

## Follicle Diameters, Egg Weight, and Egg Production Performance in Old Laying Hens Injected with Growth Hormone and Testosterone

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### ABSTRACT

Hen aging is accompanied by a disruption of productive performance, namely, egg production. The current study was carried out to investigate how exogenous Growth Hormone (GH) and Testosterone (Ts) change diameters of hierarchical follicles, egg weight, and egg production performance of old laying hens in the late phase of production. To this end, 160 HyLine W-36 laying hens (aged 73 weeks), were injected (single injection) with GH and Ts as follows: Treatment 1: 100  $\mu$ L distilled water (control group); Treatment 2: 500  $\mu$ g Ts  $\text{kg}^{-1}$  Body-Weight (BW)+50  $\mu$ g GH  $\text{kg}^{-1}$  body-weight; Treatment 3: 500  $\mu$ g Ts  $\text{kg}^{-1}$  BW+100  $\mu$ g GH  $\text{kg}^{-1}$  BW, and Treatment 4: 500  $\mu$ g Ts  $\text{kg}^{-1}$  BW+150  $\mu$ g GH  $\text{kg}^{-1}$  BW. The experiment had four replicates and 10 birds in each replicate in a completely randomized design. The diameters of Small White Follicle (SWF), Large White Follicle (LWF), the First ( $F_1$ ), Second ( $F_2$ ) and Third ( $F_3$ ) largest yellow follicles in treatment 3 were significantly larger than in the control group, in the second week after the injection. Hen-Day Egg Production percent (HDEP), egg mass, and Feed Intake (FI) of treatment 3 were significantly higher than all other groups, during the second week after the injection; besides, HDEP and FI in treatment 4 were significantly more than in the control group. These results suggest that in old laying hen, GH and Ts may positively influence follicular diameters and egg production performance.

**Keywords:** Egg parameters, Hen-day egg production, HyLine W-36 laying hens.

### INTRODUCTION

Similar to a bunch of grapes in appearance, the ovary of a laying hen is a cluster of many follicles (Alodan, 2001). Follicles are arranged in a size hierarchy ranging from 6 to 20 mm (Johnson, 2000; Alodan, 2001). Comparing the rate of follicular maturation in young and aging birds, one can notice that the former is much slower (Johnson *et al.*, 1986; Palmer and Bahr, 1992; Oguike *et al.*, 2006). The reproductive performance in females is believed to deteriorate in accordance with age. The ovaries, and particularly the follicles, are the primary targets of senescence (Lebedeva *et*

*al.*, 2010). Various factors are associated with the initiation of the gradual decline in egg production in a flock of ageing birds which include the different sizes of yolky follicles as well as the changes in the pattern of yolk accumulation into the follicles and the high rate of atresia in the small follicles in addition to the slight transverse of follicles into rapid growth phase among both the old and young hens (Oguike *et al.*, 2006); besides, more subtle changes in levels of sex steroids, namely time or amplitude of the pre-ovulatory surge of hormones, are considered to be responsible for the alterations in follicular growth of older hens (Johnson *et al.*, 1986;

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Lebedeva *et al.*, 2010). The rate at which follicles enter terminal follicular growth phase may alter the egg production rate (Reddy *et al.*, 2006). The decline in egg production rate with the layers' aging may also somehow be attributed to both an increased incidence of atresia and a reduced number of follicles reaching the final phase of rapid growth (Williams and Sharp, 1978; Palmer and Bahr, 1992; Johnson, 2000). Evidently, lower number of small follicles and the respective prevalence of atresia (Waddington *et al.*, 1985; Palmer and Bahr, 1992; Oguike *et al.*, 2006), in addition to a slower rate of follicular maturation were found in the ovaries of aged hens compared to the young ones (Johnson *et al.*, 1986; Palmer and Bahr, 1992; Oguike *et al.*, 2006).

Since increase in yolk and follicle weight may lead to enhanced egg weight (Gunawardana *et al.*, 2008), and also plays a role in proper development of embryo, follicle size, diameter and weight are focused by animal science scientists.

## MATERIALS AND METHODS

### Birds, Diet, Management and Experimental Design

In this study, 160 HyLine W-36 laying hens (73 weeks of age) were weighed individually and randomly assigned to four treatments with four replicates and 10 birds in each replicate in a completely randomized design. The birds were kept in an environmentally controlled house. Average house temperature and relative humidity were 24°C and 40–60%, respectively. Lighting regimen of 16 hours light and 8 hours dark was used. Diets were corn-soybean based, containing 16.1% crude protein and 2,800 kilocalories of metabolizable energy per kg of feed, which met or exceeded NRC requirements (NRC, 1994).

### Treatments and Injection Manner

Birds were injected (single injection) subcutaneously, at the base of the neck, with Ts and GH at body-weight-dependent dosages as follows: Treatment 1: 100 µL distilled water (control group); Treatment 2: 500 µg Ts kg<sup>-1</sup> Body-Weight (BW)+50 µg GH kg<sup>-1</sup> BW; Treatment 3: 500 µg Ts kg<sup>-1</sup> BW+100 µg GH kg<sup>-1</sup> BW, and Treatment 4: 500 µg Ts kg<sup>-1</sup> BW+150 µg GH kg<sup>-1</sup> BW. GH was prepared for injection according to manufacturer's recommendations and neutral oil was used as vehicle for Ts injection.

Based on reviewed literatures (Oades and Messent, 1981; Stephen *et al.*, 2001; Ansari-Pirsaraei, 2009), Eutropin<sup>TM</sup> (Recombinant human somatropin<sup>TM</sup>, LG Life Sciences Company, Korea) and Androne<sup>®</sup> (Testosterone Enanthate, Caspian Tamin Pharmaceutical Company, Iran) were used in the present experiment.

### Follicle Diameter Measurements and Egg Weight

Follicle diameters were measured at two stages. At the first stage, just eight h after the hormone injection, two birds from each replicate were randomly selected and slaughtered by decapitation (n= 2 × 16). Immediately after slaughtering, whole ovary and the accompanying follicular hierarchy were removed. If there was oviducal egg, it was removed, weighted and considered as one laid egg. Since it is elucidated that the follicular size is a better criterion of the follicular maturity than the weight of yolk-free mass (Etches *et al.*, 1983), the diameter of the First (F<sub>1</sub>), Second (F<sub>2</sub>), Third (F<sub>3</sub>), Fourth (F<sub>4</sub>), Fifth (F<sub>5</sub>) largest yellow follicles, Large Yellow Follicle (LYF), Small Yellow Follicle (SYF), Large White Follicle (LWF) and Small White Follicle (SWF) were measured along and across the stigma, to within±0.01 mm, by using a Vernier Calipers (Ansari-Pirsaraei *et al.*, 2008).

The second stage was carried out 14 days after the hormone injection as previously explained in the first stage. Fresh eggs were weighed (to within  $\pm 0.01$  g) individually during two weeks prior to the hormone injection and the second week after that. The Body Weight (BW) was used as a covariate in the analysis of egg weight.

### Egg Weight and Egg Production Performance

Daily egg production was recorded throughout the experiment and HDEP percent was calculated (Ansari-Pirsaraei *et al.*, 2008). HDEP percent was used as indicator for the rate of ovulation (Ebeid *et al.*, 2008). Egg production percentage and egg weight values were used to calculate egg mass (EL-Husseiny *et al.*, 2008). FI and egg weight were recorded on a weekly basis and feed conversion ratio for egg production was calculated. The equations used in the experiment are given as footnotes to Table 2.

### Statistical Analysis

All data were analyzed using General Linear Model (GLM) of SAS (SAS, 2001). Differences among means were separated with Duncan multiple range test. Differences were considered significant when  $P < 0.05$ .

## RESULTS

### Follicular Diameters and Egg Weight

The effects of hormone injection on follicular diameters and egg weight are shown in Table 1. The hormone injection increased the diameters of SWF, LWF, F<sub>3</sub>, F<sub>2</sub> and F<sub>1</sub>. Diameter of SWF in treatment 3 was significantly higher than in the control group ( $P < 0.05$ ); no significant difference was detected between the control group, treatments 2 and 4. Diameter of LWF in treatment 3 was found higher than in the control group and treatment 4 ( $P < 0.05$ ). F<sub>3</sub> was larger than in the control group and treatment 2 ( $P < 0.05$ ). The size of F<sub>2</sub> in all injected hens were greater than in the control

**Table 1.** Effect of the hormone injection on the follicular diameters and egg weight.

| Parameters                       | Treatment 1 (Control) <sup>j</sup> | Treatment 2 <sup>k</sup>        | Treatment 3 <sup>l</sup>       | Treatment 4 <sup>m</sup>        | SD <sup>n</sup> |
|----------------------------------|------------------------------------|---------------------------------|--------------------------------|---------------------------------|-----------------|
| SWF <sup>a</sup> (mm)            | 5.008 $\pm$ 0.026 <sup>b</sup>     | 5.280 $\pm$ 0.032 <sup>ab</sup> | 5.551 $\pm$ 0.015 <sup>a</sup> | 5.244 $\pm$ 0.190 <sup>ab</sup> | 0.10            |
| LWF <sup>b</sup> (mm)            | 5.780 $\pm$ 0.024 <sup>b</sup>     | 6.168 $\pm$ 0.019 <sup>ab</sup> | 6.586 $\pm$ 0.029 <sup>a</sup> | 6.015 $\pm$ 0.028 <sup>b</sup>  | 0.13            |
| SYF <sup>c</sup> (mm)            | 5.715 $\pm$ 0.029                  | 5.783 $\pm$ 0.028               | 6.121 $\pm$ 0.037              | 6.040 $\pm$ 0.034               | 0.15            |
| LYF <sup>d</sup> (mm)            | 6.268 $\pm$ 0.057                  | 6.299 $\pm$ 0.060               | 6.615 $\pm$ 0.048              | 6.430 $\pm$ 0.052               | 0.98            |
| F <sub>5</sub> <sup>e</sup> (mm) | 11.23 $\pm$ 0.01                   | 12.19 $\pm$ 0.11                | 12.74 $\pm$ 0.01               | 12.18 $\pm$ 0.16                | 1.21            |
| F <sub>4</sub> <sup>f</sup> (mm) | 16.57 $\pm$ 0.24                   | 17.18 $\pm$ 0.18                | 18.47 $\pm$ 0.18               | 17.45 $\pm$ 0.12                | 1.24            |
| F <sub>3</sub> <sup>g</sup> (mm) | 21.21 $\pm$ 0.20 <sup>b</sup>      | 21.74 $\pm$ 0.20 <sup>b</sup>   | 24.36 $\pm$ 0.25 <sup>a</sup>  | 22.36 $\pm$ 0.23 <sup>ab</sup>  | 2.01            |
| F <sub>2</sub> <sup>h</sup> (mm) | 22.90 $\pm$ 0.22 <sup>b</sup>      | 25.37 $\pm$ 0.20 <sup>a</sup>   | 26.80 $\pm$ 0.33 <sup>a</sup>  | 25.71 $\pm$ 0.21 <sup>a</sup>   | 1.98            |
| F <sub>1</sub> <sup>i</sup> (mm) | 28.70 $\pm$ 0.37 <sup>c</sup>      | 31.26 $\pm$ 0.39 <sup>ab</sup>  | 32.96 $\pm$ 0.43 <sup>a</sup>  | 30.50 $\pm$ 0.33 <sup>bc</sup>  | 2.33            |
| Egg weight (g)                   | 63.31 $\pm$ 1.73                   | 63.35 $\pm$ 2.01                | 64.12 $\pm$ 1.86               | 63.43 $\pm$ 1.49                | 0.712           |

<sup>a</sup> Small White Follicle; <sup>b</sup> Large White Follicle; <sup>c</sup> Small Yellow Follicle; <sup>d</sup> Large Yellow Follicle; <sup>e</sup> The fifth yellow follicle; <sup>f</sup> The fourth yellow follicle; <sup>g</sup> The third yellow follicle; <sup>h</sup> The second yellow follicle; <sup>i</sup> The first yellow follicle. <sup>j</sup> Injection of 100  $\mu$ l distilled water (control group); <sup>k</sup> Injection of 500  $\mu$ g Ts kg<sup>-1</sup> body-weight+50  $\mu$ g GH kg<sup>-1</sup> body-weight; <sup>l</sup> Injection of 500  $\mu$ g Ts kg<sup>-1</sup> body-weight+100  $\mu$ g GH kg<sup>-1</sup> body-weight; <sup>m</sup> Injection of 500  $\mu$ g Ts kg<sup>-1</sup> body-weight+150  $\mu$ g GH kg<sup>-1</sup> body-weight. <sup>n</sup> Standard Deviation.

Values in the same row with different superscripts are significantly different ( $P < 0.05$ ). Values are expressed as mean $\pm$ SEM (Standard Error of Mean).

**Table 2.** Effects of the hormone injection on egg production performance of the laying hens.

| Parameters   | Treatment 1 <sup>e</sup><br>(Control) | Treatment 2 <sup>f</sup> | Treatment 3 <sup>g</sup> | Treatment 4 <sup>h</sup> | SD <sup>i</sup> |
|--|---------------------------------------|--------------------------|--------------------------|--------------------------|-----------------|
| Ovulation rate <sup>a</sup><br>(HDEP) (%)                              | 60.88±2.66 <sup>c</sup>               | 60.92±2.92 <sup>c</sup>  | 64.73±3.01 <sup>a</sup>  | 62.35±2.81 <sup>b</sup>  | 0.411           |
| Egg mass <sup>b</sup><br>(g of egg hen <sup>-1</sup> d <sup>-1</sup> ) | 38.53±2.31 <sup>b</sup>               | 38.60±1.95 <sup>b</sup>  | 41.50±1.83 <sup>a</sup>  | 39.56±2.02 <sup>ab</sup> | 0.205           |
| FI <sup>c</sup> (g bird <sup>-1</sup> d <sup>-1</sup> )                | 87.93±8.75 <sup>c</sup>               | 89.57±9.42 <sup>c</sup>  | 97.26±9.89 <sup>a</sup>  | 92.95±10.02 <sup>b</sup> | 0.503           |
| FCR <sup>d</sup><br>(g of feed g <sup>-1</sup> of egg)                 | 2.361±0.701                           | 2.357±0.099              | 2.359±1.092              | 2.378±0.854              | 0.183           |

<sup>a</sup> Ovulation rate (Hen-Day Egg Production)= (Total number of eggs/Number of live layers)×100;

<sup>b</sup>Egg mass= [Egg production (%)×Egg weight (g)]/100; <sup>c</sup> FI = Feed Intake; <sup>d</sup> Feed Conversion Ratio= Feed intake/Egg mass. <sup>e</sup> Injection of 100 µl distilled water (control group); <sup>f</sup> Injection of 500 µg Ts kg<sup>-1</sup> body-weight+50 µg GH kg<sup>-1</sup> body-weight; <sup>g</sup> Injection of 500 µg Ts kg<sup>-1</sup> body-weight+100 µg GH kg<sup>-1</sup> body-weight, and <sup>h</sup> Injection of 500 µg Ts kg<sup>-1</sup> body-weight+150 µg GH kg<sup>-1</sup> body-weight. <sup>i</sup> Standard Deviation.

Values in the same row with different superscripts are significantly different (P< 0.05). Values are expressed as mean±SEM (Standard Error of Mean).

group (P< 0.05), however, no significant difference was detected between the hormone-injected hens (P> 0.05). The diameters of SYF, LYF, F<sub>5</sub> and F<sub>4</sub>, besides egg weight, were not affected significantly (P> 0.05).

### Egg Production Performance

Table 2 shows the effects of the hormone injection on egg production performance. Ovulation rate and FI in treatment 3 were higher compared to all other groups (P< 0.05); besides, ovulation rate and FI in treatment 4 differed compared with the control group (P< 0.05). Egg mass in treatment 3 was more than in the control group and treatment 2 (P< 0.05). FCR did not differ significantly (P> 0.05).

### DISCUSSION

Little research has been carried out regarding the initiation and early stages of follicular growth (Yang and Fortune, 2006). Entering an abrupt growth phase by follicles when they have a diameter of less than nine mm and contain white yolk is observed. This is aligned with the previous findings on

transformation of follicles from the resting stage to a rapid growth phase at a diameter of about five to six mm (Oguike *et al.*, 2006). It is also suggested that the transitions happening in the theca and granulosa tissues of the follicles may change the follicles from non-ovulable to ovulable stage in the hierarchy and there is even a possibility that it is responsible for follicular maturation; however, the mechanisms which cause the delay of the transverse of follicles into rapid growth phase in aged hens is not clarified (Oguike *et al.*, 2006). Each eight-mm follicle grows rapidly, three-fold increase in size (Yoshimura *et al.*, 1994), during a 25–27 hour period of time and enters the follicular hierarchy, then ovulates after complete growth and development during five to seven days (Johnson, 2000; Goerlich *et al.*, 2010). Follicular growth is affected by progesterone and androgens (Tonetta and diZerega, 1989). Gonadotropins play critical roles in terminal follicular development (Monniaux *et al.*, 1997). Initially, Ts production experiences a slight increase and then almost levels out before plunging during the last 24 hours of yolk deposition (Tonetta and diZerega, 1989). Reduction in follicular maturation rate may lead to increased pause days (Reddy *et al.*, 2006). Detailed mechanisms

regulating follicle selection and maturation are still far from understood (Johnson and Woods, 2007; McLaughlin and McIver, 2009; Onagbesan *et al.*, 2009; Goerlich *et al.*, 2010). Lots of ambiguity exists in understanding the mechanisms causing the delay of the transformation of these follicles into rapid growth phase as hens age (Williams and Sharp, 1978; Oguike *et al.*, 2006).

The earliest visible sign of primordial follicular recruitment into the growth pool is the transformation of the flattened, squamous-appearing granulosa cells into cuboidal epithelial-type cells, resulting in an increase in follicle diameter (Vendola *et al.*, 1999). Since granulosa cells of the mature preovulatory follicle contain nuclear androgen receptors, they are assumed to be target cells for Ts (Yoshimura *et al.*, 1993; Ansari-Pirsaraei *et al.*, 2008). On the other hand, the presence of androgen receptors in avian and mammalian oocytes has been demonstrated and it is assumed that Ts has a potential role in prehierarchical follicle maturation and selection for ovulation (Yoshimura *et al.*, 1993; Walters *et al.*, 2008; Goerlich *et al.*, 2010). Further, with regard to recent evidence showing the significant role of Ts in steroid production and ovulation of the preovulatory follicles, Rangel and Gutierrez (2014) believe that the role of Ts in ovulation has been redefined. They also emphasize on the stimulatory effects of Ts on progesterone production (*in vivo*), the necessity of Ts presence in preovulatory progesterone and LH peaks and the Ts and LH interaction resulting in progesterone production (Rangel and Gutierrez, 2014).

Very low density lipoprotein VLDL (whose main function is to transport triglycerides, phospholipids, and cholesterol), and vitellogenin (which is a phosphoglycolipoprotein), are the two main precursors of yolk (Shen *et al.*, 1993; Johnson, 2000). In a maturing pullet, yolk deposition is triggered by the Follicle-Stimulating Hormone (FSH) (Johnson, 2000). We have previously found that GH

plus Ts injection may increase plasma concentrations of LDL, HDL, cholesterol and also estradiol in old laying hens (Mohammadi and Ansari-Pirsaraei, 2014). Getting the signals directly from estrogen, the formation of yolk protein takes place in the liver (Gilbert, 1971) under the control of gonadotropins and ovarian-derived steroids (oestrogen, progesterone and Ts) (Shen *et al.*, 1993; Johnson, 2000). Specific dose of GH injection, through IGF systems, may increase yolk production in liver and, additionally, it could stimulate other growth factors in small follicles. For a full understanding of these mechanisms, more experiments should be done using different doses of hormones (Ansari-Pirsaraei *et al.*, 2008). On the other hand, IGF-I and FSH are proposed to be involved in regulation of the follicular hierarchy and onset of preovulatory steroidogenesis (Cassy *et al.*, 2004). Hrabia *et al.* (2012) propose that both GH and IGF-I are important stimulators of estradiol production in chicken nonhierarchical ovarian follicles (Hrabia *et al.*, 2012). Also, Hrabia *et al.* (2014) have recently suggested that GH plays a role in the development and activity of the chicken oviduct prior to the onset of egg laying. Ts, oestrogen, progesterone and gonadotropins have role in the yolk precursor transportation to the growing follicle (Shen *et al.*, 1993; Johnson, 2000).

It has been shown that progesterone injected broiler breeder hens have heavier F<sub>1</sub> follicles (Liu and Bacon, 2005). Injection of premature layers with Ts, GH and GH plus Ts, increased diameter of SWF compared with none injected layers, however, only GH plus Ts injection significantly increased the diameter of LWF and SYF. This effect is attributed to elevated growth factors, especially IGFs, in small and large growing follicles (Ansari-Pirsaraei *et al.*, 2008); these results are, in part, in line with our findings. Follicular growth may be improved as a result of androgens, progesterone (Tonetta and diZerega, 1989) and also GH functions of local production of IGF-I in ovary (Ansari-Pirsaraei, 2009), and liver



production of yolk precursors (Ansari-Pirsaraei *et al.*, 2008). Besides, Ts plays a role in yolk lipid deposition in growing follicle (Ansari-Pirsaraei, 2009). Several *in vivo* and *in vitro* investigations have revealed that GH exerts stimulatory influences on follicular growth, development and atresia blocking (Ansari-Pirsaraei *et al.*, 2008). It is reported that ovine GH could enhance the number of small follicles in the domestic hen (Williams *et al.*, 1992). It is demonstrated that egg weight may be increased due to enhanced yolk and follicle weight (Gunawardana *et al.*, 2008). Also, the significant effect of IGF-I genotype on egg weight is demonstrated (Nagaraja *et al.*, 2000). Synthesis of ovalbumin, conalbumin, ovomucoid, and lysozyme in the oviduct and vitellogenin in the liver may be increased by estradiol, which may result in enhanced egg weight (Johnson, 2000). With respect to abovementioned evidence, increase in diameters of follicle in different stages of development and egg weight was anticipated; however, it was significant only in SWF, LWF, F<sub>3</sub>, F<sub>2</sub>, and F<sub>1</sub>.

Available data suggest that GH is involved in the control of reproduction in birds. During reproductive development, GH may influence body weight leading to alteration of the number of LYF (Renema *et al.*, 1999). A relationship between GH and GH Receptor (GHR) genotypes and age at first egg and the rate of egg production is observed in laying hens (Feng *et al.*, 1997; Kuhnlein *et al.*, 1997; Lebedeva *et al.*, 2004; Ansari-Pirsaraei *et al.*, 2008). It is also demonstrated that GH local production, by means of an autocrine/paracrine mechanism, may directly stimulate progesterone production in the hen granulosa cells (Ahumada-Solórzano *et al.*, 2012); and it has recently been demonstrated that GHR mRNA is also greatly expressed in the magnum, isthmus, and shell gland of laying hens, and the possibility of significant role of GH in oviduct function of domestic hens was revealed (Hrabia *et al.*, 2013). It has also been shown that egg-laying hens have higher plasma GH concentrations and

pituitary GH mRNA expression than non-laying hens (Scanen *et al.*, 1979; Karatzas *et al.*, 1997; Lebedeva *et al.*, 2004; Ansari-Pirsaraei *et al.*, 2008) and that androgens, through nuclear and extranuclear signaling pathways, decrease number of atretic follicles. Sen *et al.* (2014) demonstrated that androgens may enhance FSH-receptor expression, resulting in follicular growth and development. It is suggested that GH and Ts, influencing the follicular growth and oviposition, alter the egg production rate (Ansari-Pirsaraei *et al.*, 2008, 2010). The effect of GH and Ts on egg production rate is attributed to stimulated IGF system (Ansari-Pirsaraei *et al.*, 2008). On the other hand, it is elucidated that the *GH* and *GHR* genes are associated with the rate of egg production (Hockinga *et al.*, 1994; Kuhnlein *et al.*, 1997; Nagaraja *et al.*, 2000) and double-yolk egg production (Hockinga *et al.*, 1994). According to the available data, the central role of estradiol in egg production is demonstrated and it would be expected to play a central role in the determination of egg mass and quality (Christians and Williams, 1999). Besides, as stated above, in our previous work, it was found that injection of old laying hens with 500 µg Ts plus 100 µg GH kg<sup>-1</sup> BW increased plasma concentration of estradiol eight h after the injection (Mohammadi and Ansari-Pirsaraei, 2014).

It is suggested that Ts modulates follicular growth and development via control of plasminogen activator. This enzyme is located in preovulatory follicles and plays a role in follicle differentiation, recruitment, ovulation, and atresia (Goerlich *et al.*, 2010). It has reported that injection of Ts to laying hens induces ovulation within eight h (Fraps, 1955). Additionally, it is reported that injection of Ts to laying hens which have a functional ovary with mature preovulatory follicles may induce ovulation (Fraps, 1955; Croze and Etches, 1980). It has been thought that preovulatory surge of Ts causes LH surge before ovulation by influencing hypothalamic–pituitary–ovarian axis, suggesting important role of Ts in ovulation

process. Furthermore, active or passive immunization against Ts leads to ovulation halt (Tanaka *et al.*, 1996; Croze and Etches, 1980; Pierce *et al.*, 2005; Ansari-Pirsaraei *et al.*, 2008, 2009). According to above-mentioned materials, improvement in ovulation rate and egg mass was anticipated.

GH plays a multifunctional role in hen's body. It may affect growth, body composition and appetite (Byatt *et al.*, 1993). We previously showed that injection of 500  $\mu\text{g Ts kg}^{-1}\text{ BW}+100\ \mu\text{g GH kg}^{-1}\text{ BW}$  and also 500  $\mu\text{g Ts kg}^{-1}\text{ BW}+150\ \mu\text{g GH kg}^{-1}\text{ BW}$  significantly increased FI during the first week after the injection. GH injection may directly influence the adrenal and also may increase Thyroxin ( $T_4$ ) in line with our previous results (Mohammadi and Ansari-Pirsaraei, 2014) and corticosterone (Scanen, 2000; Ansari-Pirsaraei, 2009). On the other hand, elevated leptin production (as an appetite increasing hormone (Niv-Spector *et al.*, 2005)) due to  $T_4$  is detected (Zou *et al.*, 2007). It is assumed that in treatments 3 and 4, GH dose was effective enough to increase FI significantly compared with the control group and treatment. The same effect was also detected in treatment 3 compared with treatment 4. These results were expected and are partly in agreement with assumed GH functions. The dual role of GH in synthesis and secretion of IGF-I by hen granulosa cell has been shown by some authors (Ansari-Pirsaraei, 2009). IGF-I may increase protein breakdown rate and play an important role in body growth and food utilization efficiency (Tomas *et al.*, 1998). In contrast with the present study, we have already shown that GH and Ts may have significant effect on FCR during the first week after the injection (Mohammadi and Ansari-Pirsaraei, 2014).

In this study, we noticed that physiological manipulation of old laying hens may improve at least some follicular diameters as well as ovulation rate and egg mass. It is notable that the present study was an attempt to shed more light on the GH and Ts roles in old laying hen reproductive system, therefore, because of the hormone residual risks, the authors do not recommend farmers

to inject the laying hens with GH and Ts. The positive effects of the enhanced GH and Ts on reproduction performance may be considered in bird selection and breeding plans to estimate future reproduction performance. Obviously, further work is needed to make such decision.

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#### REFERENCES

1. Ahumada-Solórzano, M. S., Carranza M. E., Pedernera, E., Rodríguez-Méndez, A. J., Luna M. and Arámburo, C. 2012. Local Expression and Distribution of Growth Hormone and Growth Hormone Receptor in the Chicken Ovary: Effects of GH on Steroidogenesis in Cultured Follicular Granulosa Cells. *Gen. Comp. Endocrinol.*, **175**: 297–310.
2. Alodan, M. A. 2001. Cytokine IL-1 $\beta$  Modulation of Reproductive Function in Heat Stressed Hens. PhD. Thesis, University of Nebraska, Lincoln, Nebraska.
3. Ansari-Pirsaraei, Z. 2009. Effect of Testosterone and Growth Hormone Injection on Expression of *IGF-I*, *Type-I IGFR* and *Type-II IGFBP* Genes of the Mazandaran Native Breeder Hens. PhD. Thesis, Animal Science Department, Tehran University of Tehran, Iran.
4. Ansari-Pirsaraei, Z., Mianji, G. R., Shahneh, A. Z., Zaghari, M. and Zamiri, M. J. 2010. Effect of Testosterone and Growth Hormone Injection before Puberty on Egg Quality, Egg Production and Some Blood Parameters of Native Breeder Hens. In *Proceedings International Conference on Agricultural and Animal Science (CAAS)*, World Academic Union (World Academic Press), 26-28 February, Singapore, PP. 113–117.
5. Ansari-Pirsaraei, Z., Shahneh, A. Z., Zaghari, M., Zamiri, M. J. and Mianji, G. R.



2008. Effect of Testosterone and Growth Hormone Injection before Puberty on Follicles Size, Rate of Egg Production and Egg Characteristics of the Mazandaran Native Breeder Hens. *Afri. J. Biotech.*, **7**: 3149–3154.
6. Byatt, J. C., Staten, N. R., Salsgiver, W. J., Kostelc, J. G. and Collier, R. J. 1993. Stimulation of Food Intake and Weight Gain in Mature Female Rats by Bovine Prolactin and Bovine Growth Hormone. *American J. Physiol. Endocrinol. Metab.*, **264**: E986–E992.
  7. Cassy, S., Metayer, S., Crochet, S., Rideau, N., Collin, A. and Tesseraud, S. 2004. Leptin Receptor in the Chicken Ovary: Potential Involvement in Ovarian Dysfunction of *Ad Libitum*-fed Broiler Breeder Hens. *Reprod. Biolo. Endocrinol.*, **2**: 72.
  8. Christians, J. K. and Williams, T. D. 1999. Effects of Exogenous 17 $\beta$ -Estradiol on the Reproductive Physiology and Reproductive Performance of European Starlings (*Sturnus vulgaris*). *The J. Exper. Biol.*, **202**: 2679–2685.
  9. Croze, F. and Etches, R. J. 1980. The Physiological Significance of Androgen-induced Ovulation in the Hen. *J. Endocrinol.*, **84**: 163–171.
  10. Ebeid, T. A., Eid, Y. Z., El-Abd, E. A. and ElHabbak, M. M. 2008. Effects of Catecholamines on Ovary Morphology, Blood Concentrations of Estradiol-17 $\beta$ , Progesterone, Zinc, Triglycerides and Rate of Ovulation in Domestic Hens. *Theriogenol.*, **69**: 870–876.
  11. EL-Husseiny, O. M., Abd-Elsamee, M. O., Hassane, M. I. and Omara, I. I. 2008. Response of Egg Production and Egg Shell Quality to Dietary Vegetable Oils. *Int. J. Poult. Sci.*, **7**: 1022–1032.
  12. Etches, R., MacGregor, H., Morris, T. and Williams, J. 1983. Follicular Growth and Maturation in the Domestic Hen (*Gallus domesticus*). *J. Reprod. Fertil.*, **67**: 351–358.
  13. Feng, X., Kuhnlein, U., Aggrey, S., Gavora, J. and Zadworny, D. 1997. Trait Association of Genetic Markers in the Growth Hormone and the Growth Hormone Receptor Gene in a White Leghorn Strain. *Poult. Sci.*, **76**: 1770–1775.
  14. Fraps, R. M. 1955. Egg Production and Fertility in Poultry. In "*Progress in the Physiology of Farm Animals*", (Ed.): Hammond, J.. Butterworths, London, **PP**. 671–740.
  15. Gilbert, A. B. 1971. The Endocrine Ovary in Reproduction. In "*Physiology and Biochemistry of the Domestic Fowl*". (Eds.): Bell, D. J. and Freeman, B. M.. Academic Press, London, **PP**. 1449–1468.
  16. Goerlich, V. C., Dijkstra, C. and Groothuis, T. G. G. 2010. Effects of *In vivo* Testosterone Manipulation on Ovarian Morphology, Follicular Development, and Follicle Yolk Testosterone in the Homing Pigeon. *J. Exper. Zool.*, **313A**: 328–338.
  17. Gunawardana, G. W. P., Bryant, M. M., Voitle, R. and Roland, D. A. 2008. Effect of Molting Method and Dietary Energy on Postmolt Performance of Two Strains of Single Comb White Leghorn Hens. *J. App. Poult. Res.*, **17**: 1–10.
  18. Hockinga, P. M., Bernarda, R., Wilkie, R. S. and Goddarda, C. 1994. Plasma Growth Hormone and Insulin-like Growth Factor-I (IGF-I) Concentrations at the Onset of Lay in *Ad libitum* and Restricted Broiler Breeder Fowl. *Brit. Poult. Sci.*, **35**: 299–308.
  19. Hrabia, A., Grzegorzewska, A. K. and Sechman, A. 2013. Expression and Localization of Growth Hormone Receptor in the Oviduct of the Laying Hen (*Gallus domesticus*). *Folia. Biol. (Krakow)*, **61(3-4)**: 271–276.
  20. Hrabia, A., Leśniak-Walentyn, A., Sechman, A. and Gertler, A. 2014. Chicken Oviduct. The Target Tissue for Growth Hormone Action: Effect on Cell Proliferation and Apoptosis and on the Gene Expression of Some Oviduct-specific Proteins. *Cell. Tissue. Res.*, **357(1)**: 363–372.
  21. Hrabia, A., Sechman, A. and Rzasca, J. 2012. Independent, Non-IGF-I Mediated, GH Action on Estradiol Secretion by Prehierarchical Ovarian Follicles in Chicken. In *Vitro Study Folia. Biol. (Krakow)*, **60**: 213–217.
  22. Johnson, A. L. and Woods, D. C. 2007. Ovarian Dynamics and Follicle Development. In: "*Reproductive Biology and Phylogeny of Birds*", (Ed.): Jamieson, B. G. M.. Science Publishers, UK, Plymouth, **PP**. 243–277.
  23. Johnson, A. L. 2000. Reproduction in the Female. In "*Sturkie's Avian Physiology*", (Ed.): Whittow, G. C.. Academic Press, San Diego, London, Boston, **PP**. 461–471.



24. Johnson, P. A., Dickerman, R. W. and Bahr, J. M. 1986. Decreased Granulosa Cell, Luteinizing Hormone Sensitivity and Altered Thecal Oestradiol Concentration in the Aged Hen, *Gallus domesticus*. *Biol. Reprod.*, **35**: 641–646.
25. Karatzas, C., Guemene, D., Zadworny, D. and Kuhnlein, U. 1997. Changes in Expression of the Prolactin and Growth Hormone Gene during Different Reproductive Stages in the Pituitary Gland of Turkeys. *Reprod. Nutr. Devel.*, **37**: 69–79.
26. Kuhnlein, U., Ni, L., Weigend, S., Gavora, J., Fairfull, W. and Zadworny, D. 1997. DNA Polymorphisms in the Chicken Growth Hormone Gene: Response to Selection for Disease Resistance and Association with Egg Production. *Anim. Genet.*, **28**: 116–123.
27. Lebedeva, I. Y., Lebedev, V. A., Grossmann, R., Kuzmina, T. I. and Parvizi, N. 2004. Characterization of Growth Hormone Binding Sites in Granulosa and Theca Layers at Different Stages of Follicular Maturation and Ovulatory Cycle in the Domestic Hen. *Biol. Reprod.*, **71**: 1174–1181.
28. Lebedeva, I. Y., Lebedev, V. A., Grossmann, R. and Parvizi, N. 2010. Age-dependent Role of Steroids in the Regulation of Growth of the Hen Follicular Wall. *Reprod. Biol. Endocrinol.*, **8**: 1–13.
29. Liu, H. K. and Bacon, W. L. 2005. Changes in Egg Production Rate Induced by Progesterone Injection in Broiler Breeder Hens. *Poult. Sci.*, **84**: 321–327.
30. McLaughlin, E. and McIver, S. 2009. Awakening the Oocyte: Controlling Primordial Follicle Development. *Reprod.*, **137**: 1–11.
31. Mohammadi, H. and Ansari-Pirsaraei, Z. 2014. Changes in Some Blood Parameters and Production Performance of Old Laying Hens Due to Growth Hormone and Testosterone Injection. *J. Anim. Physiol. Anim. Nutr.*, **98**: 483–490.
32. Monniaux, D., Huet, C., Besnard, N., Clément, F., Bosc, M., Pisselet, C., Monget, P. and Mariana, J. 1997. Follicular Growth and Ovarian Dynamics in Mammals. *J. Reprod. Fertil. Suppl.*, **51**: 3–23.
33. Nagaraja, S. C., Aggrey, S. E., Yao, J., Zadworny, D., Fairfull, R. W. and Kuhnlein, U. 2000. Trait Association of a Genetic Marker Near the *IGF-1* Gene in Egg-laying Chickens. *J. Hered.*, **91**: 150–156.
34. National Research Council (NRC). 1994. *Nutrient Requirements of Poultry*. 9<sup>th</sup> Revision, National Academy Press, Washington, DC, 155 PP.
35. Niv-Spector, L., Raver, N., Fri Edman Einat, M., Grosclaude, J., Gussakovsky, E., Livnah, O. and Gertler, A. 2005. Mapping Leptin-interacting Sites in Recombinant Leptin-binding Domain (LBD) Subcloned from Chicken Leptin Receptor. *Biochem. J.*, **390**: 475–484.
36. Oades, R. D. and Messent, P. R. 1981. Testosterone Administration in Chicks Affects Responding in the Presence of Task Irrelevant Stimulus Changes. *Behav. Neural Biol.*, **33**: 93–100.
37. Oguike, M. A., Igboeli, G. and Ibe, S. N. 2006. Effect of Induced-moult on the Number Small Ovarian Follicles and Egg Production of Old Layers. *Int. J. Poult. Sci.*, **5**: 385–389.
38. Onagbesan, O., Bruggeman, V. and Decuypere, E. 2009. Intra-ovarian Growth Factors Regulating Ovarian Function in Avian Species: A Review. *Anim. Reprod. Sci.*, **111**: 121–140.
39. Palmer, S. S. and Bahr, J. M. 1992. Follicle Stimulating Hormone Increases Serum Oestradiol-17 Concentrations, Number of Growing Follicles and Yolk Deposition in Aging Hens (*Gallus gallus Domesticus*) with Decreased Egg Production. *Brit. Poult. Sci.*, **33**: 403–414.
40. Pierce, A. L., Fukada, H. and Dickhoff, W. W. 2005. Metabolic Hormones Modulate the Effect of Growth Hormone (GH) on Insulin-like Growth Factor-I (IGF-I) mRNA Level in Primary Culture of Salmon Hepatocytes. *J. Endocrinol.*, **184**: 341–349.
41. Rangel, P. L. and Gutierrez, C. G. 2014. Reproduction in Hens: Is Testosterone Necessary for the Ovulatory Process? *Gen. Comp. Endocrinol.*, **203**: 250–61. doi: 10.1016/j.ygcen.2014.03.040.
42. Reddy, I. J., David, C. G. and Raju, S. S. 2006. Inter Sequence Pause Days, Egg Production, Steroid and Luteinizing Hormone in Domestic Hen (*Gallus domesticus*) immunized against cProlactin. *Int. J. Poult. Sci.*, **5**: 420–427.
43. Renema, R. A., Robinson, F. E., Proudman, J. A., Newcombe, M. and Mckay, R. I. 1999. Effects of Body Weight and Feed Allocation



- during Sexual Maturation in Broiler Breeder Hens. 2. Ovarian Morphology and Plasma Hormone Profiles. *Poult. Sci.*, **78**: 629–639.
44. Sen, A., Prizant, H., Light, A., Biswas, A., Hayes, E., Lee, H. J., Barad, D., Gleicher, N., Hammes, S. R. 2014. Androgens Regulate Ovarian Follicular Development by Increasing Follicle Stimulating Hormone Receptor and MicroRNA-125b Expression. *In Proceedings of the National Academy of Sciences of the United States of America (Proc. Natl. Acad. Sci. USA)*, **111**(8): 3008–3013. doi: 10.1073/pnas.1318978111.
  45. Statistical Analysis System (SAS). 2001. *SAS/STAT User's Guide*. 8.2 Edition, SAS Institute Inc., Cary, NC, USA.
  46. Scanes, C., Sharp, P., Harvey, S., Godden, P., Chadwick, A. and Newcomer, W. 1979. Variations in Plasma Prolactin, Thyroid Hormones, Gonadal Steroids and Growth Hormone in Turkeys during the Induction of Egg Laying and Moulting by Different Photoperiods. *Bri. Poult. Sci.*, **20**: 143–148.
  47. Scanes, C. G. 2000. Introduction to Endocrinology: Pituitary Gland. In "*Sturkie's Avian Physiology*", (Ed.): Whittow, G. C.. Academic Press, London, **PP**. 437–460.
  48. Shen, X., Steyrer, E., Retzek, H., Sanders, E. J. and Schneider, W. J. 1993. Chicken Oocyte Growth: Receptor-mediated Yolk Deposition. *Cell Tissue Res.*, **272**: 459–471.
  49. Stephen, C. Y. I., Zhang, X. and Leung, F. C. 2001. Genomic Growth Hormone Gene Polymorphisms in Native Chinese Chickens. *Exper. Biol. Med.*, **226**: 458–462.
  50. Tanaka, M., Hayashida, Y., Sakaguchi, K., Ohkubo, T., Wakita, M., Hoshino, S. and Nakashima, K. 1996. Growth Hormone-Independent Expression of Insulin-like Growth Factor I Messenger Ribonucleic Acid in Extrahepatic Tissues of the Chicken. *Endocrinol.*, **137**: 30–34.
  51. Tomas, F. M., Pym, R. A., McMurtry, J. P. and Francis, G. L. 1998. Insulin-like Growth Factor (IGF)-I but Not IGF-II Promotes Lean Growth and Feed Efficiency in Broiler Chickens. *Gen. Comp. Endocrinol.*, **110**: 262–275.
  52. Tonetta, S. A. and diZerega, G. S. 1989. Intraovarian Regulation of Follicular Maturation. *Endocrine Rev.*, **10**: 205–229.
  53. Vendola, K., Zhou, J., Wang, J., Famuyiwa, O. A., Bievre, M. and Bondy, C. A. 1999. Androgens Promote Oocyte Insulin-like Growth Factor I Expression and Initiation of Follicle Development in the Primate Ovary. *Biol. Reprod.*, **61**: 353–357.
  54. Waddington, D., Perry, M. M., Gilbert, A. B. and Hardie, M. A. 1985. Follicular Growth and Atresia in the Ovaries of Hens (*Gallus domesticus*) with Diminished Egg Production Rates. *J. Reprod. Fertil.*, **74**: 399–405.
  55. Walters, K. A., Allan, C. M. and Handelsman, D. J. 2008. Androgen Actions and the Ovary. *Biol. Reprod.*, **78**: 380–389.
  56. Williams, J., Sharp, P. J. and Goddard, C. 1992. The Effects of Growth Hormone on Ovarian Follicular Growth in Domestic Hen. *J. Reprod. Fertil.*, (Abstr.) Series No. 9-10: 103.
  57. Williams, J. B. and Sharp, P. J. 1978. Ovarian Morphology and Rates of Ovarian Follicular Development in Laying Broiler Breeder and Commercial Egg-production Hens. *Brit. Poult. Sci.*, **19**: 387–396.
  58. Yang, M. Y. and Fortune, J. E. 2006. Testosterone Stimulates the Primary to Secondary Follicle Transition in Bovine Follicles *In vitro*. *Biol. Reprod.*, **75**: 924–932.
  59. Yoshimura, Y., Chang, C., Okamoto, T. and Tamura, T. 1993. Immunolocalization of Androgen Receptor in the Small, Preovulatory, and Post Ovulatory Follicles of Laying Hens. *Gen. Comp. Endocrinol.*, **91**: 81–89.
  60. Yoshimura, Y., Tischkau, S. A. and Bahr, J. M. 1994. Destruction of the Germinal Disc Region of an Immature Preovulatory Follicle Suppresses Follicular Maturation and Ovulation. *Biol. Reprod.*, **51**: 229–233.
  61. Zou, X. T., Xu, Z. R., Zhu, J. L., Fang, X. J. and Jiang, J. F. 2007. Effects of Dietary Dihydropyridine Supplementation on Laying Performance and Fat Metabolism of Laying Hens. *Asian-Aust. J. Anim. Sci.*, **20**: 1606–1611.

## قطر فولیکولی، وزن تخم مرغ و عملکرد مرغ های تخمگذار مسن تزریق شده با هورمون رشد و تستوسترون

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### چکیده

همراه با افزایش سن مرغ صفات تولیدی، از جمله تولید تخم مرغ، کاهش می یابند. آزمایش حاضر، به صورت یک طرح کاملاً تصادفی با ۴ تیمار و ۴ تکرار و ۱۰ مرغ تخمگذار در هر تکرار، به منظور تعیین اثر تزریق هورمون رشد و تستوسترون بر قطر فولیکولی، وزن تخم مرغ و عملکرد مرغ های تخمگذار مسن در اواخر دوره تولید انجام شد. ۱۶۰ مرغ تخمگذار تجاری از نژاد های لاین W-36 در سن ۷۳ هفتگی، به صورت زیر تحت یک بار تزریق قرار گرفتند: تیمار یک (شاهد): تزریق ۱ میلی لیتر آب مقطر، تیمار دو: تزریق ۵۰۰ میکروگرم تستوسترون و ۵۰ میکروگرم هورمون رشد به ازای هر کیلوگرم وزن بدن، تیمار سه: تزریق ۵۰۰ میکروگرم تستوسترون و ۱۰۰ میکروگرم هورمون رشد به ازای هر کیلوگرم وزن بدن و تیمار چهار: تزریق ۵۰۰ میکروگرم تستوسترون و ۱۵۰ میکروگرم هورمون رشد به ازای هر کیلوگرم وزن بدن. در زمان دو هفته بعد از تزریق، قطر فولیکول های سفید کوچک، سفید بزرگ،  $F_1$ ،  $F_2$  و  $F_3$  در تیمار سه به طور معنی داری از گروه کنترل بیشتر بود. درصد تولید تخم مرغ (روز مرغ)، تولید توده ای تخم مرغ و میزان مصرف خوراک در دو هفته بعد از تزریق، افزایش معنی داری در تیمار سه نسبت به سایر گروه ها نشان دادند. همچنین درصد تولید تخم مرغ (روز مرغ) و میزان مصرف خوراک در این زمان در تیمار چهار به طور معنی داری از گروه کنترل بیشتر بود. نتایج حاصل از این آزمایش نشان داد که تزریق هورمون رشد و تستوسترون می تواند اثر مثبتی بر قطر فولیکولی و عملکرد تولید مرغ های تخمگذار مسن داشته باشد.