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Ovary and Liver *IGF-I, IGF-IR* and *IGFBP-II* Gene Expressions and Reproduction Performance of Old Laying Hens Injected with Growth Hormone and Testosterone

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ABSTRACT

This study investigated how exogenous hormones change ovary and liver *IGF-I, IGF-IR* and *IGFBP-II* gene expression and egg production performance of 160 laying hens (HyLine W-36), at 73rd week of age when subcutaneously injected with different doses of Growth Hormone (GH) and Testosterone (Ts) as follows: Treatment 1 (Tr 1): 100 µL distilled water (control group); Treatment 2 (Tr 2): Ts 500 µg kg^{-1}+GH 50 µg kg^{-1} live weight; Treatment 3 (Tr 3): Ts 500 µg kg^{-1}+GH 100 µg kg^{-1} live weight, and Treatment 4 (Tr 4): Ts 500 µg kg^{-1}+GH 150 µg kg^{-1} live weight. The birds were randomly assigned to the four experimental groups with four replicates of 10 birds each, in a completely randomized design. Fifth-largest-yellow follicle (F_5) and liver samples were taken just 8 hours after hormone injection. Production performance was measured during fourth and fifth weeks after hormone injection. Increase in liver IGF-I mRNA in Tr4 was significantly higher than that in Tr 3 and the control group. The liver *IGF-IR* gene expressions in all hormone-injected hens were significantly higher than that in the control group. The liver *IGFBP-II* mRNAs were significantly higher in Tr 2 and 4 compared with the control group and Tr 3. The expressions of IGF-I and *IGF-IR* mRNAs in F_5 wall of the layers in the control group, Tr 2 and 3, and F_5 *IGFBP-II* mRNA in Tr 3 were significantly increased compared to the other groups. Ovulation rate in Tr 3 was significantly higher than other groups. Besides, egg mass of Tr 2 and 3 was significantly more than the control group. Feed intake of Tr 3 significantly differed from the other groups. Treatment 4 had significantly higher feed conversion ratio compared with Tr 2. In conclusion, the results show the positive effects of the exogenous Ts and GH through IGF system on reproduction performance in old laying hen.

Keywords: Growth factors, Egg production, Hormone injection, Insulin-like Physiologically manipulated spent laying hen**.**

INTRODUCTION

There are lots of evidences of reproductive consequences of aging which emphasize impaired reproductive function amongst women, laboratory rodents, and hen. Aging also affects ovarian functions, and results in the initial decline in reproductive hormones and gradually leads to menopause or anestrus state (Christians and Williams, 1999). Moreover, this situation is further aggravated during the second egg production cycle of hen (Yasmeen *et al.,* 2008). For many years, the difference in ovulation rate was supposed to be related to the differences in the plasma levels of reproductive hormones such as gonadotropins (LH, FSH), progesterone and inhibins. However, the mechanisms involved in this reduction are not fully understood (Christians and Williams, 1999). It is

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suggested that the Insulin-like Growth Factors (IGF-I and -II) probably act as a local mediator for gonadotropins or GH regulatory effects on ovary. Taken together, these observations suggest that disrupted GH/IGF axis will result in poor reproductive performance (Onagbesan *et al.,* 1999). From several investigations, it can be concluded that IGF-I has a wide variety of production tissues and target cells; and in addition to the endocrine effects, it can exert autocrine and/or paracrine actions in the target tissues (Thissen *et al.,* 1994; McFarland, 1998; Fu *et al.,* 2001; Zhengwei *et al.,* 2001).

It is well known that liver-derived IGF-I exerts its endocrine action in the target tissues through the endocrine and auto/paracrine systems (Fu *et al.,* 2001; Zhengwei *et al.,* 2001; Yun *et al.,* 2005). The findings about the IGF system functions in bird's reproductive system are not as abundant as in mammals (Onagbesan *et al.,* 1999). Periodical and circadian changes occur during the reproduction cycle of female fowls which are influenced by autocrine, paracrine and endocrine actions of IGF-I and -II (Fu *et al.,* 2001). Several *in-vivo* investigations have observed effects of the gonadotropins (LH and FSH), GH and IGF injection on mammalian or avian reproductive system. These observations have led to the conclusion that IGFs have critical roles in reproductive events (Armstrong *et al.,* 1996; Ansari-Pirsaraei 2009). Measurement of *IGF-I, IGF-IR*, *Insulin Receptor (IR)* and *IGFBP-II* genes in the Japanese quail liver, testis and oviduct (using a lysate RNase protection assay) at different ages has shown that these genes were developmentally regulated in the oviduct and dramatic growth of oviduct was observed to coincide with a significant increase in the *IGF-I* gene expression. A similar expression pattern was observed in the IGF-I, IGF-IR, IGFBP-II and IR mRNAs in the oviduct (Fu *et al.,* 2001).

Altogether, the findings show that IGFs and IGFBPs exert endocrine and auto/paracrine effects on ovary under control of gonadotropins (LH and FSH) (Roberts *et al.,* 1994). These data and other studies imply that IGFs potentiate the action of gonadotropins on granulosa and theca interstitial cells *in-vitro* (Armstrong *et al.,* 1996). There is a coincidence between puberty and sexual development period and IGF-I mRNA in the oviduct of Japanese quail (Scatchard, 1949; Fu *et al.,* 2001). The aim of this study was to test the hypothesis that exogenous Ts and GH would influence ovary and liver *IGF-I, IGF-IR* and *IGFBP-II* gene expression and reproduction performance in spent laying hens. It is notable that the current experiment was a pure physiological study and the authors are well aware of the risks of drug and hormone residuals and they do not suggest the use of exogenous hormones in poultry farms.

MATERIALS AND METHODS

Experimental Design, Birds, Diets and Management

In this experiment, a total of 160 commercial (HyLine W-36) Single Comb White Leghorn hens at 73 weeks of age were used. The birds were weighed individually and randomly distributed in a completely randomized experimental design with four treatments of four replicates of 10 birds each. The birds were located in laying cages in groups of 10 in an environmentally controlled house. Environmental condition and feed program were according to White Leghorn hen breeding recommendations and were provided equally as far as possible to minimize environmental effects. To prevent the experimental birds from eating the feed of adjacent replicates, one cage in each replicate remained empty. Birds were housed under those conditions for two weeks prior to the beginning of the experiment to allow acclimation. Every day of the experimental period, all birds were checked visually for their health and welfare. Mortality rate throughout the experimental period was very low (only one), and that data were not subjected to analysis.

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The Hormones, Treatments and Injections

Eutropin™ (Recombinant human somatropin™, LG Life Sciences Company, Korea) and Androne® (Testosteron Enanthate, Caspian Tamin Pharmaceutical Company, Iran) were used in this experiment. The birds were single injected subcutaneously at the base of the neck with Ts and GH at live-weight-dependent dosages as follows: Treatment1 (Tr 1): 100 μL distilled water (control group); Treatment 2 (Tr 2): Ts 500 μg kg^{-1}+GH 50 μg kg^{-1} live weight; Treatment 3 (Tr 3): Ts 500 μg kg^{-1}+ GH 100 μg kg^{-1} live weight, and Treatment 4 (Tr 4): Ts 500 μg kg^{-1}+GH 150 μg kg^{-1} live weight. Neutral oil and distilled water, which do not exert effects on the hen's body, were used as vehicles for Ts and GH injection, respectively. As there was no necessity to inject the control group with both neutral oil and water, the birds were injected with water only, to exert any mechanical effects the injection might have (Ansari-Pirsaraei, 2009).

Slaughtering, Dissection and Tissue Collection

Three birds from each replicate unit were randomly selected and slaughtered by decapitation just eight hours after the hormone injection (n= 48). Immediately after the slaughtering, whole ovary and the accompanying follicular hierarchy and liver were removed. If there was oviducal egg, it was removed, weighted, and considered as one laid egg in that day.

Granulosa Cell Layer Isolation and Liver Samples

Since gonadotropins and growth factors exert their maximum effects on the Fifth-largest-yellow Follicle (F5), rather than First, Second, Third and Fourth-largest-yellow Follicle (F1-F4), we selected the F5 to assess the gene expression (Ansari-Pirsaraei, 2009). The fifth-largest-yellow follicle was detached from the ovary, a slit along the stigma was made to split it from the yolk, rinsed several times in distilled water to remove any adhering yolk, drained of excess water, then, the follicular wall and liver sample were immediately frozen in liquid nitrogen and stored at −70°C until used for total RNA extraction and the IGF-I, IGF-IR, IGFBP-II relative gene expression measurement (Alaw-Qotbi *et al.,* 2012).

Gene Expression Measurement

Total RNA Extraction (RNA Isolation)

All steps of the RNA isolation were performed according to the manufacture recommendations (Metabion® Company Kit).

The RNA Loading

Purified RNA was loaded on 2% agarose gel. Two different bands were detected. One of them was the most sensitive kind of RNA (rRNA) and the second one was the target mRNA which was used for reverse transcription PCR reaction.

Converting RNA to cDNA (Reverse Transcription PCR)

Since mRNA may form secondary structures and dimers which influence the reverse transcriptase enzyme ability to change mRNA to cDNA, this may reduce the RT-PCR method accuracy in various types of mRNA detection (Shimomayea and Salvato, 1989). The RNA reverse-transcription may be performed using specific primers, random hexamers and Oligo-dT primers (a short sequence of deoxy-thymine nucleotides). Messenger-RNA specific primers reduce background priming. Primers may affect the accuracy of the calculated target mRNA copy number.

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Random hexamers may result in approximately 19-fold increase in the calculated mRNA copy numbers when compared to specific downstream primers (Zhang and Byrne, 1999). QuantiTect® Reverse-Transcription commercial kit (QIAGEN Company) was used to generate cDNA (Cat. No. 205311, Lot No. 127145908). Produced cDNA was dissolved in sterilized and distilled water and frozen in liquid nitrogen and stored at −80°C until used for Real-Time PCR reaction.

Real-Time PCR Reaction (RT PCR)

Real-Time PCR reaction was performed employing QuantiFast™ SYBR® Green PCR commercial kit (QIAGEN Company). Specific primers produced according to National Centre for Biotechnology Information (NCBI). To optimize usage of the kit, the Real-Time PCR reactions were performed on the basis of 15 µL. The relative gene expression levels were detected by normalization with the level of *β*-actin.

Laying Performance

Ovulation Rate (Hen-Day Egg Production, HDEP), Egg Mass, and Egg Weight**:** Daily egg production was recorded throughout the experiment. Overall ovulation rate (HDEP) was calculated (North, 1984) during two weeks prior to hormone injection and over a period of five weeks after the hormone injection. The eggs were collected and weighed (to within±0.01 g) individually during 2 weeks prior to hormone injection and over a period of 5 weeks after the hormone injection. Subsequently, egg production percentage and egg weight values were used to calculate egg mass (EL-Husseiny *et al.,* 2008).

Feed Intake, Feed Conversion Ratio, and Mortality**:** Mortality was recorded daily and analyzed weekly throughout the experimental period. Mortality data were also considered in HDEP, feed intake, feed conversion calculations and every parameter in which mortality was involved. Weekly feed intake was recorded and feed conversion ratio was calculated.

Statistical Analysis

All data were analyzed using General Linear Model (GLM) of SAS (SAS, 2001) and the Real-time PCR software. Differences among means were separated with Duncan multiple range test. Comparative threshold Cycle Method (CT) was used to analyze the Real-time PCR raw data. Live Weights (LW) was used as covariate in the statistical analysis of egg weight. The normality of distribution was tested by using the Kolmogorov Smirnov test (Alaw-Qotbi *et al.,* 2012).

RESULTS

Gene Expressions

The effects of the hormone injection on the relative gene expression levels in the liver and F_5 are shown in Table 1. Increase in IGF-I mRNA in the liver of the birds in Tr 4 was higher than Tr 3 and the control group ($P< 0.05$). The difference between Tr 2 and 4 was not significant. The *IGF-IR* gene expression levels in the liver of all the hormone-injected hens (Tr 2, 3 and 4) were higher than the control group ($P< 0.05$). The IGFBP-II mRNA level in the liver was higher in Tr 2 and Tr 4 compared with the control group and Tr 3 ($P< 0.05$). The expressions of IGF-I and IGF-IR mRNAs in F_5 wall of the layers in Tr 4 was lower compared to the control group, Tr 2 and 3 ($P< 0.05$). Follicle IGFBP-II mRNA in F_5 were increased in Tr 3 compared to the control group, Tr 2, and 4 ($P< 0.05$). Significant difference was also observed between the control group and Tr 4.

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Table 1. Effects of the hormone injection on the gene expression levels in the liver and F_5.

	Liver			F_5		
Treatments	IGF-I	IGF-IR	IGFBP-II	IGF-I	IGF-IR	IGFBP-II
Treatment 1 (Ctrl) [a]	1.029±0.27[b]	1.035±0.29[b]	1.134±0.48[b]	1.034±0.29[a]	1.037±0.31[a]	1.122±0.63[b]
Treatment 2 [b]	2.986±1.49[ab]	4.074±0.62[a]	3.431±1.39[a]	0.784±0.41[a]	0.863±0.18[a]	0.789±0.13[bc]
Treatment 3 [c]	1.395±1.10[b]	4.133±1.08[a]	0.495±0.33[b]	1.017±0.27[a]	1.490±0.63[a]	2.276±0.80[a]
Treatment 4 [d]	5.714±0.34[a]	6.141±1.57[a]	3.388±0.70[a]	0.354±0.21[b]	0.374±0.10[b]	0.417±0.18[c]
SD [e]	1.28	2.12	1.72	0.786	0.931	1.021

[a] Injection of 100 μL distilled water (control group); [b] Ts 500 μg kg^{-1}+ GH 50 μg kg^{-1} live weight; [c] Ts 500 μg kg^{-1}+ GH 100 μg kg^{-1} live weight, and [d] Ts 500 μg kg^{-1}+GH 150 μg kg^{-1} live weight. [e] Standard Deviation. Values in the same row having different superscripts are significantly different (P< 0.05). Values are expressed as mean±SEM (Standard Error of Mean).

Reproduction Performance

The effects of the hormone injection on the egg production performance are shown in Table 2. Ovulation rate in Tr 3 was higher compared to all other groups (P< 0.05); besides, it was higher in Tr 4 than the control group (P< 0.05). Egg mass in Tr 2 and 3 were more than the control group (P< 0.05). Feed intake in Tr 3 differed compared to all other groups (P< 0.05); besides, it was higher in Tr 2 compared to the control group and Tr 4 (P< 0.05). Treatment 4 caused higher FCR compared with Tr 2 (P< 0.05). Egg weight did not differ (P> 0.05) between groups.

DISCUSSION

Gene Expressions

There are contradictory reports about *IGF* gene expression sites in birds. Researches in 1994, using RT-PCR, reported for the first time the *IGF-I* gene expression detected in bird granulosa cells. In another report, the *IGF-I* and *-II*, *IGF-IR*, *IGFBP-II* and *-V* gene expression in both granulosa and theca cells of the chicken ovary was observed (Onagbesan *et al.,* 1999). In catabolic states, hepatocytes are resistant to GH, consequently, IGF-I production is decreased by them (Pierce *et al.,* 2005). Growth

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

Treatments	Ovulation rate (HDEP) (%)	Egg mass (g of egg/hen/d)	Feed intake (g/bird/day)	FCR (g of feed/g of egg)	Egg weight
Treatment 1 (Ctrl) [a]	56.51±2.21[c]	36.63±1.78[b]	88.22±7.25[c]	2.391±0.545[a]	64.27±1.21
Treatment 2 [b]	57.72±2.72[bc]	40.75±1.99[a]	96.23±6.89[b]	2.373±0.342[b]	64.49±1.71
Treatment 3 [c]	67.57±2.43[a]	44.34±1.89[a]	105.25±8.54[a]	2.385±0.269[ab]	64.54±1.92
Treatment 4 [d]	59.27±3.05[b]	38.18±1.85[ab]	91.97±7.74[c]	2.404±0.352[a]	63.16±2.00
SD [e]	0.452	0.201	0.624	0.201	0.765

[a] Injection of 100 μL distilled water (control group); [b] Ts 500 μg kg^{-1}+ GH 50 μg kg^{-1} live weight; [c] Ts 500 μg kg^{-1}+ GH 100 μg kg^{-1} live weight, and [d] Ts 500 μg kg^{-1}+GH 150 μg kg^{-1} live weight. [e] Standard Deviation. Values in the same row having different superscripts are significantly different (P< 0.05). Values are expressed as mean±SEM (Standard Error of Mean). Ovulation rate (Hen-day egg production) = (Total number of eggs/Number of live layers)×100; Egg mass= [Egg production (%)×Egg weight (g)]/100, Feed conversion ratio= Feed intake/Egg mass.

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hormone plays a dual role in IGF-I synthesis and secretion by granulosa cells of hen (Ansari-Pirsaraei, 2009). Reduced synthesis and secretion levels of liver IGF in hypophysectomized rats can be restored to normal level by administration of GH (McFarland, 1998). Decrease in IGF-I concentration and gene expression as a result of GH injection was reported by Alaw-Qotbi *et al.* (2012), but the reason for that observation was not mentioned. Decrease in the gene expression in the current study may be attributed to the same unknown reason. It is demonstrated that LH and GH increased the production rates of IGF-I and -II by F_1 of chicken (Onagbesan *et al.,* 1999). On the other hand, currently available evidence point that both hepatic and ovarian derived IGF-I mediate effects of GH on avian folliculogenesis (Izadyar *et al.,* 1997; Bachelot *et al.,* 2002), which is in agreement with reports on the presence of high-affinity GH-binding sites, which are regulated in a tissue-specific manner in both granulosa and theca layers of hen preovulatory follicles (Lebedeva *et al.,* 2004). Additionally, either hepatic or local production of IGF-I can directly or indirectly influence GH actions on follicles. The impact of GH on gene expression of IGFs has been studied many times, whereas there are indeed few articles concerning Ts and/or GH' impact on IGFs and IGF-Rs in layer hens (Ansari-Pirsaraei, 2009). *In-vitro* experiments have shown that IGF-I is synthesized and secreted by hen granulosa cells under the influence of stimulatory effects of GH (Lebedeva *et al.,* 2004). Furthermore, increased IGF-I level has been observed in GH treated cultured chicken hepatocytes (Scanes, 2000). No significant difference was observed in plasma concentrations of total IGF-I between the three stages of the domestic hen ovulation cycle (Duclos *et al.,* 2000). There is one report on investigation of the effects of Ts and/or GH injection on IGF system of laying hens (Alaw-Qotbi *et al.,* 2012).

Insulin receptor, IGF-IR and IGF-IIR are a family of cell-surface receptors which play important roles in observed IGF effects (Feng *et al.,* 1997; Fu *et al.,* 2001). The biological actions of IGF-I are mediated mainly through the IGF-IR, and partly through the IR (Onagbesan *et al.,* 1999; Fu *et al.,* 2001; Zhengwei *et al.,* 2001). This means that physiological actions of IGF-I and –II appeared through binding IGF-I receptors. However, for IGF-I and -II bind IGF-I receptors, there is a report suggesting that affinity of IGF-I to IGF-I receptors is more than that in IGF-II (Ansari-Pirsaraei 2009). It has been shown that *IGF-IR* gene is expressed in both granulosa and theca cells of the chicken ovary (Roberts *et al.,* 1994; Armstrong and Hogg 1996; Kim *et al.,* 2004). Continuous (2.5 to 10 days) administration of recombinant chicken GH increases the circulating concentrations of IGF-I and different variant of IGFBPs (22, 28 and 36-kDa IGFBP) (Scanes *et al.,* 1999). High and low levels of IGFBPs were detected in both granulosa and theca layers of the three largest follicles of domestic hen, respectively. The findings suggest that chicken ovary produces IGFBPs in order to control the local production of IGFs (Duclos *et al.,* 1998; Duclos *et al.,* 2000). Several experiments, using RT-PCR technique, indicated that *type II* and *V binding protein* genes are expressed in both granulosa and theca cells of the chicken ovary (Allander *et al.,* 1995; Schoen *et al.,* 1995; Duclos *et al.,* 1998), which play definite roles in biological activity of IGFs, including steroidogenesis and follicular development (Shimasaki and Ling, 1991; Onagbesan *et al.,* 1999; Matsumoto *et al.,* 2000). IGFBPs may play important roles in regulating cellular responses to IGFs (Onagbesan *et al.,* 1999). IGFBPs are key modulators of the biological actions of IGF; on the other hand, the biological actions of IGF are largely dependent on binding protein interactions (Murtry 1998; Fu *et al.,* 2001; Zhengwei *et al.,* 2001). It is also suggested that IGF receptor and IGFBP concentrations play more important role in biological functions of IGF-I compared to variations in the total plasma concentration of IGF-I (Duclos *et*

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al., 2000). The results found in the present study were expected and are in line with assumed GH functions on liver and granulosa cells.

Reproduction Performance

Synthesis of progesterone and estrogen by granulosa cells is augmented by IGF-I action on proliferation of granulosa cells and amplifying effects of follicle stimulating hormone on the differentiation of granulosa cells (Bradford, 1976; Gilbert *et al.,* 1977; Fu *et al.,* 2001). It is assumed that during follicle development, IGF-I makes the granulosa cells more sensitive to FSH and LH and subsequently producing more progesterone (Onagbesan and Peddie 1995; Onagbesan *et al.,* 1999). Both IGF-I and -II increase progesterone and androstenedione production by *in-vitro*-cultured granulosa and theca cells, respectively (Onagbesan *et al.,* 1994; Williams *et al.,* 1994; Onagbesan and Peddie 1995; Roberts and Gordon 1995).

Evidence is accumulating to show that both hepatic- and ovarian-derived IGF-I probably act as a mediator in the biological action of GH on avian folliculogenesis (Izadyar *et al.,* 1997; Bachelot *et al.,* 2002; Lebedeva *et al.,* 2004). *In-vitro* experiments have indicated that IGFs play definite and critical roles in follicle development and ovary proper function in chicken (Onagbesan *et al.,* 1999). For the first time, applying Ts to stimulate ovulation was reported in 1955 and 1961 (Fraps, 1955; Tienhoven, 1961). After that, the investigations on the effects of Ts and/or GH injection on egg production rate, egg characteristics, and follicular size of laying hens in 2008 and 2009 (Ansari-Pirsaraei *et al.,* 2008; Ansari-Pirsaraei, 2009) led to the conclusion that GH and Ts, through IGF system, may affect egg production rate (Ansari-Pirsaraei *et al.,* 2008). Endocrine factors, such as steroid hormones and growth factors, regulate egg productivity in terms of the quantity of egg produced, egg weight, sexual maturity, and the number of Small Yellow Follicles (SYF) which are the most important economic traits in laying hens (Kim *et al.,* 2004). There are some reports suggesting the increase of follicle numbers in layers as a result of gonadotropins or GH injection (Johnson *et al.,* 1985; Williams *et al.,* 1992). Injection of IGF-I to sex-linked dwarf chickens, which lacked *GH receptor* gene, caused more efficient reproductive performance (Ansari-Pirsaraei 2009).

It has been elucidated that E2 and P4 exert regulatory effects on follicular development and have a positive association with egg production (Johnson and Tienhoven, 1981; Bluhm *et al.,* 1983; Kim *et al.,* 2004). On the other hand, IGF has been detected as a stimulant of P4 production in granulosa cells (Williams *et al.,* 1994; Onagbesan and Peddie 1995; Kim *et al.,* 2004) where association between IGF-I genotype and number of SYF has been observed. It is also documented that high expression of IGF-II in ovary stimulates the development of follicles (Kim *et al.,* 2004). Available data suggest that IGFs regulate egg and steroid hormone production and control ovarian function in the avian species via complex mechanisms and involve interactions with the gonadotropins (LH and FSH), GH, and maybe other growth factors (Onagbesan *et al.,* 1999). The results of several findings have shown that IGFs play definitive roles, as autocrine and/or paracrine mediators, during chicken follicle development, like they have been observed in mammals (Krishnan *et al.,* 1989; Onagbesan *et al.,* 1999; Fu *et al.,* 2001). Furthermore, it is suggested that IGFs play roles in mechanisms responsible for follicle selection to enter the ovulatory hierarchy. Increased diameter of hierarchical follicles in GH, Ts or GH plus Ts injected hens has been observed and this effect of GH and Ts is attributed to increase in growth factors especially IGF in the hierarchical follicles (Ansari-Pirsaraei *et al.,* 2008). Regulation of other growth factors is also under control of IGFs (Onagbesan *et al.,* 1999). The positive effect of GH injection, through IGF systems, on yolk production in liver and also on other growth factors in small follicles was reported (Ansari-Pirsaraei *et al.,* 2008).

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The relationships among IGF-I genotypes, endocrine factors, and egg productivity of chickens have been revealed (Kim *et al.,* 2004). It is also elucidated that the *GH* and *GH Receptor* (GHR) genes are associated with changes in the rate of egg production (Hockinga *et al.,* 1994; Kuhnlein *et al.,* 1997; Nagaraja *et al.,* 2000), and Double Yolk Egg (DYE) production (Hockinga *et al.,* 1994). Besides, the significant effect of IGF-I genotype on egg weight and specific gravity was demonstrated by Nagaraja *et al.* (2000). It has been shown that changes in the GH/IGF-I axis in *ad libitum* and restricted fed broiler can influence egg production rate (Hockinga *et al.,* 1994; Bruggeman *et al.,* 1997; Lebedeva *et al.,* 2004; Ansari-Pirsaraei *et al.,* 2008). It is assumed that IGF-I and -II exert those specific actions. With respect to the follicular development and steroidogenesis, it has been suggested that IGFs act as intra-ovarian regulators which control growth and differentiation of preovulatory follicles (Ansari-Pirsaraei, 2009). Insulin-like growth factor, Epidermal Growth Factor (EGF) and Bone Morphogenetic Proteins (BMP) regulate responsiveness of ovary to gonadotropins and mediated gonadotropin functions on follicle development and ovulation rate (Onagbesan *et al.,* 2006). The positive effects of Ts, GH and GH plus Ts on follicular size of laying hens are attributed to elevated growth factors, especially IGFs (Ansari-Pirsaraei *et al.,* 2008). Local production of IGF-I in ovary (Ansari-Pirsaraei, 2009), and liver production of yolk precursors (Ansari-Pirsaraei *et al.,* 2008) under the influence of GH may lead to increase in follicular growth.

GH has been introduced as a multifunctional hormone and may influence growth, body composition and appetite regulation (Byatt *et al.,* 1993). The chickens which were treated with antithyroid compound (propylthiouracil-PTU) showed lower levels of *GH receptor* and *IGF-I* gene expressions in liver (Akhlaghi *et al.,* 2009). The positive effects of GH on adrenal gland, T_4 and corticosterone has been demonstrated (Mohammadi and Ansari-Pirsaraei, 2014; Scanes, 2000; Ansari-Pirsaraei, 2009). T_4, in turn, increases leptin (Zou *et al.,* 2007), which is known as an appetite increasing hormone (Niv-Spector *et al.,* 2005). With respect to this evidence, increase in FI amounts in the hormone-received hens was anticipated. In such investigations, measurement of blood leptin concentration may help. The association between the *GH* and *GHR* genes and changes in body weight has been shown (Feng *et al.,* 1998). IGF-I is mainly produced by liver and it is assumed as a significant regulator of GH/IGF axis, which is the endocrine system regulating body growth in vertebrates (Pierce *et al.,* 2005).

Insulin-like growth factor-I gene expression in chicken liver has been found to be GH dependent after hatching, whereas in other tissues (except for testis) it is independent of GH and GHR before and after hatching (Tanaka *et al.,* 1996). Insulin-like growth factor-I is mainly produced by liver under the control of pituitary GH (Fu *et al.,* 2001; Zhengwei *et al.,* 2001; Pierce *et al.,* 2005). Growth hormone may increase synthesis and secretion of IGF-I by hen granulosa cells (Mohammadi and Ansari-Pirsaraei, 2014; Ansari-Pirsaraei 2009). In another experiment, it was shown that IGF-I administration may increase protein breakdown in the broilers. Besides, body growth regulation and food utilization efficiency has been assumed to fall under IGF-I control (Mohammadi and Ansari-Pirsaraei, 2014; Tomas *et al.,* 1998). In our previous work, the hormone injection significantly increased FCR during the first week after the injection (Mohammadi and Ansari-Pirsaraei, 2014). In contrast, in the present study, Tr 2 showed significantly lower FCR figures compared with the control group and Tr 4. With respect to the GH functions the results obtained in the present study were anticipated.

In this study, we noticed that hormonal manipulation of old laying hens may improve at least some egg quality and production performance parameters. Therefore, our findings suggest that reproductive hormone concentrations would be considered as selection indices in animal selection and breeding plans, in order to provide a more

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accurate estimator and predictor of future reproduction performance of commercial layers and broiler breeders. As previously stated, with regard to the risk of drug and hormone residuals, the authors strongly avoid recommending the use of exogenous hormones at farm level.

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تاثیر تزریق تستوسترون و هورمون رشد بر بیان ژن های *IGF-I*، *IGF-IR* و *IGFBP-II* در تخمدان و کبد مرغ تخمگذار و عملکرد تولید مثلی آن

ح. محمدی، و ز. انصاری پیرسرائی

چکیده

به منظور تعیین اثر تزریق تستوسترون و هورمون رشد بر بیان ژن های *IGF-I*، *IGF-IR* و *IGFBP-II* در تخمدان و کبد مرغ تخمگذار و عملکرد تولید مثلی آن در اواخر دوره تولید آزمایشی به صورت یک طرح کاملاً تصادفی با ۴ تیمار و ۴ تکرار و ۱۰ مرغ تخمگذار تجاری از نژاد های‌لاین W-36 در هر تکرار در سن ۷۳ هفتگی (اواخر دوره تولید) اجرا شد. تیمارهای آزمایشی به شرح زیر بودند: تیمار اول (شاهد): تزریق ۱ میلی‌لیتر آب مقطر، تیمار دوم: تزریق ۵۰۰ میکروگرم تستوسترون و ۵۰ میکروگرم هورمون رشد به ازای هر کیلوگرم وزن بدن، تیمار سوم: تزریق ۵۰۰ میکروگرم تستوسترون و ۱۰۰ میکروگرم هورمون رشد به ازای هر کیلوگرم وزن بدن و تیمار چهارم: تزریق ۵۰۰ میکروگرم تستوسترون و ۱۵۰ میکروگرم هورمون رشد به ازای هر کیلوگرم وزن بدن. نمونه برداری از پنجمین فولیکول زرد بزرگ (F5) و کبد درست ۸ ساعت پس از تزریق هورمون انجام شد. طی هفته های چهارم و پنجم بعد از تزریق، شاخص های عملکرد تولید مثلی محاسبه شدند. بیان ژن *IGF-I* در کبد پرنده های تیمار ۴ افزایش معنی داری نسبت به گروه شاهد و تیمار ۳ داشت. بیان ژن *IGF-IR* در کبد، در تمام گروه هایی که هورمون دریافت کرده بودند به طور

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معنی داری نسبت به گروه شاهد افزایش داشت. بیان ژن *IGFBP-II* در کبد در هر دو تیمار ۲ و ۴ نسبت به گروه شاهد و تیمار ۳ افزایش معنی داری نشان داد. بیان ژن های *IGF-I* و *IGF-IR* در فولیکول (F5) در گروه شاهد، تیمار ۲ و ۳ و بیان ژن *IGFBP-II* در این فولیکول در تیمار ۳ نسبت به گروه شاهد افزایش معنی داری نشان داد. نرخ تخمگذاری در تیمار ۳ نسبت به گروه شاهد و تیمارهای ۲ و ۴ افزایش معنی داری نشان دادند. تولید توده‌ای تخم مرغ در تیمار های ۲ و ۳ و میزان مصرف خوراک در تیمار ۳ نسبت به گروه شاهد افزایش معنی داری داشت. تیمار ۴ نیز به طور معنی داری ضریب تبدیل خوراک بالاتری نسبت به تیمار ۲ داشت. نتایج حاصل از این آزمایش نشان داد که تزریق تستوسترون و هورمون رشد، از طریق سیستم *IGF* می تواند اثرات مثبتی بر عملکرد مرغ تخمگذار در اواخر دوره تولید اعمال کند.

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