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RESEARCH PAPER

Effects of the Injection of Chicken Gonadotropin-Releasing Hormone I on Egg Fertility and Hatching Traits

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Abstract

The aim of this study was to measure the effect of chicken GnRH-I injection of roosters on egg fertility, hatching traits, and performance in layer breeder. Sixteen male and 160 female breeder chickens were divided into four experimental groups. Each group is consisting of 4 males and 40 females with four replications. The study comprised a control group, a negative control group (received 200 µl phosphate-buffered saline, PBS), a low hormone group (received 200 µl PBS + 10 µg cGnRH-I) and a high hormone group (received 200 µl PBS + 50 µg cGnRH-I). Injection of 50 µg cGnRH significantly increased the fertility percentage of laid eggs over control, PBS and low hormone injected groups after period I. Injection of either 10 or 50 µg cGnRH significantly increased the relative fertility percentages of eggs following period IV. Injection of 50 µg cGnRH induced a significant increase in the hatchability of set eggs and the hatchability of fertile eggs after periods I and II. Further research is needed to measure the impact of cGnRH on fertility and hatching rates by injecting both male and female of same breed and age with the same doses.

Introduction

Gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus causes pituitary secretion of the gonadotropins (Kaiser et al., 1995). Studies have shown that sexually active birds express a greater number of immune-reactive GnRH neurons than their sexually inactive counterparts (Sharp et al., 1990; Hahn and Ball, 1995; Parry et al., 1997; Cho et al., 1998). It has been reported that two different variants of GnRH exist in chicken: chicken GnRH-I and II (Millar and King, 1984; Miyamoto et al., 1984; Sherwood et al., 1988). The former is considered to constitute the biologically active neuropeptide that controls gonadotropin secretion (Sharp et al., 1990). Decreased hypothalamic GnRH-I secretion in older roosters correlates with lower pituitary expression of mRNA for LH and FSH compared to expression in young roosters (Avital-Cohen et al., 2013). Studies have shown that plasma testosterone concentrations diminish in aging roosters (Weil et al., 1999a; Ottinger et al., 2002). As a result, testes' weight, semen volume, and sperm concentration are generally lower in older roosters (Avital-Cohen et al., 2013). It was reported that the fertility of young roosters reached a peak of 96.2 ± 3.9% at 37 weeks of age and then their fertility began to decline. At 72 weeks, their fertility was much lower than when they were 37 weeks old (Weil et al., 1999a).

Hypothalamic secretion of GnRH is a potential regulator of pituitary FSH secretion and has a half-life of 9 minutes (Reddy et al., 2013). Reduced blood concentrations of FSH in aging roosters have been deemed responsible for diminished daily sperm production. Because the number of Sertoli cells is

stimulated by FSH and the number of sperm produced is dependent on the number of the Sertoli cells hosting spermatogonia (Weil et al., 1999b; Avital-Cohen et al., 2013). Decreased sperm output 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. Layer breeder roosters tend to become less fertile at about 70 weeks of age and this leads to their removal from the flock (Rosenstrauch et al., 1998; Weil et al., 1999b). However, male broiler breeders tend to see a dramatic reduction in fertility earlier about 45-55 weeks of age (Rosenstrauch et al., 1994; Weil et al., 1999b). Studies have shown that this occurs due to the reduction in sperm number in the ejaculate, although Sertoli cells may be crowded with excessive numbers of spermatids, which become trapped within the cytoplasmic extension owing to malformations in the Sertoli cells' ectoplasmic specialisations (Weil et al., 1996), as well as structural changes in the Leydig cells (Rosenstrauch et al., 1998). According to Sarabia Fragoso et al. (2013), a reduction in fertility occurs due to the decrease in plasma LH and testosterone concentrations. According to Weil et al. (1999a), alongside the decrease in roosters' fertility, reductions also occur in testicular weight, sperm production and testosterone levels. Similar results were obtained from another study, in which older roosts' fertility declined due to reductions in testes size, sperm concentrations and serum testosterone concentrations (Lagares et al., 2017). The reduction in aged roosters' fertility leads to diminished hatching rate and its subsequent removal from the flock. However, the poultry ındustry is currently suffering from a shortage of breeder roosters. Therefore, keeping genetically superior breeder roosters within the flock for a longer period of time, without any decrease in fertility, is economically important. The purpose of this study was therefore to determine whether injecting roosters with GnRH-I would boost egg fertility and hatching rate.

Materials and Methods

Animal, Feeding and Grouping

This study was conducted in a chicken house equipped with a fan pad cooling system, in the poultry unit of the Research and Exploration Centre, Faculty of Agriculture, Çukurova University. Sixteen male and 160 female Atak-S (BAR1, RIR1) breeder chickens were divided into 16 groups. In each group, chickens were housed in floor pens (5 m^2) under 16L: 8D light-dark cycle. Each group contained one 81-week-old male with an average body weight of 2,846 g, and ten 81-week-old females with an average body weight of 1,973 g. These groups were randomly divided into four experimental groups with four replications, comprising a control group (received no injection), a negative control group (received 200 µl phosphate-buffered saline, PBS), a low-hormone group (received 200 µl PBS + 10 µg chicken gonadotropin-releasing hormone-I (cGnRH-I) and a high-hormone group (received 200 µl PBS + 50 µg cGnRH-I. Each group consisted of 4 males and 40 females. In each group, only males were subcutaneously injected under the wing. The chickens were fed *ad libitum* with a diet containing 17% crude protein (CP) and 2,750 kcal/ kg metabolic energy (ME). Water was also provided ad libitum.

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 10 or 50 µg/200 µl with PBS. In our previous study, we injected male quails with 5 or 20 µg cGnRH and we have a significant increase in blood testosterone level in 5 µg/100 µl cGnRH injected group over PBS injected group (P=0,036) (Çiftçi et al., 2018). Chickens are 3-4 times heavier than quails, and this was the reason I have chosen the above GnRH doses (10 or 50 µg) for the injections. The amino acid sequence of cGnRH-I was Pyr-His-Trp-Ser-Tyr-Gly-Leu-Gln-Pro-Gly-NH_2 (trifluoroacetate salt). This was different from the mammalian GnRH-I at position 8, arginine being substituted by glutamine (King and Millar 1982).

Injection Procedure

Following a one-week adaptation period, roosters from each group were subcutaneously injected (under the wing) for four weeks on Thursdays at 11:00 am (one injection per week). Birds were injected with 200 µl PBS (Cat; P4417-100AB, Sigma-Aldrich Co.,3050 Spruce street, St. Louis, MO 63103 USA), 200 µl PBS containing 10 or 50 µg cGnRH-I (Cat; LHRH-012A, CPC Scientific, 1245 Ream wood Avenue Sunnyvale, CA 94089, USA).

Measurement of Fertility and Hatchability

Chicken GnRH affects pituitary secretion of gonadotropins (LH and FSH), which in turn affects the Leydig cell secretion of androgen and Sertoli cell secretion of androgen binding proteins as well as transforming beta like peptides (TGF-βs) affecting the sperm formation, maturation and consequently fertilizing ability of male gametes (Weil et al. 1999a). Hatchability depends on whether an egg was fertilized or not therefore hatchability was also measured.

One week after the last injection, eggs were collected daily and stored at 18°C and 75% relative humidity until the incubation. The time span between incubation and hatching was referred as a period. The times of incubation and hatching and the number of eggs collected and incubated in each period are displayed in Table 1.

Once hatching was complete, the fertility (fertile eggs divided by set eggs), hatchability of set eggs (hatched chicks divided by set eggs), and hatchability of fertile eggs (hatched chicks divided by fertile eggs) were determined.

Measurement of Egg Production, Feed Consumption and Female Ratio

Egg production and bird mortality were recorded daily throughout the eight-week experimental period. Feed consumption was measured every week using a digital scale (Kern DS 30K 0.1, Nuremberg, Germany), and was calculated by subtracting the remaining feed from the total feed. The sex of a day-old chick was determined according to the colour of the feathers. The female ratio was expressed as a percentage divided by the number of total hatched chicks.

Statistical Analysis

Data were analysed by analysis of variance (ANOVA) by using Minitab statistical software. Pairwise comparisons were carried out according to Tukey test with 95% confidence intervals.

Results

The effects of cGnRH injection on egg fertility are presented in Table 2. Injection of 50 µg cGnRH caused a significant increase ($P<0.05$) in the fertility of the eggs relative to the other three groups for period I, while injection of 10 µg cGnRH did not cause any significant changes relative to the control and PBS injected groups. The coefficients of variations for fertility within and between groups range between 2.29-4.39% and 2.40-4.49% respectively. In period II, 10 µg cGnRH injections induced a significant increase in egg fertility over PBS-injected group, but there were no significant differences in 10 µg cGnRH groups relative to control and 50 µg cGnRH-injected groups. For period IV, injection of 10 and 50 µg cGnRH significantly increased the egg fertility relative to control and PBS-injected groups. The egg fertility rate was significantly higher in the control and 50 µg-cGnRH-injected groups relative to the PBS- and 10 µg-GnRH-injected groups for period VI. In periods III, V and VI fertility rates were lower in PBS-injected group relative to control.

The fertility rate, for period-VI, was the lowest in 10 µg-cGnRH-injected groups. The effects of cGnRH injection on the hatchability of set eggs are displayed in Table 3.

Table 1. Incubation and hatching times and the number of eggs collected and incubated in each period

Periods	Incubation Time	Hatch Time	Egg Collected	Egg Incubated
Period-I	14.05.2018	04.06.2018	446	320
Period-II	21.05.2018	11.06.2018	646	320
Period-III	29.05.2018	19.06.2018	567	542
Period-IV	06.06.2018	26.06.2018	517	321
Period-V	11.06.2018	02.07.2018	513	320
Period-VI	20.06.2018	10.07.2018	440	320

Table 2. The fertility rates of eggs collected from experimental groups enclosed with roosters received no injection (Control), injected with PBS, 10 or 50 µg cGnRH (Mean $^{\pm}$ SE)

Fertility rates (%)					
Periods	Control	PBS	10 µg cGnRH	50 µg cGnRH	P-values
Period-I	73.84^{b} ± 2.00	76.22^{b} ± 1.36	72.47^{b} ±1.51	92.50^{a} ± 1.44	0.000
Period-II	78.78^{ab} ± 0.90	75.00^{b} ± 0.00	79.97^{a} ± 0.41	76.34^{ab} ± 1.96	0.029
Period-III	87.33^{a} ± 0.66	77.83^{b} ± 0.98	88.69^{a} ± 0.96	84.68^{a} ± 1.35	0.000
Period-IV	80.21^{b} ± 0.48	76.25^{b} ± 1.25	86.23^{a} ± 1.29	88.83^{a} ± 0.83	0.000
Period-V	78.65^{a} ± 1.47	66.27^{b} ± 2.17	76.28^{a} ± 0.93	76.22^{a} ± 1.36	0.001
Period-VI	85.11^{a} ± 1.74	73.85^{b} ± 1.86	65.26^{c} ± 1.21	84.94^{a} ± 2.02	0.000
Periods I-VI	80.65^{b} ± 0.83	74.24^{c} ± 0.51	78.15^{b} ± 0.60	83.92^{a} ± 0.35	0.000

*Data with different superscripts are statistically different

*SE: Standard error

Table 3. The hatchability of set eggs from experimental groups, enclosed with rooster received no injection (Control), injected with PBS, 10 or 50 µg cGnRH (Mean ± SE)

Hatchability of set eggs (%)					
Periods	Control	PBS	10 µg cGnRH	50 µg cGnRH	P-values
Period-I	63.77^{b} ± 0.76	61.27^{b} ± 0.77	59.95^{b} ± 0.82	82.50^{a} ± 1.44	0.000
Period-II	63.77^{b} ± 0.76	63.75^{b} ± 1.25	61.27^{b} ± 0.77	70.09^{a} ± 1.43	0.001
Period-III	78.02^{a} ± 1.06	67.17^{c} ± 1.92	74.22^{ab} ± 1.04	70.08^{bc} ± 0.50	0.000
Period-IV	72.88^{b} ± 0.88	66.25^{c} ± 1.25	64.95^{c} ± 0.72	78.79^{a} ± 0.56	0.000
Period-V	72.49^{a} ± 1.03	53.75^{c} ± 0.72	67.52^{b} ± 1.08	64.96^{b} ± 0.72	0.000
Period-VI	77.53^{a} ± 1.17	61.23^{c} ± 0.45	46.10^{d} ± 1.36	72.52^{b} ± 0.85	0.000
Periods I-VI	71.41^{a} ± 0.23	62.24^{b} ± 0.67	62.33^{b} ± 0.81	73.16^{a} ± 0.49	0.000

*Data with different superscripts are statistically different

*SE: Standard error

The hatchability of set eggs was significantly higher in the 50 µg-cGnRH-injected group compared to the other treatment groups in periods I and II. The coefficients of variations within and between the groups ranged between 2.45-3.22 and 2.51-3.65%, respectively. The number of hatched set eggs was the lowest in the PBS-injected group relative to the other groups in periods III and V. Injection of PBS significantly reduced the number of hatched set eggs relative to the other groups in period V. The number of hatched set eggs was highest in the 50 µg-cGnRH-injected groups in period IV. The number of hatched set eggs fell in period VI in the PBS- and 10 µg-cGnRH-injected groups as compared with control and 50 µg-cGnRH-injected groups.

The effects of GnRH on the number of hatched fertile eggs are displayed in Table 4. The number of hatched fertile eggs increased (P<0.05) in the 50 µg cGnRH-injected groups relative to the PBS-injected groups in period I. The highest hatchability rate of fertile eggs was observed in the 50 µg cGnRH-injected group in period II. In period III, the number of hatched fertile eggs was significantly higher in the control group than in the GnRH-injected groups. In period IV, the injection of 10 µg cGnRH caused a significant reduction in the number of hatched fertile eggs compared with the other treatment groups. The number of hatched fertile eggs in period V was significantly higher in the control group compared with the PBS-injected group. In period VI, the number of hatched fertile eggs fell significantly in the 10 µg cGnRH-injected groups relative to other groups due to the reduction in the hatchability of fertile eggs.

Through the periods I-VI, injection of PBS decreased fertility (Table 2), as compared with other groups, but PBS injection did not negatively affect the hatchability of those fertile eggs (Table 4), while injection of 10 µg cGnRH did not cause important differences in fertility rates as compared with the control group but caused a decrease in the hatchability those fertile eggs as compared with other groups (Table 2 and 4). The data indicating the effect of cGnRH on female ratios are displayed in Table 5. According to the analysis of variance, female ratios significantly fell (P<0.05) in the 50 µg-cGnRH-injected group compared with the PBS- and 10 µg-cGnRH-injected groups in period I. In period II, the female ratio was the highest in the 50 µg-cGnRH-injected groups. The female ratio was the highest in the 10 µg-cGnRH-injected groups and the lowest in the 50 µg-cGnRH-injected groups in period III. The female ratio was the highest in the 50 µg-cGnRH-injected group compared with the control and PBS-injected groups, after period IV. The female ratio was the highest in the 10 µg-cGnRH-injected group relative to the other treatment groups in period V, and the differences were not statistically significant (P=0.179) in period VI. Within group and between group coefficients of variation for female ratio ranged between 2.68-8.42 and 3.17-8.73%, respectively. The differences in daily egg production and feed consumption were not statistically significant (Tables 6 and 7).

In overall, injection of GnRH caused some degree of increase in fertility rate and hatchability of fertile eggs. Injection of 50 µg GnRH caused better effect than the effect of 10 µg GnRH, while there were no differences in female ratios, egg production and daily feed consumption (g/bird) in period 1 and 2 (Table 5, 6 and 7). Injection of PBS caused some degree of adverse effect on fertility rate, hatchability of set eggs and hatchability of fertile eggs.

Discussion

In all vertebrate species including poultry, reproduction is determined by the hypothalamo-pituitary-gonadal axis. This axis controls the fertility of an individual, crucial to the economic viability of the industry (Ippala et al., 2016). According to a study, reduction in fertility occurs due to the decrease in plasma LH and testosterone concentrations (Weil et al., 1999a), which is under the control of hypothalamic GnRH secretion. Decrease in fertility of the older roosters might be a result of the decreased secretion of GnRH.

Table 4. The hatchability of fertile eggs weekly collected from groups enclosed with roosters received no injection (Control) or injected with PBS, 10 or 50 µg cGnRH (Mean ± SE)

Hatchability of fertile eggs (%)					
Periods	Control	PBS	10 µg cGnRH	50 µg cGnRH	*P-values*
Period-I	86.55^{ab} ± 2.63	80.49^{b} ± 2.20	82.81^{ab} ± 1.76	89.18^{a} ± 0.17	0.034
Period-II	80.94^{bc} ± 0.31	85.00^{b} ± 1.67	76.62^{c} ± 1.18	91.87^{a} ± 1.46	0.000
Period-III	89.34^{a} ± 0.75	86.28^{ab} ± 1.94	83.68^{b} ± 0.73	82.81^{b} ± 1.17	0.013
Period-IV	90.89^{a} ± 1.53	86.87^{a} ± 0.21	75.37^{b} ± 1.19	88.71^{a} ± 0.30	0.000
Period-V	92.31^{a} ± 2.80	81.27^{b} ± 1.58	88.54^{ab} ± 1.61	85.28^{ab} ± 1.39	0.010
Period-VI	91.18^{a} ± 1.70	83.05^{a} ± 1.90	70.72^{b} ± 3.09	85.52^{a} ± 2.22	0.000
Periods I-VI	88.53^{a} ± 0.78	83.82^{b} ± 1.11	79.62^{c} ± 0.99	87.23^{ab} ± 0.60	0.000

*Data with different superscripts are statistically different
*SE: Standard error

Table 5. Female ratios following the incubations of eggs collected from the groups enclosed with roosters received no injection (Control) or injected with PBS, 10 or 50 µg cGnRH (Mean±SE)

Female ratios (%)					
Periods	Control	PBS	10 µg cGnRH	50 µg cGnRH	P-values
Period-I	46.96^{bc} ± 2.53	55.13^{a} ± 2.01	52.10^{ab} ± 1.22	43.93^{c} ± 1.21	0.004
Period-II	49.04^{b} ± 1.84	41.19^{c} ± 1.82	50.96^{b} ± 0.96	57.14^{a} ± 0.00	0.000
Period-III	45.26^{b} ± 0.91	45.44^{b} ± 0.90	56.48^{a} ± 0.77	41.67^{c} ± 0.00	0.000
Period-IV	47.50^{b} ± 0.83	47.25^{b} ± 2.33	53.71^{ab} ± 3.46	60.33^{a} ± 0.77	0.003
Period-V	55.14^{b} ± 0.92	51.14^{b} ± 2.18	68.54^{a} ± 1.51	53.71^{b} ± 3.46	0.001
Period-VI	43.54^{a} ± 1.36	48.86^{a} ± 1.94	48.47^{a} ± 2.79	50.13^{a} ± 2.00	0.179
Periods I-VI	47.90^{b} ± 2.31	48.17^{b} ± 2.80	55.04^{a} ± 3.78	51.15^{ab} ± 3.75	0.001

*Data with different superscripts are statistically different
*SE: Standard error

Table 6. Daily egg production per hen in control and treatment groups throughout the eight weeks' experimental period (Mean ± SE)

	Egg production (hen/day, %)				
Weeks	Control	PBS	10 µg cGnRH	50 µg cGnRH	P-values
W-0	41.79^{a} ±5.57	40.36^{a} ± 3.61	39.29^{a} ± 3.76	41.79^{a} ± 3.10	0.966
W-1	55.71^{a} ± 2.92	58.57^{a} ± 6.25	57.14^{a} ± 4.84	59.29^{a} ± 8.76	0.976
W-2	53.57^{a} ± 2.64	50.36^{a} ± 6.32	46.43^{a} ± 2.89	52.14^{a} ±7.34	0.790
W-3	48.93^{a} ± 6.10	50.00^{a} ± 6.32	44.29^{a} ± 2.54	51.43^{a} ± 1.01	0.775
W-4	47.86^{a} ± 6.81	47.86^{a} ± 1.89	45.36^{a} ± 0.90	42.14^{a} ± 6.74	0.821
W-5	40.00^{a} ± 5.56	45.36^{a} ± 5.42	39.64^{a} ± 4.79	37.50^{a} ± 5.80	0.754
W-6	41.07^{a} ± 4.96	46.07^{a} ±2.13	39.64^{a} ± 3.89	35.36^{a} ± 3.57	0.299
W-7	36.79^{a} ± 6.10	45.71^{a} ± 3.45	41.07^{a} ± 4.72	35.00^{a} ± 3.38	0.313
W-8	37.86^{a} ± 7.39	37.86^{a} ± 7.20	41.43^{a} ± 2.02	37.86^{a} ± 6.71	0.969
W0-8	44.84^{a} ± 4.11	46.91^{a} ± 4.46	43.81^{a} ± 2.58	43.61^{a} ± 3.96	0,925

*Data with different superscripts are statistically different
*SE: Standard error

Table 7. Daily feed consumption by per bird, a week after the last injection of experiment (Mean ± SE)

	Daily feed consumption (g/bird)				
Weeks	Control	PBS	10 µg cGnRH	50 µg cGnRH	P-values
W-1	121.07^{a} ± 1.97	123.28^{a} ± 1.49	120.71^{a} ± 3.87	121.27^{a} ± 2.92	0.908
W-2	120.84^{a} ± 2.03	120.65^{a} ± 2.74	116.69^{a} ± 3.40	120.03^{a} ± 3.36	0.731
W-3	113.57^{a} ± 2.66	110.73^{a} ± 1.72	115.70^{a} ± 3.96	117.95^{a} ± 6.29	0.639
W-4	110.69^{a} ± 0.66	105.26^{a} ± 1.29	115.80^{a} ± 1.77	114.33^{a} ± 5.68	0.124
W-5	111.15^{ab} ±2.93	110.67^{ab} ±3.76	114.29^{a} ± 0.90	104.12^{b} ± 1.36	0.081
W-6	101.74^{a} ± 2.54	107.78^{a} ± 5.38	112.22^{a} ± 3.69	109.59^{a} ± 4.39	0.365
W-7	106.98^{a} ± 1.86	111.44^{a} ± 2.64	113.38^{a} ± 3.26	111.35^{a} ± 3.65	0.493
W-8	115.75^{a} ± 1.25	117.43^{a} ± 0.87	117.37^{a} ± 3.99	113.60^{a} ± 1.64	0.615
W 1-8	112.73^{a} ± 1.68	113.41^{a} ± 0.89	115.77^{a} ± 2.36	114.03^{a} ± 2.35	0.715

*Data with different superscripts are statistically different

*SE: Standard error

Therefore, 80 weeks old roosters were injected with 10 or 50 µg cGnRH. In overall, injection of 10 or 50 µg cGnRH caused significant increases in egg fertility over PBS-injected groups (P<0.05). The fertility rates were slightly decreased in 10 or 50 µg cGnRH-injected groups as compared to control group in periods V and VI due to the high amount of experimental error. In period VI, the fertility rate was the lowest in 10 µg cGnRH group due to the higher amount of error. Injection of 50 µg cGnRH caused better beneficial effect on fertility rate and as well as on hatchability of set eggs (Table 2 and 3). The decreases in hatchability of set eggs in periods V and VI were because of the higher experimental errors (Table 3). Close results were obtained following the broiler roosters' injection with a short-acting GnRH analogue (Buserelin acetate, 0.0042 mg/mL) by Hezarjaribi et al. (2016). According to their results, GnRH stimulated significant increases (P<0.05) in the percentages of fertile eggs and hatchability over the control group (Hezarjaribi et al. 2016). In this study, the control group (natural group) was not injected in the same way as the control groups in Hezarjaribi et al. (2016) study. Indeed, the key differences between the two studies comprise the dose and form of GnRH applied. Here we can state that the injection of GnRH (especially in high doses) increased the egg fertility rate. In this study, the injection of PBS caused a significant decrease in egg fertility after period V, relative to the control group. Indeed, PBS decreases the motility of sperm cells. In a study, different diluents (Such as saline, PBS, autologous prostate secretion or a modified TRIS buffer) were used to dilute canine semen in order to find out the best one that influences sperm motility and viability at a lower extent (Schäfer-Somi and Aurich, 2007). Dilution of canine semen with PBS caused a significant decrease in total motility as compared with sperm cells diluted in saline and in other diluents (Schäfer-Somi and Aurich, 2007). The motility of sperm cells is one of the important factors affecting the fertility potential of an individual. Hatching is primarily dependent on whether an egg is fertilized or not. Therefore, it is a broadly male-dependant phenomenon. This is the reason why commonly observed hatching losses in broiler breeds after 45 weeks of age isoften associated with the fertility of roosters (Sarabia Fragoso et al. 2013). In one study, the reproductive performances of young (35 weeks old) and aging (73 weeks old) broiler roosters were compared. It was reported that testes' weight, semen volume, sperm concentration, hypothalamic mRNA expression of GnRH-I, pituitary expression of mRNA for LH, FSH and plasma testosterone concentration were all lower in ageing roosters relative to their younger counterparts (Avital-Cohen et al., 2013). Thus, aging is associated with diminished GnRH secretion, resulting in reduced hatching rate.

In this study, injection of 50 µg cGnRH increased the number of hatched set eggs (Table 3). This was partly due to the increase in egg fertilization rate owing to the cGnRH injection (Table 2). In this study, 81-weeks- old male layer breeders were subcutaneously injected with 10 or 50 µg cGnRH. The dose, application route and form of GnRH all varied. Hezarjaribi et al. (2016) have also reported an increase in hatchability following injection of 60-weeks-old broiler roosters with 0.3 mL GnRH analogue buserelin acetate (0.0042 mg/mL). The results of the present study demonstrate that the hatchability of set eggs significantly increased (P<0.05) following the injection of 50 µg cGnRH (Table 3). Injection of 50 µg cGnRH induced a substantial increase in the hatchability of fertile eggs relative to the PBS-injected group and other groups in periods I and II, respectively (Table 4). Here, it is likely that GnRH has a positive impact on hatching. In mammals, it was reported that the treatment of hypophysectomised mice with a GnRH agonist increased the hatching rate (Yang et al., 1995). In this study, it was found that injection of 10 µg cGnRH caused a better effect in female ratio as compared to PBS and control groups (Table 5).

The coefficient of variation within and between groups ranged between the 2.68-8.42 and 3.17-8.73% respectively. It is difficult to compare the results of this study with those of similar studies due to the general lack of the data presenting the effect of cGnRH on sex ratio in avian species. Nevertheless, a relationship between hormone and secondary sex ratio has been reported in Holstein dairy cows (Emadi et al., 2014). It is physiologically accepted that the amount of estradiol produced in domestic mammals is relative to the amount of GnRH reaching the pituitary gland. Therefore, a cow's phenotypic response to estradiol may in turn be related to its response to GnRH. According to Emadi et al. (2014), estradiol benzoate administration increased the likelihood of calves being male. When day length shortens, melatonin secretion in domestic mammal increases and this increase is accompanied by an increase in GnRH secretion. According to one study, male-to-female ratio was higher in cows conceived in the summer, autumn, and winter than in cows conceived in the spring (Youssefi et al., 2013). This, in turn, indicates the relationship between GnRH and sex ratios. However, we cannot conclusively state that GnRH injection has a positive or negative impact on female ratios owing to different animal species and the administration of different hormones in different form, dose and application route.

In this study, no significant differences have been observed, in terms of daily egg production ($P>0.05$, Table 6), as a result of response to the cGnRH injections. This is because only roosters were injected. In literature, there are plenty of reports indicating the presence of a positive relation between the blood concentration of GnRH and the egg-laying performance of hens (Sharp et al., 1990; Ippala et al., 2016; Cowan et al., 2014). Also, no differences have been observed, in daily feed consumption per bird, between cGnRH-injected groups and others, (Table 7). Hence, the appetite of birds was not altered by GnRH injections.

Conclusion

The results demonstrate that the injection of older roosters with cGnRH (especially in high dose, 50 µg) has positive impacts on egg fertility and hatching rates. The decrease in fertility of older rooster results with decrease in fertility and hatching rates, which cause the removal of the roosters from the flock. But poultry industry is toughly suffering from the shortage of supplying the breeder rooster on time at required number. Thus, keeping the genetically superior breeder rooster within the flock longer, without any decrease in fertility, is economically important.

It is quite early to say that injection of older rooster with cGnRH-I extents the duration of their fertility. Further research needed to measure the impact of cGnRH on fertility and hatching rates by injecting both male and female of same breed and age with the same doses.

References

Avital-Cohen, N., Heiblum, R., Argov-Argaman, N., Rosenstrauch, A., Chaiseha, Y., Mobarkey, N., Rozenboim, I. (2013). Age-related changes in gonadal and serotonergic axes of broiler breeder roosters. Domestic Animal Endocrinology, 44, 145-150. doi:10.1016/j.domaniend.2013.01.002

Cho, R. N., Hahn, T. P., Macdougall-Shackleton, S., Ball, G. F. (1998). Seasonal variation in brain GnRH in free-living breeding and photorefractory house finches (Carpodacus mexicanus). General and Comparative Endocrinology, 109, 244-250. doi: 10.1006/gcen.1997.7027

Cowan, M. L., Martin, G. B., Monks, D. J., Johnston, S. D., Doneley, R. J. T., Blackberry, M. A. (2014). Inhibition of the reproductive system by deslorelin in male and female pigeons (Columba livia). Journal of Avian Medicine and Surgery, 28, 102-108. doi:10.1647/2013-027

Çiftci, H.B., Aktan, S., Başkar, V.C., Yildirim, İ., Aygün, A. (2018). The Effects of Chicken GnRH on Serum Testosterone Concentration and Egg Fertility in Japanese Quail. Journal of Animal Science and Products (JASP), 1, 84-92. https://dergipark.org.tr/tr/download/article-file/898753

Emadi, S., Rezaei, A., Bolourchi, M., Hovareshti, P., Akbarinejad. V. (2014). Administration of estradiol benzoate before insemination could skew secondary sex ratio toward males in Holstein dairy cows. Domestic Animal Endocrinology, 48, 110-118. doi: 10.1016/j.domaniend.2014.03.001

Hahn, T. P., Ball, G. F. (1995). Changes in brain GnRH associated with photorefractoriness in house sparrows (Passer domesticus). General and Comparative Endocrinology, 99, 349-363. doi: 10.1006/gcen.1995.1119

Hezarjaribi, A., Rezaeipour, V., Abdollahpour, R. (2016). Effects of intramuscular injections of vitamin E-selenium and a gonadotropin releasing hormone analogue (GnRHa) on reproductive performance and blood metabolites of post-molt male broiler breeders. Asian Pacific Journal of Reproduction, 5, 156-160. https://doi.org/10.1016/j.apjr.2016.01.013

Ippala, J. R., Mishra, A., Mondal, S., David, G. & Awachat, V. (2016). Different wavelengths of light on GnRH mRNA, LH, steroid hormones and egg lay in the ageing hens (Gallus gallus domesticus). International Journal of Bioassays, 5, 4597-4602. doi: 10.21746/ijbio.2016.06.003

Lagares, M., Ecco, R., Martins, N., Lara, L., Rocha, J., Vilel, A. D., Barbosa, V., Mantovani, P., Braga, J. Preis, I. (2017). Detecting reproductive system abnormalities of broiler breeder roosters at different ages. Reproduction in Domestic Animals, 52, 67-75. doi: 10.1111/rda.12804

Kaiser, U. B., Sabbagh, E., Katzenellenbogen, R. A., Conn, P. M. Chin, W. W. (1995). A mechanism for the differential regulation of gonadotropin subunit gene expression by gonadotropin-releasing hormone. Proceedings of the National Academy of Sciences of the United States of America, 92, 12280-12284. doi: 10.1073/pnas.92.26.12280

King, J. A., Millar, R. P. (1982). Structure of chicken hypothalamic luteinizing hormone-releasing hormone. II. Isolation and characterization. The Journal of Biological Chemistry, 257, 10729–10732. https://doi.org/10.1016/S0021-9258(18)33883-3

Millar, R., J. King. J. (1984). Structure-activity relations of LHRH in birds. The Journal of Experimental Zoology, 232, 425-430. doi:10.1002/jez.1402320307

Miyamoto, K., Hasegawa, Y., Nomura, M., Igarashi, M., Kangawa, K., Matsuo, H. (1984). "Identification of the second gonadotropin-releasing hormone in chicken hypothalamus: evidence that gonadotropin secretion is probably controlled by two distinct gonadotropin-releasing hormones in avian species. Proceedings of the National Academy of Sciences of the United States of America, 81, 3874-3878. doi: 10.1073/pnas.81.12.3874

Navara, K. J. (2010). Programming of offspring sex ratios by maternal stress in humans: assessment of physiological mechanisms using a comparative approach. Journal of Comparative Physiology B, 180, 785-795. doi: 10.1007/s00360-010-0483-9

Ottinger, M., Kubakawa, K., Kikuchi, M., Thompson, N., Ishii, S. (2002). Effects of exogenous testosterone on testicular luteinizing hormone and follicle-stimulating hormone receptors during aging. Experimental Biology and Medicine (Maywood), 227, 830-836. doi: 10.1177/153537020222700917

Parry, D. M., Goldsmith, A. R., Millar, R. P. & Glennie, L. M. (1997). Immunocytochemical Localization of GnRH Precursor in the Hypothalamus of European Starlings during Sexual Maturationand Photorefractoriness. Journal of Neuroendocrinology, 9, 235-243. doi: 10.1046/j.1365-2826.1997. 00575.x

Reddy, G. R., Xie, C., Lindaman, L. L., Coss, D. (2013). GnRH increases c-Fos half-life contributing to higher FSHβ induction. Molecular Endocrinology, ;27, 253-265. doi: 10.1210/me.2012-1168.

Rosenstrauch, A., Degen, A. A., Friedländer, M. (1994). Spermatozoa retention by Sertoli cells during the decline in fertility in aging roosters. Biology of Reproduction, 50, 129-136. doi:10.1095/biolreprod50.1.129

Rosenstrauch, A., Weil, S., Degen, A. A., Friedländer, M., 1998. Leydig cell functional structure and plasma androgen level during the decline in fertility in aging roosters. General and Comparative Endocrinology, 109, 251-258. doi:10.1006/gcen.1997.7029

Sarabia Fragoso, J., Pizarro Díaz, M., Abad Moreno, J., Casanovas Infesta, P., Rodriguez-Bertos, A., Barger, K. (2013). Relationships between fertility and some parameters in male broiler breeders (body and testicular weight, histology and immunohistochemistry of testes, spermatogenesis and hormonal levels). Reproduction in Domestic Animals, 48, 345-352. doi: 10.1111/j.1439-0531.2012. 02161.x

Schäfer-somi, S., Aurich, C. (2007). Use of a new computer-assisted sperm analyzer for the assessment of motility and viability of dog spermatozoa and evaluation of four different semen extenders for predilution. Animal Reproduction Science, 102, 1-13. doi: 10.1016/j.anireprosci.2005.03.019

Sharp, P., Talbot, R., Main, G., Dunn, I., Fraser, H., Huskisson, N. (1990). Physiological roles of chicken LHRH-I and-II in the control of gonadotrophin release in the domestic chicken. The Journal of Endocrinology, 124, 291-299. doi: 10.1677/joe.0.1240291

Sherwood, N. M., Wingfield, J. C., Ball, G. F., Dufty, A. M. (1988). Identity of gonadotropin-releasing hormone in passerine birds: comparison of GnRH in song sparrow (Melospiza melodia) and starling (Sturnus vulgaris) with five vertebrate GnRHs. Generai and Comparative Endocrinology, 69, 341-351. doi: 10.1016/0016-6480(88)90024-x

Vizcarra, J., Kirby, J., Kreider, D. (2010). Testis development and gonadotropin secretion in broiler breeder males. Poultry Science, 89, 328-334. doi:10.3382/ps.2009-00286

Weil, S., Degen, A. A., Rosenstrauch, A., Friedländer. M. (1996). Intratesticular spermatozoa retention in low fertility ageing roosters is related to malformations of Sertoli cell ectoplasmic specializations. The Journal of Experimental Zoology, 275, 317-325. https://doi.org/10.1002/(SICI)1097-010X(19960701)275:4<317::AID-JEZ10>3.0.CO;2-7

Weil, S., Rozenboim, I., Degen, A. A., Dawson, A., Friedländer, M., Rosenstrauch, A. (1999a). Fertility decline in aging roosters is related to increased testicular and plasma levels of estradiol. General and Comparative Endocrinology, 115, 23-28. doi: 10.1006/gcen.1999.7276

Weil, S., Degen, A. A., Friedländer, M., Rosenstrauch, A. (1999b). Low fertility in aging roosters is related to a high plasma concentration of insulin and low testicular contents of ACTH and lactate. General and Comparative Endocrinology, 115, 110-115. doi:10.1006/gcen.1999.7291

Yang, B. C., Uemura, T., Minaguchi, H. (1995). Effects of a gonadotropin releasing hormone agonist on oocyte maturation, fertilization, and embryonal development of mice. Journal of Assisted Reproduction and Genetics, 12, 728-732. doi: 10.1007/bf02212901

Youssefi, R., Vojgani, M. Gharagozlou, F., Akbarinejad, V. (2013). More male calves born after Presynch-Ovsynch protocol with 24-hour timed AI in dairy cows. Theriogenology, 79, 890-894. doi: 10.1016/j.theriogenology.2013.01.007.