Association among Egg Productivity, Granulosa Layer IGF-I, and Ovarian IGF-I in Korean Native Ogol Chicken

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ABSTRACT : There exists considerable evidence that insulin-like growth factor-I (IGF-I) is involved in the regulation of ovulation rate and follicle development. IGF-I is believed to modulate the effects of gonadotropins on follicular growth and cell differentiation via paracrine and autocrine mechanisms. Therefore, this study was performed to relate the expression of IGF-I on ovaries and follicles with egg productivity at 60 wk. The egg productivity of 70 KNOC was recorded from 20 to 60 wk. Blood was taken every 10 wk and ovaries and follicles were taken at 60 wk. Serum IGF-I and IGF-I of ovaries and follicles were measured by radioimmunoassay. Based on egg production levels up to 60 wk and ovarian IGF-I expression at 60 wk, respectively. Chickens were divided into two groups, high and low. Egg production and serum IGF-I in the high IGF-I group were higher than those in the low group. Moreover, the IGF-I expression of follicles in the high ovarian IGF-I expression group was higher than that in the low group. These finding are consistent with the report that IGF-I indirectly regulates ovulation in chickens, suggesting that this regulation may play an important role in improved egg productivity. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 3 : 325-330)***

Key Words : IGF-I, Egg productivity, Ovary, Follicle, KNOC

INTRODUCTION

Korean Native Ogol Chicken (KNOC), a protected species under the Korean government's Protected Species Act No. 265, is a dual purpose (egg and meat) chicken with low egg productivity. Few studies on the relationships between these characteristics and endocrine factors in KNOC have been reported. Although some progress had been made in understanding the effect of insulin-like growth factor-I (IGF-I) on avian metabolism, very little is known about the function of IGF-I in the regulation of reproduction. Recently, there were reports on the important roles of IGF-I in the egg productivity of layers (McMurtry et al., 1997) and the association of IGF-I with the egg productivity of KNOC (Kang et al., 2000).

IGF-I is a nonglycoprotein whose structure is homologous to pro-insulin (Humbel, 1995). The mature chicken IGF-I (7 kDa) contains 70 amino acids, eight of which differ from those of human, porcine, or bovine IGF-I (Ballard et al., 1990). Due to the considerable similarity of mammalian and avian IGF-I, a great divergence in the functionality of IGF-I would not be expected. However, the evidence from mammalian studies has shown that the minor, but unique, differences that do exist between species in IGF biochemistry can be of great physiological significance. For example, more IGF is present in the free from in chickens, and to date no unique IGF-II receptor has been identified in birds (Spencer et al., 1996; McMurtry et al., 1998).

In mammals, IGF-I has well-documented functions as autocrine and paracrine regulators of growth and differentiation of ovarian granulosa and theca cells (Giudice, 1992; Bergh et al., 1993; Spicer and Echternkamp, 1995). There appear, however, to be some species differences in the sites of production and effects (Spicer and Echternkamp, 1995).

In mammals, the ovary is a major site of the hormonally regulated production of IGF-I (Guidice, 1992). IGF-I has also been shown to potentiate the action of gonadotrophins on granulosa and theca interstitial cells *in vitro* (Mondschein et al., 1989). On the other hand, little is known about the ovarian IGF system in poultry. Studies have demonstrated the expression of genes encoding IGF-I in both the granulosa and theca cells (Roberts et al., 1994; Amstrong and Hogg, 1996). Onagbesan et al. (1999a) has confirmed that the IGF-I gene is expressed in both theca and granulosa cells and has shown that the amount of IGF-I synthesized varies with follicular size and hormonal stimulation in poultry. These observations indicate that IGF-I acts as intra-ovarian regulators that modulate the growth and differentiation of ovarian follicles in chickens. However there has been no report detailing the role, or even the presence, of ovarian IGF-I in KNOC. Therefore, more basic research regarding the of functions IGF-I in KNOC is required to further understand their intra- and inter-species differences. So the objectives of the present study were to correlate ovarian IGF-I expression at 60 wk to both serum IGF-I concentration and follicular levels, and to investigate the association of egg productivity with ovarian IGF-I expression.

MATERIALS AND METHODS

Animals

Seventy unselected female KNOC were purchased from the KNOC Breeding Farm in Yeonsan, Korea. They were

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raised at the Korea University Animal Breeding Center with a diet of 16% crude protein, 3% crude fat, 7% crude fiber, 15% crude ash, 3% Ca, 0.45% P, 0.58% methionine+cystine, 2.75 Mcal/kg metabolizable energy ad libitum. All chicks were housed in individual cages and received 15 hr of light/d for 17 weeks followed by 15 min increase in light/wk up to 17 h of light/d (Ohh, 1988). Egg productivity was recorded daily from the onset of laying until the age of 60 wk. Average egg productivity (sexual maturity, number produced, and egg weight) was calculated every 5 wk until 60 weeks of age.

Blood collection and serum preparation

Blood (3-5 ml) was taken from the wing vein every 10 wk from 20 wk to 60 wk, and blood collection was conducted from 11:00 to 15:00. In order to obtain serum, blood samples were stood at room temperature for 2 h and centrifuged for 20 min at 1,000×g, and the supernatant was stored at -70°C until used.

Protein extraction form the ovary and follicle granulosa layers

The first (F1), second (F2), third (F3), fourth (F4), and fifth (F5) of the follicles were removed from the ovary. The vascular and connective tissues surrounding the follicles were removed with forceps and an incision was made in the follicle to allow most of the yolk to flow out. The granulose layer was carefully separated from the theca layer as described by Roberts (1994). The ovaries and granulosa layers were washed 3 times in ice-cold Ringer solution (125 mM NaCl, 1 mM $CaCl_2 \cdot 2H_2O$, 5 mM KCl; pH 7.5) to remove any adhering yolk. These samples were homogenized in pre-chilled RIPA buffer (150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris; pH 8.0) containing protease inhibitors (100 mM PMSF, 1.0 mg/ml aprotinin) and incubated at 4°C for 30 min. The samples were centrifuged at 10,000×g in a pre-chilled centrifuge at 4°C for 15 min. The supernatant from the ovary lysate was collected and stored at -70°C until being analyzed for IGF-I.

IGF-I radioimmunoassay (RIA)

Recombinant chicken IGF-I (GroPep. Pty Ltd., Australia) was iodinated by the chloramine-T method (Lee and Henricks, 1990). Iodinated IGF-I was purified on a Sephadex G-50 column and aliquots were stored -20°C until used.

Ovarian, follicular, and serum IGF binding proteins were removed using an acid-ethanol method (Daughaday et al., 1980). Briefly, each sample was acidified with acid-ethanol (87.5% ethanol, 12.5% HCl) and stood for 30 min at room temperature. Then the samples were centrifuged at 1,800×g for 30 min and neutralized with 0.2 ml of 0.855 M Tris-base. IGFBPs removed supernatant was mixed 0.1 ml of RIA buffer (30 mM sodium phosphate, 0.02% protamine sulfate, 10 mM EDTA, 0.05% Tween-20, 0.02% sodium azide; pH 7.5) incubated with rabbit anti-human IGF-I polyclonal antiserum (GroPep Pty., Ltd.; final dilution of 1:10,000) and 15,000 cpm [^{125}I] IGF-I in RIA buffer for 16-18 h at 4°C. Then 0.1 ml of goat anti-rabbit IgG antibody (GroPep Pty., Ltd.) was added and the mixture was incubated for 1 h followed by an additional 1 h incubation with 0.1 ml of normal rabbit serum at 4°C. After addition of 1 ml RIA buffer, the tubes were centrifuged for 10 min at 3,000×g at 4°C. The supernatant was aspirated and the pellet was counted in a gammer-counter. The amount of IGF-I was determined by logit-log plots and the intra-assay coefficient of variation was 7.8%.

Selection for experimental groups

Chicks were divided into high and low groups based on their ovarian IGF-I levels at 60 wk. The criterion used for selection between the high and low groups as 20% in both the upper and lower classes.

Statistical analysis

The data were statistically analyzed using the Duncan method of one way ANOVA and the Pearson's correlation coefficients procedure in the SAS package (1995).

RESULTS

Ovarian IGF-I expression at the age of 60 weeks

The criterion for ovarian IGF-I concentrations between the selected groups is shown in Table 1. Seventy unselected seventy KNOCs were divided into the two groups depending on their ovarian IGF-I expressions at 60 wk. The high IGF-I group had higher than 3.21 ng/mg expression and the low IGF-I group had less than 1.79 ng/mg ($p<0.05$). The two groups comprised the upper and lower 20% of the unselected group, respectively.

Serum IGF-I concentrations in the high and low ovarian IGF-I groups

The changes in the serum IGF-I concentrations of the selected and unselected groups during the egg production period are shown in Table 2. In the unselected group, the

Table 1. Ovarian IGF-I expression at the age of 60 weeks in Korean Native Ogol Chick[1)]

Trait	Selected by ovarian IGF-I		Total (n=70)
	High group (n=15)	Low group (n=15)	
Ovarian IGF-I (ng/mg)	3.21[a]±0.47	1.79[b]±0.12	2.48±0.53

[1)] All values are expressed as mean±SD.
[2)] Means with different superscripts differ ($p<0.05$).

Table 2. Comparison of serum IGF-I concentration between the high and low ovarian IGF-I groups[1)]

Groups	No. of chicks	Serum IGF-I (ng/ml)				
		20 wk	30 wk	40 wk	50 wk	60 wk
High (>3.21 ng/mg)	15	37.9±13.2	36.2±9.7	37.5±6.6	32.5[a]±12.5	31.0[a]±12.7
Low (<1.79 ng/mg)	15	31.4±12.2	34.2±6.4	36.7±8.8	14.8[b]±8.9	17.0[b]±6.9
Total	70	34.3±10.0	33.4±7.6	36.0±7.5	25.5±13.8	23.8±11.0

[1)] All values are expressed as mean±SD.
[2)] Means with different superscripts differ ($p<0.05$).
[3)] Chicks were divided into high and low groups based on ovarian IGF-I concentration.

maximum serum IGF-I concentration was found at 40 wk, and thereafter the level decreased. The concentrations of serum IGF-I in the high group were higher than those in the low group throughout the wholel period. In particular, the serum IGF-I concentration were significantly different between the high and low groups at 50 and 60 wk (32.5± 12.5 and 14.8±8.9 ng/ml, 31.0±12.7 and 17.0 ± 6.9; $p<0.05$). Overall, the concentration of serum IGF-I was greater at 40 wk of age than at any other age.

Comparison of IGF-I expression in the granulosa layer between the high and low ovarian IGF-I groups

Table 3 shows a comparison of the granulosa layer IGF-I concentrations in both groups. Although the two groups did not show any statistical difference in IGF-I expression, the high group tended to show higher levels than the low group except for F3 and F4. It seems that IGF-I expression in the granulosa layer varies depending upon the follicular developmental stage.

Comparison of egg productivity between the high and low ovarian IGF-I groups

Table 4 and 5 show the changes in age of the first egg laid (sexual maturity), egg production and egg weight in the selected and unselected groups. In the unselected group,

Table 3. Comparison of IGF-I expression in granulosa layer between the high and low ovarian IGF-I groups[1)]

Groups	No. of chicks	Granulosa layer IGF-I (ng/mg)				
		F1	F2	F3	F4	F5
High (>3.21 ng/mg)	15	9.0 ±1.3	6.3 ±2.8	5.2 ±1.5	5.7 ±1.9	6.9 ±3.2
Low (<1.79 ng/mg)	15	8.8 ±2.4	5.6 ±1.3	5.7 ±2.7	6.2 ±1.8	3.7 ±3.4
Total	70	8.9 ±2.6	6.0 ±1.7	5.4 ±3.0	5.9 ±2.1	4.8 ±3.5

[1)] All values are expressed as mean±SD.

Table 4. Comparison of age first egg laid between the high and low ovarian IGF-I groups[1)]

Trait	High group (n=15)	Low group (n=15)	Total (n=70)
AFE[2)] (day)	183.5±0.47	203.3±25.4	194.6±21.9

[1)] All values are expressed as mean±SD.
[2)] Age of first egg laid.

sexual maturity was achieved at the age of 194.6 days (Table 4), and the maximum egg production of approximately 20 ea was found at 35-55 wk (Table 5). Similarly, egg weight gradually increased until 50 wk (50.5 g) and then declined. In the comparison of the age of the first egg laid between the two groups, the high group showed approximately 20 days faster than the low group (Table 4). In the comparison of egg production between the two groups, a significant difference ($p<0.05$) was detected at 45 wk. Moreover, total egg production in the high group was remarkably higher than that of the low group (129.3 vs 76.5 ea; $p<0.005$). On the other hand, the egg weight increased until 50 wk and decreased afterwards in both groups, but no statistical difference in egg weight was detected.

Correlation coefficient of ovarian IGF-I with serum IGF-I, granulosa layer IGF-I, and egg productivity in KNOC

The correlation coefficients of ovarian IGF-I with serum IGF-I, granulosa layer IGF-I, and egg productivity are shown in Table 6. Serum IGF-I and egg production showed positive correlations with ovarian IGF-I. In particular, ovarian IGF-I had a significant correlation with serum IGF-I (at 50 wk, 0.39294; $p<0.05$; at 60 wk, 0.47030; 0.0001). On the other hand, ovarian IGF-I had a negative correlation with egg weight during the whole period. With granulosa layer, ovarian IGF-I had a negative, but insignificant, correlation.

DISCUSSION

The number of eggs laid by a hen is determined by the number of follicles destined for ovulation and by the capacity of the oviduct to transform the ova into hard-shelled eggs. The ovary of layer chickens contains in general five to six hierarchical yellow follicles, which allow a mature follicle to ovulate on successive days for extended periods. It also contains a number of small and large white follicles, from which recruitment of only one follicle to the hierarchy is made on a daily basis (Onagbesan et al., 1999b).

KNOC is a breed that expresses the characteristics of both broiler and layer, with low egg productivity. The egg production of KNOC is approximately 122 ea until 60 wk

Table 5. Comparison of egg productivity (egg production number and egg weight) between the high and low ovarian IGF-I groups[1)]

Traits	Groups	No. of chicks	Weeks							Total
			~25	~30	~35	~40	~45	~50	~55	~60	
AEN[2)] (ea)	High (>3.21 ng/mg)	15	1.3 ±1.4	14.1 ±6.8	20.3 ±7.1	22.4 ±3.8	18.7[a] ±7.3	21.5 ±6.8	18.3 ±7.3	12.9 ±6.4	129.3 ±20.9
	Low (<1.79 ng/mg)	15	0.8 ±1.2	8.6 ±7.4	18.7 ±9.3	17.4 ±9.2	11.8[b] ±8.5	16.9 ±8.9	16.5 ±9.4	8.2 ±5.7	76.5 ±55.1
	Total	70	3.4 ±2.0	11.8 ±7.0	19.2 ±7.2	20.1 ±6.0	17.3 ±6.8	20.3 ±6.8	18.1 ±7.6	11.9 ±5.7	106.8 ±43.8
AEW[3)] (g)	High (>3.21 ng/mg)	15	38.7 ±2.5	43.8 ±3.1	47.0 ±2.6	48.7 ±2.3	50.0 ±2.7	50.8 ±2.5	49.1 ±2.8	49.6 ±2.6	47.8 ±2.4
	Low (<1.79 ng/mg)	15	41.7 ±2.0	42.7 ±7.8	48.5 ±2.7	47.9 ±4.5	48.7 ±5.3	51.9 ±2.8	48.1 ±6.3	48.2 ±6.2	47.5 ±3.6
	Total	70	40.5 ±2.7	43.7 ±4.0	47.3 ±2.5	48.6 ±2.8	49.7 ±3.2	50.5 ±2.7	48.8 ±3.6	48.8 ±3.6	47.8 ±2.6

[1)] All values are expressed as mean±SD.
[2)] Average egg production number.
[3)] Average egg weight.
[ab] Mean with different superscripts differ ($p<0.05$).

Table 6. Pearson's correlation coefficient of ovarian IGF-I with serum IGF-I, granulosa layer IGF-I, and egg productivity in KNOC

Traits	Weeks				
	20	30	40	50	60
Serum IGF-I (ng/ml)	0.02748	0.19328	0.09179	0.39294*	0.47030**
AFE[1)] (day)	-0.20786				
Egg production (ea)	-	0.18918	0.08564	0.03094	0.18770
Egg weight (g)	-	-0.23958	-0.14377	-0.10557	-0.03209
	F1	F2	F3	F4	F5
Granulosa layer	-0.07787	-0.14244	-0.17823	-0.04283	0.18511

[1)] Age of first egg laid.
* $p<0.05$, ** $p<0.0001$.

(Kang et al., 2001), which is far less than that of the White Leghorn (>350) (Robert, 1996). Moreover, the average egg weight of KNOC is approximately 50 g from sexual maturity to 60 wk (Kang et al., 2001), which is lighter than that of the White Leghorn (>60 g) (Su and Silversides, 1996). These suggest that KNOC needs to be improved for egg productivity as a layer.

Onagbesan et al. (1999a) documented the modulating role of IGF-I in the growth and differentiation of chicken ovarian cells. In the present study, after the age of first egg laid, it was found that serum IGF-I and egg production in KNOC reached a peak at the same age (40 wk) and declined afterwards (Tables 2 and 5). Moreover, our result of a similar tendency of these traits in the high ovarian IGF-I expression group support the previous report by Onagbesan et al. (1999a).

The hierarchical system of rapid follicular development in the domestic hen makes it a potentially useful system to study the factors that regulate follicular development, particularly at the ovarian level by an autocrine or paracrine mechanism. Although the regulation of steroidogenesis has been characterized in chicken granulosa and theca cells (Etches, 1990), the involvement of peptide growth factors has not been much studied. There is a complex intraovarian IGF system in the mammalian ovary (Adashi et al., 1992), but this has not been identified in birds.

The IGF-I expressions in the granulosa layers, from F1, F2, and F5 (Tables 1 and 3) were higher in the high ovarian IGF-I group than in the low group. These results indicate the involvement of ovarian IGF-I in the regulation of follicular development in KNOC. Similar studies were done by Onagbesan et al. (1999a) but, in their results, the medium-sized follicles produced higher amounts of IGF-I than the larger F1 or F2 follicles. In addition, when stimulated by hormones (LH, FSH, and GH), the larger follicles tended to secrete higher amounts of IGF-I than did the medium-sized follicles (F3 and F4). This may represent changes that occur in the follicles in response to temporal functional requirements (Onagbesan et al., 1999a). In the present study, the IGF-I concentrations in the granulosa layers of the follicles (F1-F5) ranged from 9.0 to 3.7 ng/mg in both group (Table 5). In the previous report (Onagbesan et al., 1999a), basal IGF-I produced by cultured granulosa cells of the four largest follicles (F1 to F4) in ISA Brown layers were approximately 3-4 ng/ug DNA. In addition, the report by Roberts et al. (1994) showed that the IGF-I concentration in the granulosa layer was higher than that in the theca layer (0.82 vs 0.31 pmol/g wet wt). These indirectly imply that the concentration of IGF-I is influenced by some species differences in the mechanism and site of production and mechanisms.

In a previous report (Kang et al., 2000), serum IGF-I was positively associated with egg production and negatively related to egg weight. In this study, a similar result with egg production was obtained for ovarian IGF-I

(Table 5). Moreover, ovarian IGF-I has a positive correlation with serum IGF-I at 50, 60 wk (Table 6). Thus, these results suggest that both ovarian and serum IGF-I regulate egg production and IGF-I certainly acts as autocrine and/or paracrine as well as endocrine in KNOC.

Evidence based on gene expression, secretion, and action of IGF-I in both the granulosa and theca cells of chickens strongly suggest that IGF-I plays important roles in the control of the reproductive functions of the avian ovary (Onagbesan et al., 1999b). The control mechanism is complex and there seems to be differential effects between strains and nutritional states. To unravel this complex mechanism and to improve reproductive efficiency, especially in KNOC, further investigations are necessary.

In conclusion, the expression of ovarian IGF-I in KNOC was examined to investigate for possible correlation with egg productivity. IGF-I seems to regulate ovulation and follicle development in KNOC, based on the positive association of IGF-I with egg production. Further studies on other endocrine regulators related to egg productivity are required to fully understand the endocrine mechanism of egg productivity in KNOC.

IMPLICATION

Until now, few studies have investigated egg productivity by monitoring egg productivity related markers, such as endocrine factors (growth factors and steroid hormones). Although KNOC had lower egg productivity than other layers, the basic patterns of endocrine factors were similar. The results of this study suggest that selection by IGF-I expression would be beneficial for improving egg production in layers including KNOC. In order to improve egg productivity, several further studies at the gene level and of other related endocrine factors are required.

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