GnRH ANALOGUE (BUSERELIN ACETATE) MODULATES REPRODUCTIVE PERFORMANCE IN 'OLD HENS'

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SUMMARY

To test the influence of a GnRH analogue (buserelin acetate, BA) on the reproductive activity of aging hens, a total of sixty, 50-wk-old Alexandrian hens were randomly and equally distributed among three treatment groups. Two groups received weekly intramuscular injections of 0.1 or 0.2 mL of the GnRH analogue solution, providing 0.42 and 0.84 µg of buserelin acetate, respectively, for 1 month. The control group received 0.15 mL of physiological saline. Reproductive parameters (egg production, egg weight and egg mass) and plasma FSH, LH and Estradiol (E_2) were measured. Overall, low- and high-BA treatments, respectively, resulted in 58% and 30% increases in LH concentrations; 77% and 50% increases in FSH concentrations; and 41% and 8% increases in E_2 concentrations; compared to control. Weekly egg production increased by 37% and 29% with low- and high-BA, respectively. Egg weight on the other hand responded differently to treatments, as it declined to 97% of control. It can be concluded that in Alexandrian laying hens the decrease in egg production associated with advancing age can be attributed to reduction in plasma LH; hence treating aging hens with the GnRH analogue was capable of increasing egg production.

Key words: GnRH, old hens, reproduction, egg production, estradiol, LH, FSH

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) controls the reproductive physiology and behavior of vertebrates by stimulating synthesis and release of gonadotropin from the pituitary gland (Ubuka *et al.*, 2008). It plays a key role in the control of sexual maturity, ovulation rate, incubation, photorefractoriness, and reproductive aging, which all appear to be related to alterations in GnRH regulation (Mans and Taylor, 2008). In the hen, egg production declines with advancing age, and comparing hens at peak of egg laying with hens at end of the laying year showed that plasma LH and FSH were lower in old than in young laying hens (Ciccone *et al.*, 2005). There is a lack of information available concerning the effect of a GnRH analogue on local hens; however, it was shown to improve reproductive status of Alexandrian cockerels (Elnagar, 2009).

The objective of this study was to determine whether the GnRH analog, Buserelin Acetate (BA), can reverse the declines in plasma LH and FSH and the reductions in egg production associated with aging in Alexandrian hens.

MATERIALS AND METHODS

The present study was carried out at the poultry research center, Faculty of Agriculture, Alexandria University, using Alexandrian hens at the end of the production season (50 wk of age).

GnRH analogue solution:

Gonadotropin-releasing hormone analogue solution used in this study is a veterinary therapeutic product sold as a ready-to-use injection solution of a synthetic releasing hormone that releases luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the interior pituitary. It consists of Buserelin Acetate (0.0042 mg/mL), purchased from Intervet Ltd. PO Box 4079 Auckland, New Zealand.

Experimental design:

A total of sixty, 50-wk-old Alexandrian hens were randomly and equally distributed among three treatment groups with two replicates each (10 birds per group). Treatments (TRT) consisted of one month of weekly intramuscular (i.m.) injections, of 0.1 or 0.2 mL of the GnRH analogue solution, providing 0.42 (low) and 0.84 (high) µg of BA, respectively, followed by a month of recovery (REC). Control birds received weekly i.m. injections of 0.15 mL of physiological saline (0-BA) for one month, followed by a month of recovery. Feed and water were provided on an *ad libitum* basis throughout the study.

Data collected:

Blood samples (10 from each treatment group) were collected biweekly for biochemical analysis. Monitoring birds revealed that most of them lay their eggs between 10 and 11 am, so blood samples collection started about 6 am, assuming that is the time when tested hormones peak and consequently, sample timing was not a factor in the statistical analysis.

Radioimmunoassay (RIA) was used for FSH, LH and E_2 determinations (Follett *et al.*, 1972; Akiba *et al.*, 1982 and Krishan *et al.*, 1993). Weekly egg production was recorded, eggs were weighed, and egg mass (egg weight x egg number) was calculated.

Statistical analysis:

Data were analyzed using SAS (SAS 1996) program, using general linear model. Significant differences among treatment means were separated using Duncan's multiple range procedure (Duncan 1955).

Results and Discussion

Gonadotrophins (LH and FSH):

Overall, low and high BA treatments resulted in a 58% and 30% increase, respectively (p=0.0001) in LH concentrations (9.8$\pm$0.643 and 8.1$\pm$0.270 ng/ml) compared to control (6.2$\pm$0.221 ng/ml) (Table 1). Results showed that LH in control birds did not differ between TRT and REC periods. Low-BA boosted LH during the TRT period to 72% higher than control and it remained 42% higher during the REC period (p=0.0441) (Table 1). The effect of high-BA was less profound, with increases of 28% and 34% over control during the TRT and REC periods, respectively (Table 1) these concentrations did not differ from low-BA concentrations during REC.

Table 1. Mean (± SE) of reproductive status parameters of old hens treated with 0(control), 0.42 (low) and 0.84(high) μg "Buserelin Acetate"/hen/week, overall and during one month of treatment (TRT) and one month of recovery (REC)

Item		LH (ng/mL)	FSH (ng/mL)	E_2 (pg/mL)	Egg number (hen/wk)	Egg weight (g)	Egg mass (g)
Control (0 BA)		6.2 ± 0.221^{C}	1.01 ± 0.097^{C}	355 ± 18^{B}	3.83 ± 0.20^{C}	56.67 ± 0.20^{A}	219 ± 8^{C}
0.42 μg.(low)		9.8 ± 0.643^{A}	2.80 ± 0.094^{A}	501 ± 29^{A}	5.23 ± 0.19^{A}	55.24 ± 0.21^{B}	288 ± 10^{A}
0.84 μg. (high)		8.1 ± 0.270^{B}	2.53 ± 0.067^{B}	383 ± 35^{B}	4.95 ± 0.19^{B}	54.91 ± 0.18^{B}	271 ± 10^{B}
***P* value**		0.0001	0.0001	0.0067	0.0001	0.0001	0.0001
Control (0 BA)	TRT	6.4 ± 0.056^{C}	0.80 ± 0.049^{D}	351 ± 31^{B}	4.10 ± 0.27^{C}	57.46 ± 0.28^{A}	236 ± 12^{C}
	REC	5.9 ± 0.433^{C}	1.21 ± 0.057^{C}	360 ± 26^{B}	3.56 ± 0.27^{D}	55.77 ± 0.37^{B}	202 ± 10^{D}
0.42 μg. (low)	TRT	11.0 ± 0.644^{A}	2.97 ± 0.092^{A}	515 ± 32^{A}	5.33 ± 0.28^{A}	55.55 ± 0.26^{BC}	296 ± 15^{A}
	REC	8.4 ± 0.088^{B}	2.63 ± 0.086^{B}	488 ± 17^{A}	5.11 ±0.26^{AB}	54.88 ± 0.35^{CD}	279 ± 14^{AB}
0.84 μg. (high)	TRT	8.2 ± 0.503^{B}	2.52 ± 0.101^{B}	277 ± 12^{B}	4.94 ± 0.27^{B}	54.58 ± 0.27^{D}	270 ± 15^{B}
	REC	7.9 ± 0.300^{B}	2.55 ± 0.112^{B}	490 ± 22^{A}	4.95 ± 0.27^{B}	55.25± 0.24^{BCD}	274 ± 15^{B}
***P* value**		0.0441	0.0063	0.0192	0.0132	0.0001	0.0012

^{A,B,C} Different letters within a column denote significant differences between treatments (P<0.05)

Measurements of LH at two-wk intervals are shown in Fig 1, with the highest LH in response to low-BA at 4 wk and the lowest LH occurring in the control group at the end of the experimental period (p=0.0064). It should be noted that LH decreased in all groups over the experimental period, and that LH concentrations in response to high-BA were always intermediate to the other two groups.

Regarding FSH concentrations, the values shown in Table 1 represent increases (p=0.0001) over control of 177% and 150% for low- and high-BA, respectively, FSH in low-BA birds was 271% over control during TRT and 117% during REC, and increases in response to high-BA compared to control were 215% and 111% during TRT and REC, respectively. As with LH, the FSH peak for all groups occurred at wk 4 in response to low-BA. However, FSH was lowest in control hens at the onset of the study, increasing over the 8-wk period (p=0.0001). This age-related increase in FSH concentrations in the control hens may be related to decreases in pulse frequency of GnRH, which stimulates release of FSH but not LH (Dalkin *et al.*, 1989; Kaiser, 1997 and Ciccone, 2005). High-BA treatment-induced FSH concentrations were always intermediate to the other two groups (Fig 2). Which supports previous findings indicating that [D-Leu6,Pro9]-GnRH N-ethylamide treatment increased plasma concentrations of LH in Taihe hens (Wang *et al.*, 1994).

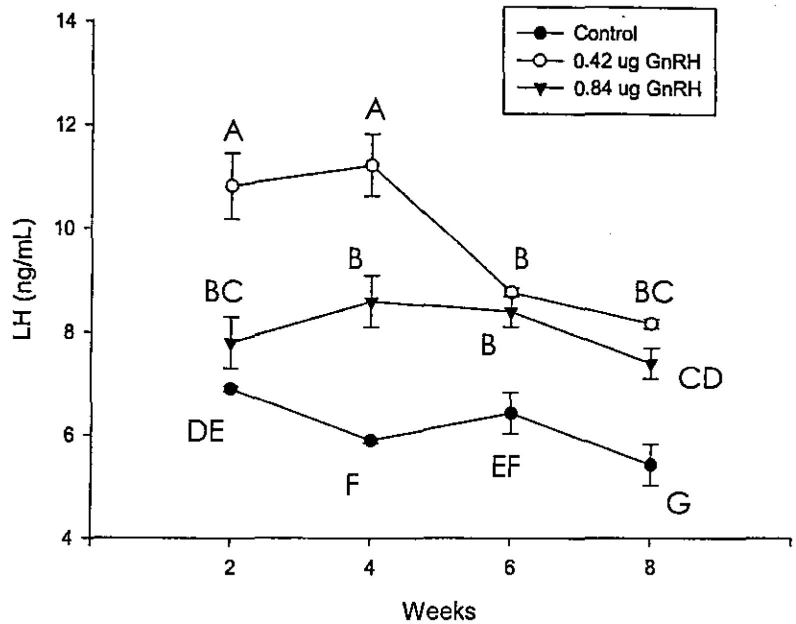

Figure 1. Effect of weekly injection (i.m.) of 0, 0.42, or 0.84 μg Buserelin Acetate (BA) on plasma LH of 50-wk-old Alexandria hens

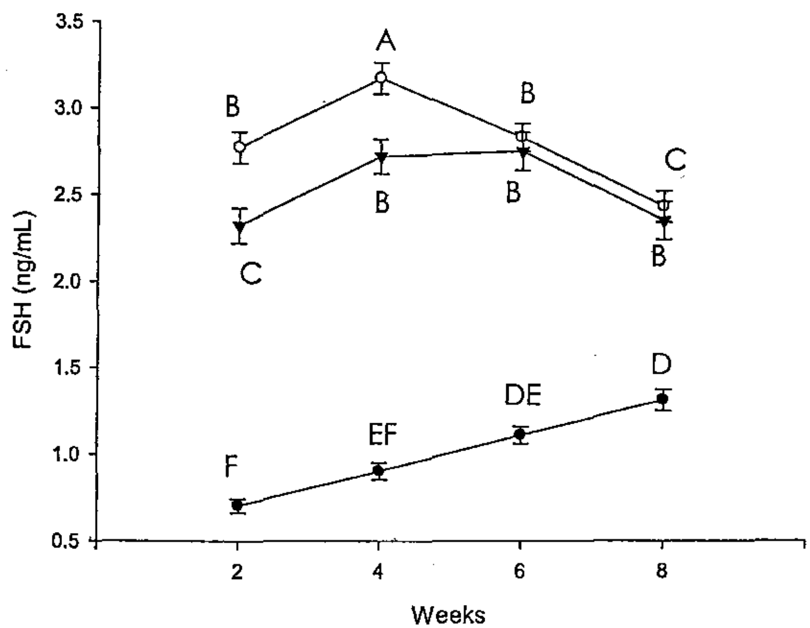

Figure 2. Effect of weekly injection (i.m.) of 0, 0.42, or 0.84 μg Buserelin Acetate (BA) on plasma FSH of 50-wk-old Alexandria hens

Estradiol:

Overall, low-BA resulted in a 41% (p=0.0067) increase in E_2 compared to control hens, as indicated by the values shown in Table 1. The 8% increase in E_2 in response to high-BA did not differ from control. Concentrations of E_2 in control hens did not change over the course of the experiment. Low-BA resulted in elevation of E_2 over control by,47 and 36% during TRT and REC, respectively (p=0.0192). The response of E_2 to high-BA differed considerably from LH and FSH, with no effect seen during TRT but a 36% increase over control during REC.

In control hens, E_2 concentrations did not change over experimental time (Fig 3). As with LH and FSH, the response of E_2 to low-BA was greater than to high-BA (p=0.0034). Unlike the other two hormones, however, E_2 in response to high-BA was lower than the other two groups, including control (Fig 3). Whether this was a direct effect of BA on E_2 or an indirect effect mediated through LH (Fig 1) is uncertain; however, high doses of GnRH have been shown to decrease LH (Peters *et al.*, 1994; Ulker *et al.*, 2001 and Sato *et al.*, 2005), so the possibility of an indirect effect exists.

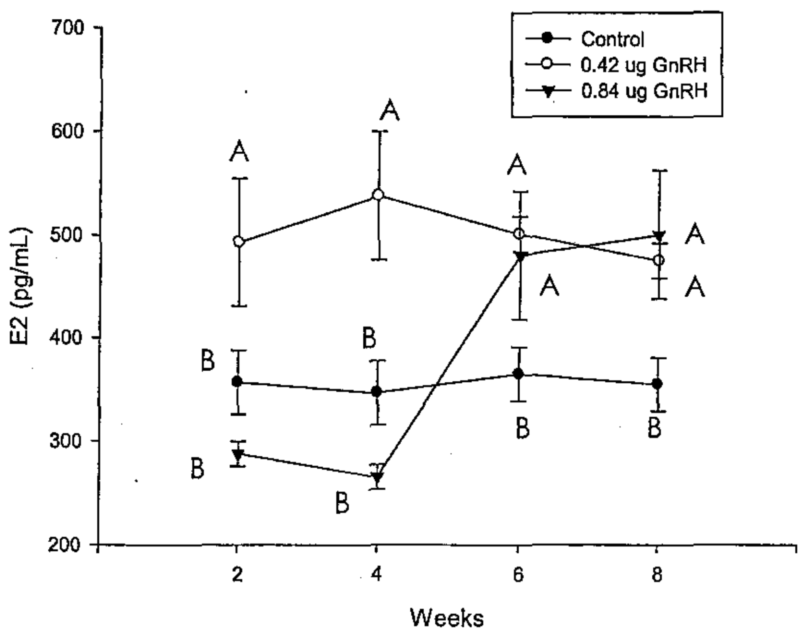

Figure 3. Effect of weekly injection (i.m.)of 0, 0.42, or 0.84 µg Buserelin Acetate (BA) on plasma estradiol (E_2) of 50-wk-old Alexandria hens

These findings are in harmony with previous findings, when young Taihe hens were implanted with capsules releasing GnRH, plasma estradiol concentration was higher in the GnRH group than in the control group, and it was concluded that GnRH capsouls stimulated sexual precocity and ovulation in young Taihe hens (Wang *et al.*, 1996) .

The profound effect of the lower dose of BA over the higher one observed in this study may possibly be attributed to the fact that high doses of GnRH may result in reduced storage of LH in the pituitary gland and in decreased numbers of GnRH receptors on pituitary cells, resulting in suppressed gonadotropin secretion from the pituitary gland (Peters *et al.*, 1994; Ulker *et al.*, 2001 and Sato *et al.*, 2005).

Egg Production:

Overall,egg production increased (p=0.0001) as a result of low- and high-BA treatments by 37% and 29%, respectively, over control (Table 1). Among control birds, egg production declined (p=0.0132) over the eight wk of the study by 15%, whereas low-BA boosted egg production over control by 30% during TRT period and the effect rose to 44% above control during the REC period (p=0.0132). As with LH and FSH, high-BA resulted in egg production levels that were intermediate to the other two but not different from the low-BA REC level (Table 1). The weekly egg production peak (Fig 4) occured simultaneously with the peaks of LH, FSH and E_2 (Fig 1,2,3) at the 4th wk of treatment with low-BA (p=0.0001). The lowest egg production was recorded by the control group at the end of the experimental period.

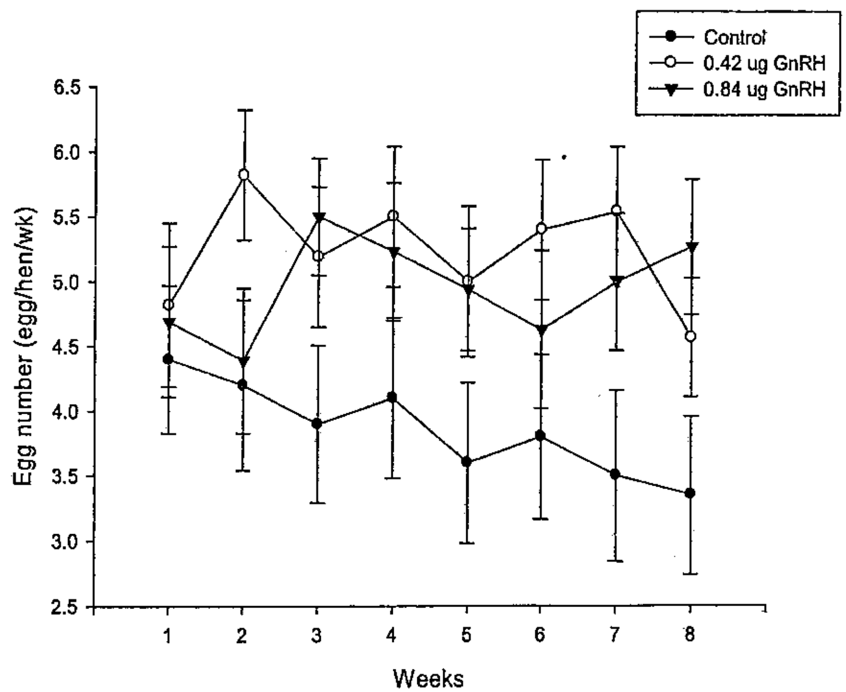

Figure 4. Effect of weekly injection (i.m.) of 0, 0.42, or 0.84 µg Buserelin Acetate (BA) on egg production of 50-wk-old Alexandria hens

These findings are in agreement with the findings of Costantini *et al.* (2009), who reported that when Budgerigar (captive-bred endangered birds) were treated with slow-release GnRH analogue they showed higher rates of egg-laying. Also, when young Taihe hens were implanted with capsules releasing GnRH,. the laying rate increased significantly (Wang *et al.*, 1996).

Egg weight on the other hand responded differently to BA treatments as it declined (p=0.0001) to 97% of control egg weight (Table 1). Among control birds, egg weight declined by 3%, (p=0.0001) over the course of the study. Low-BA resulted in egg weights that were 3% and 2% lower and high-BA resulted in egg weights that were 5% and 1% lower than control during TRT and REC, respectively (p=0.0001). As shown in Fig. 5, the highest egg weights were recorded for the control group during the first 4 wk of the experimental period.

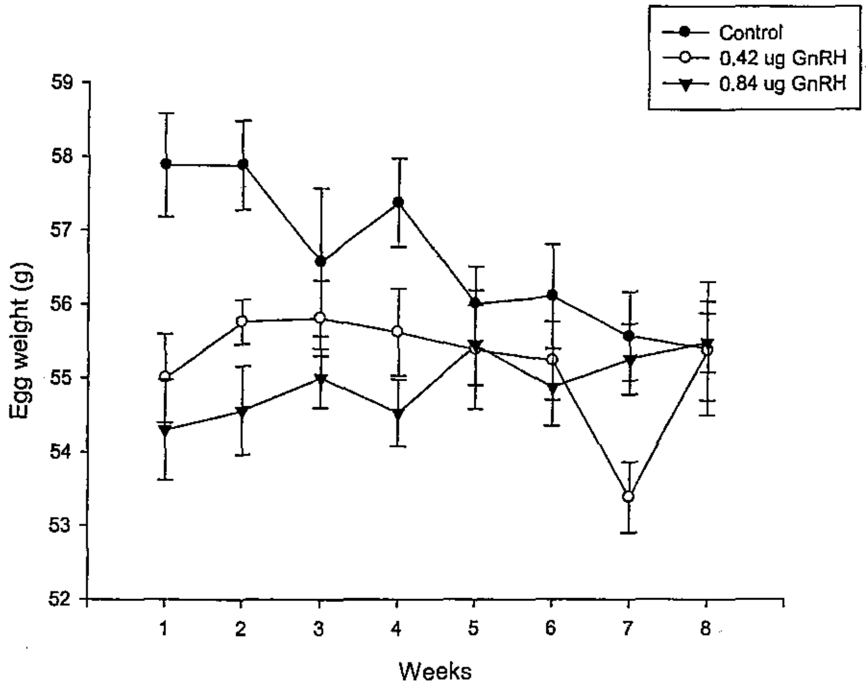

Figure 5. Effect of weekly injection (i.m.) of 0, 0.42, or 0.84 µg Buserelin Acetate (BA) on egg weight of 50-wk-old Alexandria hens

Overall, low- and high-BA treatments resulted in a 32% and 24% increase, respectively (p=0.0001) in egg mass compared to control (Table 1). Egg mass in control birds decreased by 14% (p=0.0012) over the 8 wk study, whereas low-BA increased egg mass by 25% over control values during TRT and by 38% during REC (p=0.0001). The effect of high-BA was less profound, as egg mass increased 14% and 36% over control during TRT and REC, respecively. As shown in Fig. 6, weekly egg mass was highest (p=0.0001) in low-BA hens after 2 wk of treatment, whereas the lowest was recorded by control birds at the end of the experimental period.

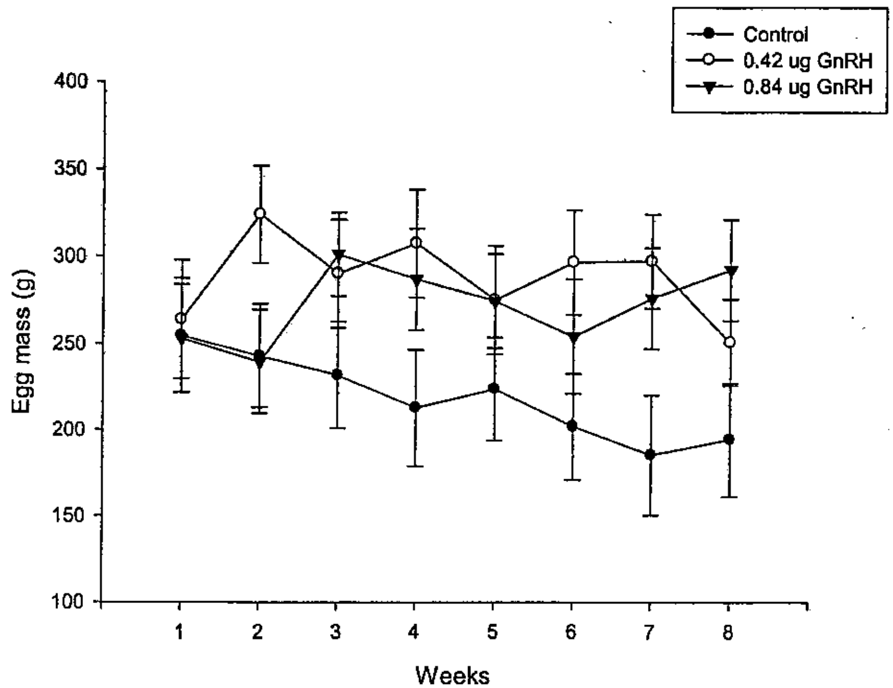

Figure 6. Effect of weekly injection (i.m.) of 0, 0.42, or 0.84 µg Buserelin Acetate (BA) on egg mass of 50-wk-old Alexandria hens.

It can be concluded that in Alexandrian laying hens the decrease in egg production associated with age may be attributed to a reduction in plasma LH, despite increasing FSH and stable E_2. It should be noted that hens in this 8-wk study were all near the end of the laying period, so no hormone or production comparisons could be made with early-lay birds. It appears, based on the BA treatment results of this study that a low dose of this GnRH analogue may be a useful tool for treating hens at the end of the production curve to improve end-of-lay production.

REFERCENCES

Akiba Y., L.S. Jensen, C.R. Bart, R.R Kraeling, 1982. Plasma estradiol, thyroid hormones and liver lipids content in laying hens fed different caloric diets. J. Nutr., 112: 299-308.

Ciccone N.A, P.J. Sharp, P.W. Wilson, I.C. Dunn, 2005. Changes in reproductive neuroendocrine mRNAs with decreasing ovarian function in aging hens. Gen. Comp. Endocrinol. 144 (1): 20-27.

Costantini V, Carraro C, Bucci FA, Simontacchi C, Lacalandra GM, Minoia P, 2009. Influance of a new slow-release GnRH analouge implant on reproduction in the Budgerigar (*Melopsittacus undulatus,* Shaw 1805). Anim. Rep. Sci. 111(2/4): 289-301.

Dalkin A.C., D.J. Haisenleder, G.A. Ortolano, T.R. Ellis, J.C. Marshall, 1989. The frequancy of gonadotropin-releasing-hormone stimulation differently regulates gonadotropin subunit messenger ribonucleic acid expression. Endocrionl. 125: 917-924.

Duncan D.B., 1955. Multiple range and multiple F tests. Biomet. 11: 1-42.

Elnagar Samar A., 2009. Response of Alexandria cockerels reproductive status to GnRH (Receptal) injection. Int. J. Poult. Sci. 8(3): 242-246.

Follett B.K., C.G. Scanes, F.J. Cunningham, 1972. A radioimmunoassay for avian luteinizing hormone. Endocrinol. 52: 359-378.

Kaiser U.B., A. Jakubowiak, A. Steinberger, W.W. Chin, 1997. Differential effects of gonadotropin-releasing hormone (GnRH) pulse frequency on gonadotropin subunit and GnRH receptor messenger ribonuclic acid levels in vitro. Endocrinol. 138: 1224-1231.

Krishnan K.A., J.A. Proudman, D.J. Blot and J.M. Bolir, 1993. Development of an homologous radioimmunoassay for chicken follicle stimulating hormone and measurement of plasma FSH during the ovulatory cycle. Comp. Physiol. 105A: 729-734.

Mans C. and W.M. Taylor, 2008. Update on neuroendocrine regulation and medical intervention of reproduction in birds. Vet. Clin. Exot. Anim. 11: 83-105.

Peters K.E., E.G. Bergfeld, A.S. Cupp, F.N. Kojima, V. Mariscal, T. Sanchez, M.E. Wehrman, H.E. Grotjan, D.L. Hamernik, R.J Kittok and J.E. Kinder, 1994. Luteinizing hormone has a role in development of fully functional lutea (CL) but is not required to maintain CL function in heifers. Biol. Reprod. 51: 1248-1254.

SAS institute 1996. SAS® Users's Guide: Statistics. SAS Institute Inc., Cary, NC.

Sato T., K. Nakada, Y. Uchiyama, Y. Kimura, N. Fujiwara, Y. Sato, M. Umeda and T. Furukawa, 2005. The effect of pretreatment with different doses of GnRH to synchronize follicular wave on superstimulation of follicular growth in dairy cattle. J. Reprod. Sci. 51: 573-578.

Ubuka T., N.L. McGuire, R.M. Calisi, N. Perfito and G.E. Bentley, 2008. The control of reproductive physiology and behavior by gonadotropin-inhibitory hormone. Integ. Comp. Biol. 48(5): 560-569.

Ulker H., B.T. Gant, D.M. de Avila and J.J. Reeves, 2001. LHRH antagonist decreases LH and progesterone secretion but does not alter length of estrous cycle in heifers. J. Anim. Sci. 79:2902-2907

Wang G., D. Huang, W. Zhao, J. Zhu, B. Fan, X. Zhang, and Jiangsu, 1996. Ovulation of Taihe hens stimulated by slow-release gonadotropin capsules. J. Agric.Sci. 12(3): 36-40.

Wang G., Z. Li, J. yan, Z. Xie, W. Zhao, J. Zhu and D. Huang, 1994. A study of the reproductive endocrinology of ovulation induced by [D-Leu6,Pro9]-GnRH N-ethylamide in laying Taihe hens. Acta Agir. Nucl. Sin. 8(4): 226-232.

مشابه محرر الهرمون الجنسي (GnRH) المعروف بخلات البيوسرلين يعدل الاداء التناسلي "للدجاجات المسنة"

سمر علي النجار

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أجريت التجربة لاختبار تأثير مشابه الهرمون الجنسي المعروف بخلات البيوسرلين علي النشاط الإنتاجي للدجاجات العتاقي. تم استخدام 60 دجاجة اسكندراني عمر 50 أسبوع وتم توزيعها عشوائيا إلي ثلاثة معاملات : دجاجات المجموعة الثانية والثالثة تم حقنهم تحت الجلد أسبوعيا بتركيز 1. و 2. مل من (GnRH) والذي يوفر 0.42 و 0.84 ميكروجرام من خلات البيوسرلين علي التوالي وذلك لمدة شهر بينما دجاجات المجموعة الأولي كانت بمثابة الكنترول أو المقارنة. استمرت التجربة شهرين (شهر معاملة وآخر استرجاع) وذلك لمعرفة التأثير علي الصفات الإنتاجية مثل (إنتاج البيض- وزن وكتلة البيض) وتقدير هرمونات التبويض والهرمون المنبة لنمو الحويصلات المبيضية وهرمون الأسترادايول في الدم وكانت أهم النتائج المتحصل عليها هي:

أدت المعاملة بخلات البيوسرلين الي زيادة 58 و 30 % في تركيزات هرمون التبويض وهرمون نمو الحويصلات المبيضية ،كما أدت المعاملة إلي زيادة من 177 و 150 % وأيضا زيادة 41 و 8 % في تركيز هرمون الأستروجين وذلك مع الجرعات المنخفضة والعالية من خلات البيوسرلين وذلك بالمقارنة بالكنترول علي التوالي.

إنتاج الدجاج الأسبوعي يظهر إختلافات معنوية نتيجة المعاملة بخلات البيوسرلين حيث ارتفعت بنسبة 42 و35 % وذلك مع الجرعات المنخفضة والعالية علي التوالي ومن ناحية اخري فإن وزن البيض يظهر اختلافات نتيجة المعاملة حيث انخفض بشكل ملحوظ ليصل إلي 97% من وزن بيض المجموعة المقارنة مع الجرعتين

نستطيع أن نقول أن إنخفاض إنتاج البيض في دجاجات أسكندرية المرتبط بتقدم العمر يعزي إلي انخفاض تركيز هرمون التبويض وهرمون نمو الحويصلات المبيضية في البلازما وبالتالي فإن معالجة الدجاجات في نهاية منحني إنتاج البيض بالحقن بمحرر الهرمون الجنسي (GnRH) يكون قادر علي زيادة تركيز البلازما من هرمون التبويض والهرمون المنبة لنمو الحويصلات المبيضية وبالتالي إنتاج البيض.