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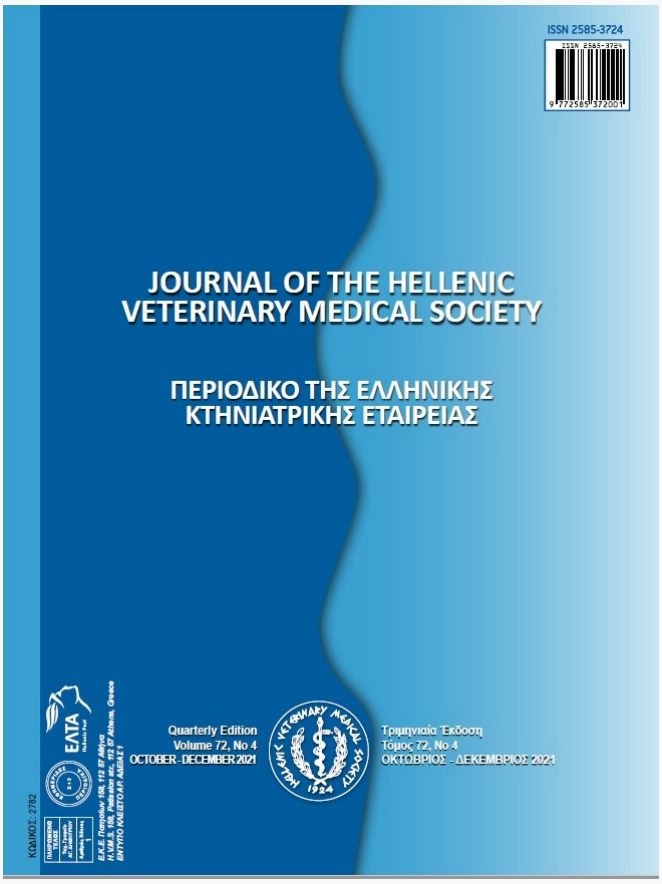

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Research article
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The effects of cGnRH on gonadotropin secretion and hatching traits in Japanese quail

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ABSTRACT: The aim of this study was to measure the effect of chicken Gonadotropin Releasing Hormone-I (cGnRH-I) on male fertility potential. Male and female quails were placed in cages(1 male and 5 females) and devoted into four experimental groups: a) control group - no injection, b) negative control group - 200 µl standard saline was injected, c) 5µg cGnRH group - 5µg cGnRH-I was injected and d) 20µg cGnRH group - 20µg cGnRH-I was injected. Each group was consisting of scattered six cages. In each cage only males were subcutaneously injected under the wing (3 injections - 1 week apart from each other). A fourth injection was administered at the end of egg collection and one hour later blood was collected and serum concentrations of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), prolactin and testosterone were measured. Egg fertility, hatchability and weekly egg production per hen were also measured. Injection of cGnRH-I did not increase pituitary gonadotropin secretion (LH or FSH); however, serum LH concentration non-significantly reduced in negative control group. A significant decrease in serum testosterone concentration was observed in negative control group compared to 20 µg cGnRH injected group. Fertility and hatchability of total set eggs were lower in negative control group compared to other groups. Egg production in control group was significantly decreased, probably due to the non-significant suppression of prolactin. Embryonic mortality (hatchability of fertile eggs) non-significantly increased in control and negative control groups compared to GnRH injected groups. It seems that cGnRH has a positive effect on fertility and hatchability; however, more studies are needed with older males to confirm our findings.

***Keywords*:** Egg, Fertility, Hatchability, Quail, Testosterone

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INTRODUCTION

Naturally, quails are seasonal breeders. They breed when there is enough food, water, light, temperature and security for the mother to raise the offspring (Ubuka et al., 2013). Gonadal development occurs in spring, in response to increasing day length. If the day length is over 11.5 h, rapid gonadal development occurs. Reproductive cycle terminates in late summer when the day length decreases below 14.5 h causing the complete regression of gonads (Nicholls et al., 1988). If the short day length is artificially increased (16h light and 8h dark) then a complete return to maturity occurs (Nicholls et al., 1988). Quails remain in breeding continuously by keeping them on unchanging 16h light and 8h dark cycle after the short days.

Quails use photoperiodic information directly through photoreceptors located within the mediobasal hypothalamus, which regulates the synthesis of Gonadotropin Releasing Hormone (GnRH) (Dawson, 2015). GnRH regulates reproduction through the regulation of the pituitary gonadotropins secretion *via* GnRH receptors on the gonadotropes in anterior pituitary gland (Kaprara and Huhtaniemi, 2018).

In birds, two distinct forms of cGnRH (cGnRH-I and cGnRH-II) have been characterized, and a third immune-reactive form similar to lamprey GnRH-III has also been reported (Bédécarrats et al., 2006). Chicken GnRH-I was first chromatographically isolated from chickens' hypothalamus (Miyamoto et al., 1982). Its structure and amino acid sequence were further determined (King and Millar, 1982; Miyamoto et al., 1983). It was shown that cGnRH-I differs from the mammalian GnRH-I by the substitution of Arg with a Gln at position 8. Miyamoto et al., (1984) identified a second form of GnRH (cGnRH-II) from chicken hypothalamic extracts. ThecGnRH-II differs from cGnRH-I at position 5, 7 and 8 (Bédécarrats et al., 2006).

In quails and chickens, in vitro as well as in vivo experiments have shown that both cGnRH-I and II stimulate the release of LH and FSH (Hattori et al., 1986). In contrast to mammalians, LH and FSH are synthesized from different gonadotropes in anterior pituitary gland, in poultry (Bédécarrats et al., 2006). Both LH and FSH act on the gonads to stimulate gametogenesis and sex steroid secretion (Ubuka et al., 2013).

It was reported that photo-stimulation causes an increase in GnRH release within 23 h in in vitro condition in quails (Millam et al., 1984; Perera and Follett, 1992). Photo stimulation causes substantial increase in LH and FSH secretion in male quails leading to the increase in steroid synthesize and the attainment of the puberty in about 5 weeks (Ubuka et al., 2013). According to Follet (1976), LH, FSH and testosterone could be detected in birds at short-day but their concentrations were greatly increased following photo-stimulation. In quail, LH pulses were observed only after exposure to long days with a frequency of 1 pulse per 80 min (Urbanski, 1984). In spring, LH levels first rose when the day length reached 11.9 h; however, in late summer the first signs of a decrease were detectable when the photoperiod decreased to 14.7 h and levels became basal when it reached the14 h (Robinson and Follett, 1982).

Aging in male quails and chickens causes reduction in GnRH-I release (Ottinger et al., 2004; Avital-Cohen et al., 2013). There is significant decrease in quail egg fertility and hatchability from the week 56 of age and onwards (Ottinger et al., 1983). According to Ottinger et al. (2002) aging in quails causes morphological abnormalities in the testes and the decrease in LH and FSH receptor expressions leading to the loss of fertility. However, in young photo-regressed males, testosterone implants stimulated the increased LH binding; whereas they did not affect FSH binding. The response to testosterone treatment was reduced by aging (Ottinger et al., 2002).

Reduction in GnRH-I secretion, in ageing roosters, has been associated with the lowest LH and FSH secretion and a reduction in plasma testosterone concentration (Ottinger et al., 2002; Weil et al., 1999). As a result, testes weight, semen volume and sperm concentration found generally lower compared to that of young roosters (Avital-Cohen et al., 2013). 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 egg fertility (Rosenstrauch et al., 1994; Vizcarra et al., 2010).

Egg fertility constitutes an economically important factor in the poultry industry due to its effect on chick hatching. Reduction in egg fertility leads to diminished hatching rate and causes the removal of the males from the flock. Poultry industry is current-

ly suffering from a shortage of breeder males. Therefore, keeping genetically superior breeder males within the flock for a longer period of time, without any decrease in fertility, is economically important. Thus, the main aim of this study was to evaluate the effects of cGnRH-I injection on fertility of male Japanese quails (*Coturnix japonica*).

MATERIAL AND METHODS

Animal, housing, feeding and adaptation period

Japanese quails (*Coturnix Japonica*) used in this study were housed and treated according to the animal right committee act no 5199, which was published in 25509 numbered official paper of the state, on 01 July 2004. The related certificate from Selçuk University, School of Agriculture, Animal Right Committee allowing animal experimentation was obtained (Certificate no: 2019/2-003). On 5th of December 2019, fortyweeks old male (n=24, average body weight= 213.20 ± 6.35) and female quails (n=120, average body weight= 238.31 ± 10.28) were randomly selected and housed in numbered (From 1 to 24) cages (25 x 35 x 48 cm) under 16L: 8D light-dark cycle. Birds were placed in cages as one male and five females in the poultry yard of the School of Agriculture, Selçuk University. Along the course of the study, birds were fed *ad libitum* with diet containing 20% CP, 2901 kcal/kg metabolic energy, 2.5%Ca, 0.35% available P, 1.055% LIMS and 0.45% M. Water was freely available.

Preparation of cGnRH-I

Five mg cGnRH-I (Cat: ab143495, Abcam, Discovery Drive, Cambridge Biomedical Campus, Cambridge, CB2 0AX,UK) was dissolved in pre-prepared isotonic saline (OSEL Pharmacy and Trade Incorporation, Beykoz, İstanbul-Turkey) and then its concentration was arranged to 5 or 20 µg/200 µl. The amino acid sequence of injected cGnRH-I was different from the mammalian GnRH-I at position 8 as the Arg has been substituted with the Gln. The amino acid sequence of cGnRH-I used in this study was pGlu-His-Trp-Ser-Tyr-Gly-Leu-Gln-Pro-Gly-NH2.

Grouping and injection

Along the study, birds were kept in numbered cages. Four weeks before the first injection on 30th of January2020,groups are randomly formed. Each group was consisting of six scattered cages. Two weeks before the start of injections, all groups were compared with each other to see if there were any differences in terms of egg laying, fertility and hatchability parameters. No statistically important differences were found. In each group, only males were subcutaneously injected under the wing. Birds were devoted into four experimental groups: a) control group -which was received no injection, b) negative control group, which was subcutaneously injected with 200 µl standards aline, c) 5µg cGnRH group, which injected with 200 µl standard saline containing 5 µg cGnRH and d) 20 µg cGnRH group, which injected with 200 µl standard saline containing 20 µg cGnRH. Birds were injected four times. The first injection was conducted when birds were 48 weeks old at 10:00 am on 30thJanuary 2020, the second injection was conductedat10:00 am on 6thFebruary and the third injection was conducted at 10:00 am on 13th February 2020. After the completion of egg collection and incubation works, the fourthnjection was donet 10:00 am on 3rd April 2020 and one hour later blood was collected for serum extraction and hormone analysis.

Egg collection, incubation and the measurement of egg laying performance

Eggs were daily collected at 09:30 am and weekly incubated (from Friday to Thursday), from the 7th of February 2020 to 3rd of April 2020, and the cage number was plotted on each egg collection viol without knowing which viol belongs to which group. Egg viols were daily placed in an egg collection cupboard (YMK-FN-685722,Qualitec, Fevzi Çakmak Mahallesi, Modesa Sanayi Sitesi 10735 Sk. No:13 Karatay/Konya-Turkey) in which temperature and humidity set to 15 ºC and 75%. Eggs were daily kept in this condition until the weekly incubation on Fridays. Two hours before the incubation the temperature of egg collection cupboard was brought to 25 ºC. Eggs were weekly incubated on Friday at 4pm in an incubator (T1600 S, Cimuka, 1214. Sokak 21/3 Ostim, Yenimahalle/Ankara-Turkey); temperature and humidity set to 37.6 ºC and 57% for 14 days. After 14 days, eggs were incubated in a hutching machine (T2400 H, Cimuka); temperature and humidity set to 37.2 ºC and 74% for 3 days. The time period between the incubation and the hatching named as one period and there were eight periods in total. The numbers of eggs collected and incubated in each week have been shown on Table 1. Daily egg number from each cage was recorded and weekly egg production for each cage and per quail calculated.

Table 1. The numbers of collected and incubated eggs in each week

Weeks	Number of egg collected	Number of egg incubated
Week-I	523	488
Week -II	472	439
Week -III	462	422
Week -IV	537	489
Week -V	531	490
Week -VI	522	483
Week -VII	530	488
Week -VIII	507	470

The determination of the number of hatched, fertilized and unfertilized eggs

After each period, the number of hatched eggs was determined by counting shells of hatched eggs. The number of unfertilized eggs and the number of not hatched eggs bearing an embryo were determined after breaking the eggs. The eggs not containing an embryo were accepted as unfertilized. The number of fertile eggs was determined by counting the number of hatched eggs plus the number of not hatched eggs bearing an embryo.

Measurement of embryonic mortality

The number of eggs not hatched, but bearing an embryo at different developmental stages were designated as mortal embryos. Embryonic mortality was devoted into three distich stages as mentioned below (Aygun et al., 2012).

Embryos dead on 1-9 days of incubation: Distinguished by the formation of eye and the absence of feather. The embryos bearing without feather were considered as dead between 1-9 days of incubation.

Embryos dead on 10-16 days of incubation: Distinguished by the presence of feather and yolk outside. Embryos bearing feather and the yolk outside the body were considered as dead between 10-16 days of incubation.

Embryos dead on 17-18 days of incubation: Distinguished by well-developed embryo and the absence of yolk outside.

Measurement of fertility and hatchability

The number of fertile Eggs: F

The number of hatched eggs: H

The number of not hatched eggs bearing an embryo: E

The number of total incubated eggs: I

$$F = H + E$$

Fertility (%) = (F/I) x100

Hatchability of total set eggs (%) = (H/I) x 100

Hatchability of fertile eggs (%) = (H/F) x100

Measurement of weekly egg production

Eggs produced from numbered cages were daily recorded and weekly egg produced from each cage divided by 5 to calculated weekly egg production per hen.

Measurement of weekly food consumption

The amount of food daily added to feed box was weighted by using a balance (DS-30, 99 Reagent Lane, Fair Lawn, NJ 07410, USA). The amount of food added was recorded along a week and subtracted from the reaming food in food box then divided by 6 to calculate weekly food consumption per bird.

The fourth injection, blood collection and serum extraction

The forth injection was carried out subcutaneously under the wing on Friday3rd April 2020 at 10:00 a.m. One hour later, birds were bought to the abattoir beside to the poultry yard. Birds were individually placed in a transparent plastic box attached to a CO_2 cylinder, equipped with a manometer. Gas flow rate to box arranged to 10L/min and covered by the lid. After 60-65 second bird was become unconscious and taken out form the box for bleeding. A small cut was given on the neck to see the jugular vein. A sterile 2 mL single use syringe provided with a sterile 22G hypodermic needle was used for blood collection from the jugular vein (Setecoject, Lot: A1606105, Set Medical Equipment Manufacturing and Trade Corporation, Mareşal Fevzi Çakmak Caddesi. No:18, 34522 Esenyurt/İs-

tanbul- Turkey). About 2 mL blood was collected and placed in a 5 mL sterile vacuumed blood tube containing gel and clot activator (Disera Medical Equipment Corporation, Lot: 2365.0043.19, İbni Melek OSB Mahallesi, TOSBİ Yol 4 Sokak No: 29 Tire Organize Sanayi Bölgesi 35900 Tire/ İzmir-Turkey). The tubes were transferred to the biotechnology lab of Animal Science department for serum extraction within 2 hours. Serum extracted by centrifugation at 4500 R.P.M for 20 minutes by using a bench top centrifuge (Nüve, Serial no: 02-0090, Esenboğa yolu, Akyurt o6287 Ankara- Turkey). Extracted serum was placed in sterile cryovials (Tarsons, Lot: 059D-10-200619, Jasmine Tower, Suite 213 31 Shakespeare Sarani, Kolkata-700 017, India) with cage number printed on and kept in a deep freezer set at -30ºC (Raypa, Serial no: 344 1/021, Avenida Del Vallès, 322 Polígono Industrial 'Els Bellots'08227 Barcelona-Spain) until analysis.

Hormone analysis

Serum concentration of hormones was quantitatively determined in accord to the procedure supplied with the related ELISA Kits (My BioSource, Inc. P.O. Box 153308 San Diego, CA 92195-3308 USA). Optical density values were measured by using an ELISA plate reader set to 450 nm wavelength. Optical densities were corrected by discarding the blank and then a standard curve was drawn; the curve was fit by using four parameter logistic regressions. Unknowns were compared with standard curve and their concentrations were plotted as ng/mL of blood.

Serum testosterone concentration was determined by using chicken testosterone (T) ELISA Kit (Inc. Cat: MBS703019). Serum LH concentration was measured by using chicken LH ELISA kit (Inc. Cat:MBS281782). Serum prolactin concentration was measured by using chicken prolactin ELISA kit (Inc. Cat: MBS739982) and serum FSH concentration was determined by using chicken follicle stimulating hormone (FSH) ELISA kit (Inc. Cat: MBS260290).

Statistical analysis

The data was analyzed by analysis of variance (ANOVA) by using Minitab statistical software. All data are presented as mean ±SEM. Pairwise comparisons were conducted according to the Tukey test, with 95% confidence intervals.

RESULTS

Serum hormone concentrations, fertilities of the incubated eggs, hatchability of the total set eggs, hatchability of the fertile eggs, weekly egg production per hen and weekly food consumption per bird are presented on Tables 2, 3, 4,5, 6 and 7 respectively. Injection of cGnRH-I did not increase pituitary gonadotrophin secretion (Table 2). The fertility of incubated eggs and the hatchability of total set eggs through the period I-VIII was lower in saline injected group (Table 3 and 4). There were no differences in hatchability of fertile eggs between the groups (Table 5). Through the weeks I to VIII, weekly egg production was lower in control group (Table 6). Weekly food consumption was higher in saline injected group compared to 5 µg cGnRH injected group (Table 7).

Table 2. Measured serum hormone concentrations (ng/mL) in experimental groups after the injections

	Experimental Groups				
Hormones	Control	Negative Control	5 µg cGnRH	20 µg cGnRH	*P-value*
LH	0.22 ± 0.06^{A}	0.08 ± 0.02^{A}	0.15 ± 0.04^{A}	0.22 ± 0.07^{A}	0.176
FSH	0.02 ± 0.00^{A}	0.03 ± 0.00^{A}	0.03 ± 0.00^{A}	0.03 ± 0.01^{A}	0.241
Testosterone	2.53 ± 0.52^{AB}	1.21 ± 0.32^{B}	2.79 ± 0.47^{AB}	3.38 ± 0.74^{A}	0.058
Prolactin	18.54 ± 6.89^{A}	39.35 ± 7.58^{A}	23.11 ± 4.99^{A}	25.39 ± 8.05^{A}	0.209

*Data were displayed on the table as Mean ± SEM

A,B Data with different superscripts in the same line are statistically different (P=0.058)

Table 3. The fertility of incubated eggs (%)

	Experimental Groups				
Periods	Control	Negative Control	5 µg cGnRH	20 µg cGnRH	*P-value*
Period-I	64.91 ± 12,08^{A}	65,52 ± 7,92^{A}	73,61 ± 4,39^{A}	84,31 ± 4,92^{A}	0.302
Period-II	74.68 ± 7.00^{AB}	53,02 ± 6.38^{B}	77.74 ± 5.69^{A}	78.29 ± 3.12^{A}	0.016
Period-III	73.50 ± 5,27^{A}	55.36 ± 12,24^{A}	81.84 ± 5.39^{A}	74.34 ± 5.60^{A}	0,129
Period-IV	75.82 ± 7.57^{A}	57.76 ±6.33^{A}	75.94 ± 7.56^{A}	69.49 ± 6.76^{A}	0.256
Period-V	59.08 ± 11.11^{A}	53.68 ± 10.35^{A}	77.24 ± 6.70^{A}	68.20 ± 12.28^{A}	0.410
Period-VI	75.29 ± 8.22^{A}	64.48 ± 9.69^{A}	69.80 ± 9.50^{A}	73.94 ± 9.08^{A}	0.837
Period-VII	74.95 ± 6.15^{A}	56.27 ± 5.47^{A}	73.42 ± 8.89^{A}	73.88 ±11.16^{A}	0.344
Period-VIII	79.72 ± 6.96^{A}	57.97 ± 10.38^{A}	76.65 ± 7.13^{A}	61.57 ± 13.46^{A}	0.334
Period I- VIII	72.24 ± 2.87^{A}	58.01 ± 2.97^{B}	75.78 ± 2.37^{A}	73.00 ± 3.08^{A}	0.000

*Data were displayed on the table as Mean ± SEM
^{A,B} Data with different superscripts in the same line are statistically different

Table 4. The hatchability of total set eggs (%)

	Experimental Groups				
Periods	Control	Negative Control	5 µg cGnRH	20 µg cGnRH	P-Value
Period-I	55.78 ± 10.73^{A}	59.94 ± 8.15^{A}	60.30 ± 6.68^{A}	70.98 ± 5.42^{A}	0.588
Period-II	59.73 ± 8.54^{A}	47.42 ± 6.56^{A}	62.62 ± 9.63^{A}	62.03 ± 6.58^{A}	0.505
Period-III	44.85 ± 4.93^{AB}	40.45 ± 9.06^{B}	70.68 ± 5.98^{A}	57.85 ± 5,93^{AB}	0.019
Period-IV	68.80 ± 7.22^{A}	42.64 ± 9.52^{A}	57.19 ± 10.33^{A}	54.82 ± 3.63^{A}	0.438
Period-V	52.42 ± 8.77^{A}	40.18 ± 8.37^{A}	63.8 ± 8.80^{A}	60.34 ± 10.60^{A}	0.299
Period-VI	59.17 ± 9.15^{A}	51.65 ± 10.22^{A}	61,38 ±7.74^{A}	62.57 ±7.10^{A}	0.810
Period-VII	64.38 ± 6.83^{A}	42.90 ± 3.54^{A}	60.32 ± 8.61^{A}	52.01 ± 9.53^{A}	0.217
Period-VIII	70.53 ± 7.61^{A}	36.23 ± 8.11^{B}	74.7 ± 3.74^{A}	51.6 ±11.73^{AB}	0.013
Period I- VIII	58.46 ± 2.86^{A}	45.18 ± 2.86^{B}	63.89 ± 2,71^{A}	59.02 ± 2.75^{A}	0.000

*Data were displayed on the table as Mean ± SEM
^{A,B} Data with different superscripts in the same line are statistically different

Table 5. The hatchability of fertile eggs (%)

	Experimental Groups				
Periods	Control	Negative Control	5 µg cGnRH	20 µg cGnRH	P-Value
Period-I	88.08 ± 4.76^{A}	90.99 ± 4.11^{A}	80.67 ± 5.03^{A}	84.05 ± 3,27^{A}	0.379
Period-II	77.50 ± 6.10^{A}	89.72 ± 4,72^{A}	78.17 ± 9.84^{A}	78.61 ± 6.67^{A}	0.576
Period-III	61.16 ± 6.38^{B}	77.08 ± 4.96^{AB}	86.69 ± 6.00^{A}	78.64 ± 7.22^{AB}	0.056
Period-IV	79.18 ± 4.18^{A}	71.86 ± 12.00^{A}	79.79 ± 6.38^{A}	80.34 ± 3.35^{A}	0,827
Period-V	77.56 ±5.74^{A}	74.75 ± 3.67^{A}	80.97 ± 8.83^{A}	90.40 ± 2.88^{A}	0.277
Period-VI	76.81 ± 4.73^{A}	77.77 ± 5.91^{A}	89.61 ± 3.34^{A}	86.16 ± 3.53^{A}	0.154
Period-VII	85.64 ± 3.96^{A}	77.26 ± 3.72^{A}	81.63 ± 5.69^{A}	72.34 ± 8.08^{A}	0,398
Period-VIII	90.13 ± 4.19^{A}	59.79 ± 5.23^{B}	89.37 ± 2.02^{A}	86.24 ± 5.70^{A}	0.000
Period I- VIII	79.51 ± 2.07^{A}	77.40 ± 2.41^{A}	83.36 ± 2.17^{A}	82.10 ± 1.94^{A}	0.208

*Data were displayed on the table as mean ± SEM
^{A,B} Data with different superscripts in the same line are statistically different

Table 6. Weekly egg production per quail

	Experimental Groups				
Weeks	Control	Negative Control	5 µg cGnRH	20 µg cGnRH	P-Value
Week-I	4.23 ± 0,63^{A}	4.47 ± 0.42^{A}	4.37 ± 0.28^{A}	4.37 ± 0.45^{A}	0.998
Week -II	3.60 ± 0.54^{A}	3.93 ± 0.35^{A}	4.13 ± 0.46^{A}	4.07 ± 0.44^{A}	0.845
Week -III	3.00 ± 0.32^{A}	4.17 ± 0.31^{A}	4.00 ± 0.39^{A}	4.23 ± 0.45^{A}	0.102
Week -IV	3.80 ± 0.40^{A}	4.43 ± 0.38^{A}	4.67 ± 0.48^{A}	5.00 ± 0.13^{A}	0.168
Week -V	3.93 ± 0.46^{A}	4.57 ± 0.29^{A}	4.63 ± 0.42^{A}	4.57 ± 0.26^{A}	0.512
Week -VI	3.30 ± 0.47^{B}	4,53 ± 0.29^{AB}	4.63 ± 0.24^{A}	4.93 ± 0.28^{A}	0.012
Week -VII	3.77 ± 0.57^{A}	4.43 ± 0.19^{A}	4.77 ± 0.29^{A}	4.70 ± 0.31^{A}	0.233
Week -VIII	3.70 ± 0.45^{A}	4.17 ± 0.56^{A}	4.57 ± 0.30^{A}	4.47 ± 0.29^{A}	0.470
Week I- VIII	3,67 ± 0.17^{B}	4.34 ± 0.12^{A}	4.45 ± 0.13^{A}	4.57^{A} ± 0.11	0.000

*Data were displayed on the table as mean ± SEM
^{A,B} Data with different superscripts in the same line are statistically different

Table 7. Weekly food consumption per quail (gr)

	Experimental Groups				
Weeks	Control	Negative Control	5 µg cGnRH	20 µg cGnRH	P-Value
I	248.72 ± 5.80^{A}	249.79 ± 7.67^{A}	231.78 ± 5.43^{A}	245.15 ± 3.60^{A}	0.139
II	272.49 ± 11.71^{A}	263.04 ± 10.80^{A}	253.94 ± 11.30^{A}	260.99 ± 9.19^{A}	0.672
III	296.17 ± 15.79^{A}	298.64 ± 20.27^{A}	271.22 ± 16.11^{A}	292.49 ± 23.43^{A}	0.736
IV	269,83 ± 12.20^{A}	274.58 ± 9.86^{A}	242.91 ± 13.24^{A}	261.25 ± 15.07^{A}	0.335
V	235.15 ± 18.07^{A}	265.28 ± 18.88^{A}	234.95 ± 10.86^{A}	248.55 ± 9.75^{A}	0.449
VI	242.53 ± 14.15^{A}	276.07 ± 17.01^{A}	239.05 ± 5.88^{A}	250.33 ±3.27^{A}	0.133
VII	247.51 ± 12.20^{A}	282.20 ± 26.07^{A}	237.08 ± 6.31^{A}	250.12 ± 7.50^{A}	0.212
VIII	230.65 ± 11.16^{AB}	272.88 ± 16.48^{A}	226.60 ± 6.39^{B}	251.97 ± 7.16^{AB}	0.028
I- VIII	255.38 ± 5.23^{AB}	272.80 ± 5.83^{A}	242.19 ±3.84^{B}	257.61 ± 4.33^{AB}	0.000

*Data were displayed on the table as mean ± SEM
^{A,B} Data with different superscripts in the same line are statistically different

DISCUSSION

In the present study, injection of cGnRH-I did not increase LH and FSH secretion. Several studies have shown a little or no in vivo effect of cGnRH-I on FSH secretion in non-photo-stimulated immature intact chickens (Bruggeman et al., 1998; Dunn et al., 2003; Proudman et al., 2006) or in young cockerels (Krishnan et al., 1993). However, both cGnRH-I and II significantly increased LH secretion and cGnRH-II was more potent than cGnRH-I (Proudman et al., 2006). In this study, serum concentration of LH is measured. If the pulse frequency and the pulse amplitude of a hormone change, it is reflected as a change in serum concentration of that hormone. It is impossible to take more than one blood sample from a quail. In this study, male quails were injected in order from cage 1 to cage 24. Blood was collected in order from cage 1 to cage 24 one hour after the last quail was injected. The transport of quails to the abattoir, the preparation for bleeding and the blood collection gave enough time for a LH pulse to occur. In this study, LH secretion did not change ($P > 0.05$) by cGnRH-I injection. This could be because we applied long day photoperiod (8 hours dark and 16 h light) rhythm. Long day photoperiod rhythm caused an increase in endogen cGnRH-I release. In culture condition, it was reported that GnRH release increased from super-fused hypothalamic slices in photo-stimulated quail (Millam et al., 1984; Perera and Follett, 1992). Therefore, cGnRH concentration was already high in their blood and injecting extra cGnRH did not cause significant differences in blood concentrations of LH and FSH at the time of blood collection.

Serum testosterone concentration was increased ($P = 0.058$, close to significant) after the injection of 20 µg cGnRH as compared to that of negative control group. This might be a result of a non-significant decrease in serum LH concentration in negative control group, which has not been treated with cGnRH. There were no significant differences in serum prolactin concentrations among the experimental groups, but serum prolactin concentration was numerically

higher in negative control groups as compared to other groups. This is not a consequence of a decreased serum testosterone concentration due to the saline injection, since plasma prolactin concentrations were found to be similar in intact and castrated male quail (Boswellet al., 1995). Furthermore, an inverse relationship was reported between serum LH concentration and serum prolactin level in European quail *(Coturnix coturnix japonica)* (Camper and Burke, 1977). In the negative control group, serum LH concentration was non-significantly reduced, and this might be a reason for a non-significant increase in serum prolactin concentration.

The fertility of incubated eggs and the hatchability of total set eggs through the period I-VIII were lower in saline injected negative control group compared to other groups. However, these significant differences were obvious only in few cases (fertility - period II or hatchability period III and VIII between negative control and 5μg GnRH groups) probably because of increased SEM. The decrease in fertility and hatchability might be due to non-significant decrease in serum concentration of LH, and to the significant decrease in serum testosterone concentration in negative control group compared to that of 20 μg cGnRH injected group. A strong positive correlation between serum concentration of LH and testosterone was found previously (Ottinger et al., 1983). Therefore, the reason for decreased serum testosterone concentration ($P < 0.05$) might be the reflection of the non-significant decrease in serum LH concentration due to the lack of GnRH injection in negative control group. Hatching is depending on whether an egg is fertilized or not fertilized. Therefore, it is a broadly male dependent phenomenon. The decreased fertility might affect hatchability and it could be due to numerically increased serum prolactin concentration in negative control group. The adverse effect of prolactin on egg fertility in Japanese quail has already been reported (Renzoni, 1970).

The hatchability of fertile eggs was similar to all groups through I-VIII period. Decrease in hatchability of fertile eggs is a consequence of increased embryonic mortality. Increase in embryonic mortality might be a result of decreased sperm quality rather than the number of sperm inseminated. In broiler breeder hens, no relation has been reported between inseminated sperm number and embryonic mortality (Van Wambeke, 1984). A correlation has been reported between semen quality and the testosterone response to GnRH in cockerels (Barna and Mézes, 1994). Therefore, it could be assumed that decrease in sperm quality in control group may lead to the increase in the number of dead embryos, which caused the decrease in hutching. However, in this study this was obvious only in few periods; it seems that hatchability of fertile eggs did not affected by the injection of GnRH in the present study.

In control (natural) group, egg production was significantly lower through the period I-VIII and only in period VI compared to other groups. This might be a result of non-significant decrease of prolactin in control group. In birds, a positive relation between serum prolactin concentration and egg production has been reported (Goldsmith and Hall, 1980; Jensen et al., 2019). In control group, males received no injection. Therefore, prolactin concentrations weren on-significantly reduced. Semen was transferred to females via the mating and this probably resulted the significant reduction in egg laying. Follicle-stimulating hormone is crucial for steroidogenesis, follicular recruitment, growth, and selection (Li et al., 2011). There is a positive relation between FSH concentration and egg laying. Blood concentration of FSH increases during the laying period? (Li et al., 2011). Weekly food consumption per quail increased in negative control group as compared to 5 μg cGnRH injected group. The increased appetite in negative control group might be a result of suppressed GnRH secretion. The role of cGnRH in food intake was studied in the goldfish. Intracerebroventricular administration of cGnRH induced a decrease of food consumption in a dose-dependent manner (Matsuda et al., 2008). Non-significant reduction in serum LH concentration could be an indication that GnRH secretion was suppressed in negative control group.

CONCLUSION

According to our results, the injection of cGnRH significantly increased egg fertility compared to the negative control group. Furthermore, the hatchability of total set eggs was lower in negative control group, as compared to other groups, due to the decreased egg fertility. This is probably caused by the reductions in serum LH (non-significant) and testosterone (significant) concentrations. The decrease in hatchability of fertile eggs is a reflection of embryonic mortality. In overall, through the Period-I-VIII, no significant differences, in terms of embryonic mortality, were noted among the groups. In this study, we injected cGnRH at 48 weeks old males; thus, it is difficult to say that

cGnRH injection increases fertility potential of older males. More studies are needed with older males to confirm.

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CONFLICT OF INTEREST

None declared by the authors.

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