receptal from 40-48 weeks of age had significantly higher motility as it increased to reach 133 and 129% of control respectively ($p \leq 0.0001$), meanwhile, birds treated with 0.4 ml of Receptal had similar sperm motility as the controls (Fig. 4). Interaction of treatments with months showed significant effect ($p \leq 0.0009$), where the highest motility 133% of control was observed with the lowest Receptal level by the second month and the lowest motility 85% of control was observed with the highest Receptal level by the second month (Fig. 6).

This effect was also reported by Bhaskaran and Dubey (2004 and 2005) when Holstein Friesian and Jersey bulls were treated with GnRH. Also Fathi *et al.* (2000) observed increase in motility of naked neck cocks sperms when they were treated with GnRH.

Blood analysis: Table 1 elucidates effects of GnRH analogue injection on some blood biochemical constitutes. Overall, blood total lipids was not affected by Receptal treatment whereas, cholesterol has increased significantly in a dose dependent manner to reach 115, 117 and 119% of control with the three Receptal levels, respectively. Total protein did not show significant differences except with the medium dose of Receptal as it increased to reach 112% of controls level, although it has increased numerically with the low and high doses of Receptal. Albumin levels increased significantly with the treatments and reached it's highest values with the highest Receptal dose (123% of control). Similar results were reported by Singh *et al.* (2006) in buffaloes treated with Receptal as total serum protein increased significantly. Also Khasatiya *et al.* (2005) reported an increased total protein in buffaloes injected with 5 ml Receptal. On the other hand, Patel and Dhami (2005) reported significant increases in both cholesterol and triglycerides after Holestein-Friesian cows were treated with Receptal.

Seminal plasma: Seminal plasma constitutes showed a reflection of the blood status Table 1. Receptal treatment resulted in a dose dependent increase in seminal plasma total lipids as it increased by 23, 39 and 61% cholesterol also increased by 21, 31 and 12% with the low, medium and high dose of Receptal, respectively. Seminal plasma total protein decreased with the low and high doses of Receptal and increased significantly with the medium dose to reach 121% where albumin increased by 41, 32 and 15% with the low, medium and high dose of Receptal, respectively.

It can be concluded that although the natural gonadotrophin releasing hormones in chickens differ than mammals, the synthetic mammalian GnRH was capable of improving 40 week old cockerels reproductive status as it increased circulating testosterone, ejaculated volume, sperm concentration and sperm motility.

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