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The Effects of Chicken GnRH on Serum Testosterone Concentration and Egg Fertility in Japanese Quail

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ABSTRACT

The decrease in fertility of aged cocks results with decreased hatching rate. Male fertility is under the control of the hypothalamus-pituitary and testicular axis. Therefore, the objective of this study is to measure the effect of chicken GnRH-I (cGnRH-I) on serum testosterone concentration and egg fertility. Twenty weeks old male and female Japanese quail were kept in cages and fed *ad libitum*. Quails were randomly divided into 4 groups as phosphate buffered saline group (PBS), 5 µg cGnRH-I group, 20µg cGnRH-I group and group received no injection (Naturel group, N group). Each group included 8 replicates and each replicate made up randomly selected 8 females and 2 males. Only male birds were subcutaneously injected once a week for three weeks with 200 µl as PBS, 200 µl PBS containing 5 or 20µg cGnRH-I. One week after the last injection, eggs from each group were weekly collected and incubated for 11 days and broken on day 12 then the numbers of fertilized and unfertilized eggs were determined. Blood samples from the males were obtained from the jugular vein. Serum was extracted from the clotted blood by centrifuging. Total serum testosterone levels were measured by competitive immunochemistry. Injection of 5µg cGnRH increased serum testosterone concentration over PBS injected group other differences were not statistically different. Injection of cGnRH did not cause significant changes in fertility rates. To conclude, 20 weeks old male quails were injected with cGnRH-I and giving extra GnRH-I did not cause any difference in fertility, since there was plenty of GnRH in their blood.

Japon Bıldırcınlarında Tavuk GnRH'nin Serum Testesteron Konsantrasyonu ve Yumurtalarda Döllülük Üzerine Etkileri

MAKALE BİLGİSİ

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Araştırma Makalesi

ÖZET

Yaşlı erkeklerin fertilitesindeki düşüş çıkış oranında azalmayla sonuçlanır. Erkeğin fertilitesi hipotalamo-hipofisyal ve testis eksenin kontrolü altındadır. Böylece bu projenin amacı tavuk GnRH-I (cGnRH-I)'in serum testosteron konsantrasyonuna ve yumurta döllülük oranına etkisini ölçmektir. Yirmi haftalık erkek ve dişi Japon bıldırcınları kafeslere konuldu ve *ad libitum* beslendiler. Bıldırcınlar random olarak; fosfatla tamponlanmış tuz çözeltisi (PBS), 5 µg cGnRH-I, 20µg cGnRH-I enjektedilen grup ve enjeksiyon yapılmayan doğal grup (N) olmak 4 gruba ayrıldı. Her grup 8 alt grupa ayrıldı ve

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Anahtar Kelimeler

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her bir alt gruba 8 dişi 2 erkek konuldu. Sadece erkekler haftada bir kez olmak üzere üç hafta boyunca 200 µl PBS, 5 veya 20 µg cGnRH-I içeren 200 µl PBS ile enjekte edildiler. Son enjeksiyondan bir hafta sonra yumurtalar haftalık toplandı, 11 gün boyunca kuluçka makinasında tutuldu ve 12. günde kırılarak döllü ve dölsüz yumurtalar belirlendi. Kan numuneleri erkelerin jugular damarından alındı. Serum; pıhtılaşan kan örneklerinden santrifüjle ayrıldı. Serumdaki toplam testesteron kompetetif ümmünokimya yöntemiyle ölçüldü. 5µg cGnRH enjeksiyonu serum testesteron konsantrasyonunu PBS enjekte dilen gruba kıyasla artırırken diğer faklar istatistiksel olarak önemli bulunmadı. GnRH enjeksiyonu fertilite oranında önemli değişmeye sebep olmadı. Bu çalışmada 20 haftalık genç bıldırcınlara cGnRH enjeksiyonu yapıldı. Hayvanlar genç oldukları için kanlarında yeteri kadar GnRH vardı ve fazladan GnRH enjekte etme bir farka sebep olmadı.

Introduction

It was firstly proposed that a factor from the hypothalamus is released into the anterior pituitary gland to stimulate gonadotropin secretion by Geoffrey Harris, John Everett, and Charles Sawyer between 1930-1940 (Terasawa *et al*., 2010).This hypothalamic factor was initially named as Luteinizing Hormone Releasing Hormone (LHRH) because of its preferential positive effect on luteinizing hormone (LH) secretion rather than the secretion of Follicle Stimulating Hormone (FSH) (McCann *et al*., 1960). However, injection of a specific LHRH antagonist suppressed both LH and FSH secretion. Therefore, it was named as gonadotropin releasing hormone (GnRH). Gonadotropin releasing hormone was firstly purified from pig, ovine and bovine hypothalamus (Kochman and Domański, 1969; Schally *et al*., 1971) and its molecular structure, as a decapeptide, was firstly explained by Andrew Schally and his team in 1971 (Schally *et al*., 1971). This GnRH is accepted as mammalian GnRH and designated as GnRH-I. The discovery of GnRH-I led to extensive research in this field and it is still an active area of research. Empirical studies on different protocohordata and vertebrata species have shown the presence of two distinct varieties of GnRH identified in chicken brain (named as chicken GnRH-I and II). Chicken GnRH-I (cGnRH-I) rather than chicken GnRH-II (cGnRH-II) is considered to be the biologically active neuropeptide controlling gonadotropin secretion (Sharp *et al*., 1990). In addition to these, more than 30 different varieties of GnRH were identified (Lescheid *et al*., 1997; White *et al.,* 1998; Latimer *et al*., 2001). In-situ hybridization, northern transfer, inmmunehistochemichal and western blotting techniques have shown that hypothalamus and pituitary are not only the places where GnRH and GnRH receptors are expressed, they are also expressed in extra hypothalamic sites such as in leydig cells, seminiferous tubules, Sertoli cells, and in developing germ cells (Bahk *et al.,* 1995; Bull *et al*., 2000; Ramakrishnappa *et al.*, 2005; Anjum *et al*., 2012).

Male fertility is under the control of the hypothalamus-pituitary and testicular axis and it is an economically

important factor affecting the hatching rate. The fertility of layer breeder roosters starts to decline after about 70 weeks of age (Rosenstrauch *et al*., 1998; Weil *et al*., 1999a). However, the fertility of male broiler breeders declines earlier about 45-55 weeks of age (Rosenstrauch *et al*., 1994; Weil *et al*., 1999a). In Japanese quail, fertility decreases from the week 56 onwards (Ottinger *et al.*, 1983).

Experimental studies have shown that the decrease in fertility occurs due to the reduction in sperm number in the ejaculate (Weil *et al*., 1996), as well as structural changes in the Leydig cells (Rosenstrauch *et al*., 1998). According to another study reduction in fertility occurs due to the decrease in plasma LH and testosterone concentrations (Weil *et al.*, 1999b). The decrease in fertility of aged males results with decreased hatching rate and this lead to the removal of the males from the flock. But, poultry ındustry is toughly suffering from the shortage of supplying the breeder males on time at required number. Thus, keeping the genetically superior breeder males within the flock longer, without any decrease in fertility, is economically important. Also, the physiology of mammalian GnRH-I is well known, but there has been scarce knowledge about the physiology of chicken cGnRH-I. Therefore, the objective of this study was to measure the effect of cGnRH-I on quail serum concentration of testosterone and fertility.

Material and methods

Animal, feeding and grouping

Twenty weeks old male (n=64) and female (n=256) Japanse quail (*Coturnix japonica*), reared from the same hatch at Animal Research and Exploration Centre, Süleyman Demirel University, Isparta-Turkey. During the course of the study, birds were kept in 60 x 35 x 97 cm scattered cages (Cimuka BYK-03-4K, Ankara-Turkey) under 16L:8D light–dark cycle and fed *ad libitum* with a diet supplying 16 % crude protein (CP) and 2750 kcal/kg metabolic energy (ME). Birds were randomly divided into 4 groups as Phosphate Buffered Saline (PBS) group, 5 µg cGnRH group, 20µg cGnRH group and group received no injection (Naturel group, N group). Each group consisted of 8 replications and each replication made up randomly selected 8 females and 2 males.

Preparation of PBS and cGnRH-I

Two PBS tablets (Cat; P4417-100AB, Sigma-Aldrich Co.,3050 Spruce street, St. Louis, MO 63103 USA) were dissolved in 400 ml of deionized water (pH: 7.4) and subsequently sterilized by filtering through a corning 500 ml bottle-top 0.22 µm vacuum filter (Cat: 430521, Corning Incorporated, Corning. NY 14831, USA). Five mg cGnRH-I (Cat; LHRH-012A, CPC scientific, 1245 ream wood avenue Sunnyvale, CA 94089, USA) was dissolved in PBS and its concentration was arranged to 5 or 20 µg/200 µl with PBS.

Injection

After a one week adaptation period, only male birds (n=2, from each replication) were subcutaneously

injected, once a week (Tuesday at 10:00) for three weeks with PBS (Cat; P4417-100AB, Sigma-Aldrich Co.,3050 Spruce street, St. Louis, MO 63103 USA), PBS containing 5µg cGnRH (Cat; LHRH-012A, CPC Scientific, 1245 Ream wood Avenue Sunnyvale, CA 94089, USA), PBS containing 20µg cGnRH (Cat; LHRH-012A, CPC Scientific) and a group received no injection (Natural group, N).

Measurement of fertility

One week after the last injection, eggs from each group were weekly collected and incubated in an incubator (Cat; 44194949, Cimuka Hb700c, Ankara-Turkey) for 11 days. Incubated eggs were broken on day 12 and the numbers of fertilized and unfertilized eggs were determined by looking at the development of embryos.

Blood collection

Three hours after the last injection, male quails were individually taken into the abattoir behind the poultry yard and blood samples (about 3 ml) were obtained by cutting the jugular vein with a scalpel blade and poured into 5ml tubes (BD-Belliver Industrial Estate, Cat: 367955, Plymouth UK) containing gel and clot activator at room temperature. Then the birds were decapitated by cutting the neck.

Serum extraction and the *measurement of serum testosterone concentration*

Serum was extracted from the clotted blood by centrifuging at 4000 RPM for 10 minutes at room temperature by using a centrifuge (Nüve, NF 200, Serial no: 02.12766, Ankara, Turkey). Separated serum was kept at -30 ºC in a deep freezer and one week after the blood collection, serum samples (n=64) were taken out from the -30ºC deep freezer (Raypa ACH 284, Galileo Equipments, S.L., 28108 Madrid, Spain). Total serum testosterone levels were measured by competitive immunochemistry with chemiluminescence technology (ADVIA Centaur TSTO Ready Pack; Siemens Healthcare Diagnostics Inc. 511 Benedict Avenue Tarrytown, NY 10591-5005, USA).

Statistical analysis

All data are presented as mean + SEM and analysed by one-way analysis of variance (ANOVA) using Minitab statistical software (Minitab 15). Pairwise comparisons were conducted according to the Tukey test, with 95% confidence intervals.

Results

Injection of **5**µg cGnRH-I increased serum testosterone concentration over PBS injected group (P=0,036) other differences were not statistically different (P=0,117) (Figure 1). Injection of cGnRH did not cause major changes in fertility rates (P>0.05). Not important differences were found in fertility rate between the experimental groups in first (P=0.574), second (P=0.25) and third week (P=0,387) after the last injection (Figure 2).

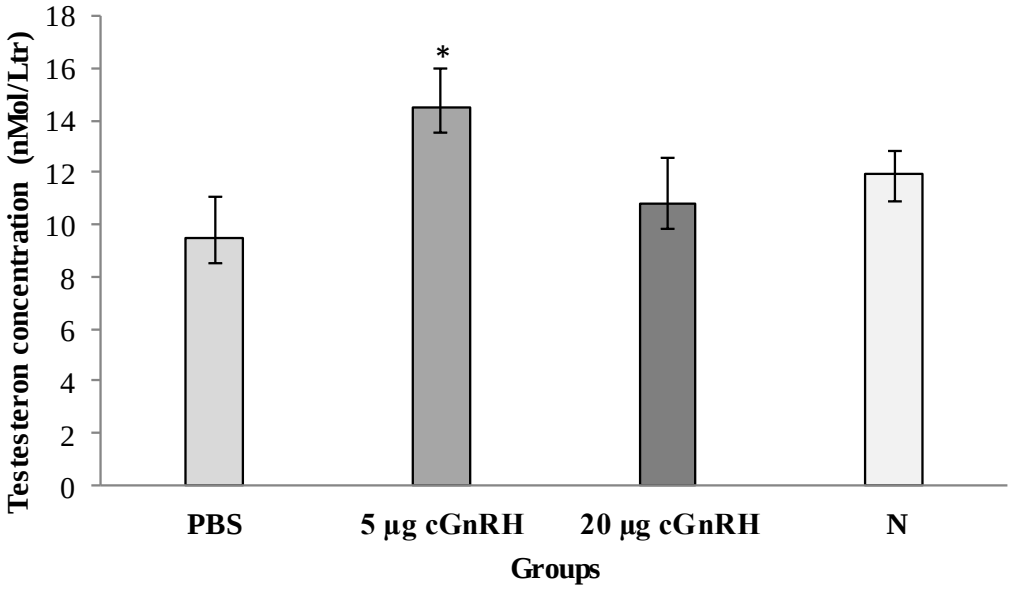

Figure 1. Serum testosterone concentration of Japanese quails 3h after the injections. Star (*) indicates that the difference is significant (P= 0,036) as compared with PBS injected group. Other differences were not significant (P=0,117).

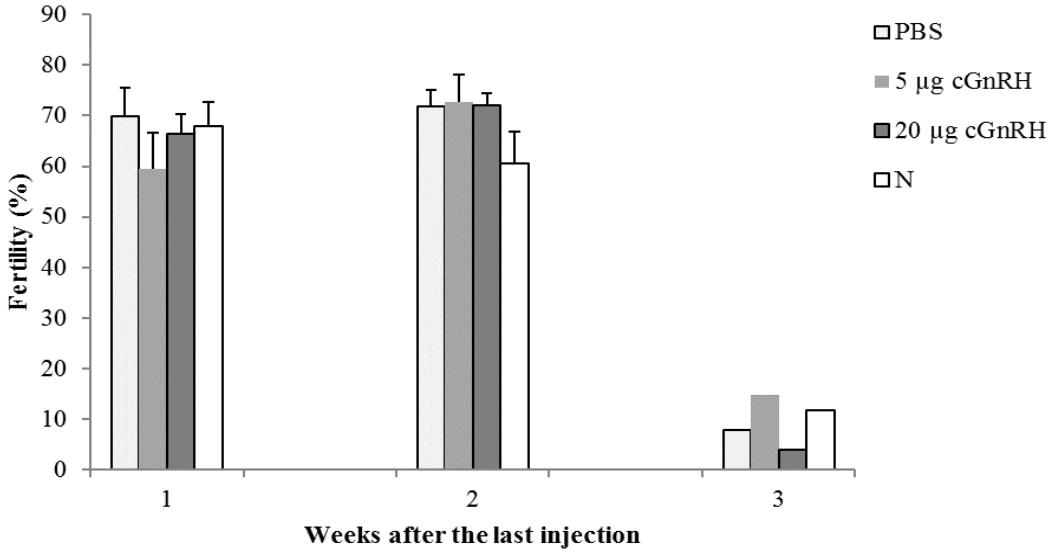

Figure 2. The fertility rates in 1st, 2nd and 3rd weeks after the last injection. Differences were not statistically significant (P>0.05).

Discussion

Injection of 5µg cGnRH-I significantly increased serum concentration of testosterone over PBS injected group (P<0.05). Serum testosterone concentration was the lowest as compared with natural group.

It indicates that PBS has adverse effect on blood testosterone concentration. This is probably the reason why injection of 5μg cGnRH-I caused a significant increase in serum testosterone concentration over PBS injected group. In a study, conducted by Qasimi *et al*. (2018), PBS injected 15 to 100 d ay old group of male quail chicks were kept as a control group, while the other group of the same age quails were injected with 10 μg cGnRH-I. According to their results, injection of cGnRH-1 increased plasma LH and testosterone concentration over the PBS injected control (Qasimi *et al*., 2018). Even there is age and dose difference, almost the similar results obtained in this study. Here, when the injection dose of GnRH-I increased to 20 μg serum concentrations of testosterone were non-significantly decreased as compared with 5 μg cGnRH-I and natural group (N). It is more likely that the testosterone secretion response to cGnRH depends on the dose applied. Low dose (5 μg) has positive effect on serum testosterone concentration, while high dose (20 μg) has negative effect. In this study, we could not see any substantial difference in fertility, because we injected 20 weeks old male quails. So, they were young and have enough amount of GnRH in their blood to increase luteinizing hormone and testosterone secretion for spermatogenesis and fertility. Fertility decreases in Japanese quail from the week 56 onwards (Ottinger *et al*., 1983). If we had got injected male quails older than 56 weeks, the difference in fertility would have be significant.

Empirical studies have demonstrated that sexually active birds express more immunoreactive GnRH neurons than sexually inactive counterparts (Sharp *et al*., 1990; Hahn and Ball, 1995; Parry *et al*., 1997; Cho *et al.*, 1998). Lower hypothalamic GnRH-I secretion in ageing roosters has been associated with the lower pituitary expression of mRNA for LH and FSH relative to that of young roosters. Consequently, testes weight, semen volume, sperm concentration and plasma testosterone concentrations are generally lower in older roosters (Avital-Cohen *et al*., 2013). Luteinizing hormone (LH) binds to its receptor on Leydig cells and causes the secretion of testosterone (Avital-Cohen *et al*., 2013). Studies have shown that plasma testosterone concentrations diminish in ageing roosters (Weil et al., 1999b; Ottinger *et al*., 2002). Reduced blood concentrations of FSH in ageing roosters have been deemed responsible for diminished daily sperm production, which is in turn strongly associated with decreased fertility (Rosenstrauch *et al*., 1994; Weil *et al*., 1996; Vizcarra *et al*., 2010).

In this study, male quails were culled just after the third injection and the absence of males dramatically decreased the fertility rate in third week. In this area, the data are scarce and therefore it is difficult to compare the present result with the results obtained from preliminary studies. Because, this is the first study assessing the impact of cGnRH on serum testosterone concentration and fertility rate in quail.

As a conclusion, young male quails injected with cGnRH-I and giving extra GnRH-I did not cause any

difference in fertility, since there was plenty of GnRH in their blood. If we had have got injected male quails older than 56 weeks, the results would have been significant.

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