Follicular Development and Expression of the Messenger Ribonucleic Acid for the Inhibin/Activin Subunits in Two Genetic Lines of Turkey Hens that Differ in Total Egg Production

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ABSTRACT The characterization of the follicular hierarchy and the expression of the mRNA for the inhibin/activin subunits was investigated in the follicles of 2 lines of turkey hens selected for over 40 generations for increased egg production (Egg line) or increased body weight (Growth line). The follicular hierarchies of 6 hens from the Egg and Growth lines were characterized in middle (45 wk of age) and late production (58 wk of age). Relative follicular weights for individual hierarchical follicles (>12 mm), pooled small yellow follicles (5 to 12 mm), and large white follicles (2 to 5 mm) were calculated. Total RNA was extracted for Northern blot analysis from individual granulosa cell layers of the F1 through F4 follicles, and from the combined granulosa and theca layers of small yellow follicles and large white follicles from an additional 6 hens from each genetic line. Egg line hens displayed a more distinct follicular size hierarchy than Growth line hens at 45 and 58 wk. Although total follicular weight relative to body size was greater at 45 and 58 wk of age for the Egg line hens than the Growth line hens, the total number of hierarchical follicles was greater in the Growth line hens at 45 and 58 wk of age. Expression of follistatin and the inhibin β_B-subunit was highest in nonhierarchical follicles, whereas the expression of the inhibin α- and β_A-subunits was highest in the hierarchical follicles. The inhibin α- and β_A-subunit mRNA expression pattern in the 4 largest follicles of the Growth line hens was not similar to the Egg line hens or characteristic of laying hens that have a high rate of egg production. The unusual inhibin subunit mRNA expression in the largest hierarchical follicles of the Growth line hens may account for their development of an abnormal follicular size hierarchy and for their poor egg production.

Key words: turkey, follicle, inhibin, activin

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INTRODUCTION

Inhibin is a heterodimeric protein composed of an α- and β-subunit. Depending upon which of the 2 distinct but similar β-subunits (β_A and β_B) is combined with the α-subunit, inhibin exists as inhibin-A (α-β_A) or inhibin-B (α-β_B). In contrast, activin may form as a homodimer or heterodimer of the β-subunits, resulting in the homodimers activin-A (β_A-β_A) and activin-B (β_B-β_B) or the heterodimer activin-AB (β_A-β_B). Activin and inhibin are bound by follistatin, a soluble-binding protein that binds both hormones through their common β-subunit (Nakamura et al. 1990). Follistatin binds activin with a greater affinity than inhibin (Shimonaka et al., 1991; Krummen et al., 1993) and neutralizes almost all of the biological actions of activin upon binding (Mather et al., 1993). In addition to modulating FSH secretion from the pituitary, inhibin and activin have been associated with a multitude of reproductive functions as reviewed by Halverson and DeCherney (1996), Mather et al. (1997), Woodruff (1998, 2002), Welt et al. (2002), and Phillips and Woodruff (2004).

The laying hen ovary provides a unique model for studying follicular development due to the organization of its yolk-filled preovulatory follicles into a hierarchy according to size. The largest follicle, which will be ovulated within 24 to 26 h, is designated as the F1 follicle, and the second largest follicle, which will be ovulated 24 to 26 h after the F1 follicle, is the F2 follicle, and so on. The smallest hierarchical preovulatory follicle matures each day from a pool of small yellow follicles (**SYF**) that are 5 to 12 mm in diameter, which in turn mature from a pool of large white follicles (**LWF**) that are less than 5 mm in diameter.

The mRNA and protein expression of the inhibin/activin subunits and follistatin are well characterized in the laying hen ovary (Davis and Johnson, 1998; Lovell et al., 1998, 2003; Johnson et al., 2005). In general, the granulosa cells of the largest follicles produce inhibin-A, whereas the small follicles produce inhibin-B (Lovell et al., 1998, 2003; Johnson et al., 2005). Specifically, the F1 follicle is

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the primary source of inhibin-A (Lovell et al., 1998). Activin-A production is greater in the theca cells than in the granulosa cells (Lovell et al., 1998). Activin-A production steadily increases in a follicle as it matures from the small prehierarchical stage through to the F4 stage. As follicular maturation continues beyond the F4 stage, however, activin-A production rapidly declines (Lovell et al., 2003). Follistatin is produced by theca and granulosa tissue, and production by the granulosa tissue is greatest in the prehierarchical follicles (Lovell et al., 2003).

Although inhibin and activin production have been well characterized in highly productive laying hen strains, little focus has been given to production of these hormones in poultry species with poor egg production. Although the inhibin α- and β_A- subunits have been cloned in the turkey (Ahn et al., 2001), a comprehensive examination of the expression of the inhibin subunits during follicular development has not been completed. The Ohio Agriculture Research and Development Center maintains 2 genetic lines of turkey hens differing greatly in BW and egg production. Egg line was selected by McCartney et al. (1968) from an established random bred control line (McCartney, 1964), whereas growth line was selected for increased 16-wk BW (Nestor, 1977). The Egg line was originally selected based on the total number of eggs produced by dams for 84 d (generations 1 through 3). Selection was subsequently based on 180-d egg production for generations 4 through 26 and for 250-d egg production for subsequent generations (Nestor et al., 1996). Selection for increased BW in the Growth line has reduced egg production in the hens of this genetic line due to a decrease in the intensity of lay as measured by average clutch size (Nestor et al., 1996, 2000). In contrast, selection for increased egg production in the Egg line has greatly reduced broodiness and vastly increased the intensity of lay in the hens of this genetic line (Anthony et al., 1991; Nestor et al., 1996).

Research utilizing the Egg and Growth lines of hens provides a unique opportunity to characterize the mRNA expression of the inhibin/activin subunits and follistatin in birds with poor egg production compared with laying hens as well as the opportunity to compare expression of these subunits in hens of the same species with vastly different egg production rates. Therefore, the current study was conducted to characterize the follicular size hierarchy of these 2 genetic lines of turkey hens and to characterize mRNA expression of the inhibin/activin subunits and follistatin in the preovulatory follicles of these hens.

MATERIALS AND METHODS

Birds

Egg line and Growth line poults were hatched at the Ohio Agriculture Research and Development Center and shipped immediately to the North Carolina State University Turkey Educational Unit. The turkeys were raised in floor pens and provided 10 h of light per d until 25 wk of age when the hours of light were reduced to 8 h. At 31 wk of age the turkey hens were moved to breeding pens (6 birds per pen) and photo-stimulated for reproduction by providing 15.5 h of light per d. Each breeding pen was equipped with a nest box. The turkeys were provided with free access to appropriate commercial diets and water at all times through rearing and production. All animal procedures were approved by the North Carolina State University Animal Care and Use Committee.

Characterization of Follicular Hierarchies

At 45 and 58 wk of age, 6 hens from the Egg and Growth lines were selected and weighed. The hens were killed by electrocution 2 to 4 h prior to ovulation. The entire ovary was removed from each bird, and follicles were separated into hierarchical follicles (>12 mm), SYF (5 to 12 mm), or LWF (2 to 5 mm). Weights were determined for individual hierarchical follicles, pooled SYF, and pooled LWF. The following year (2004) the follicular weights of all the hierarchical, SYF, and LWF were again determined for 6 Egg line and 6 Growth line hens at 45 and 58 wk of age.

For the second collection (58 wk of age) of follicles during the first year (2003) of the experiment, the F1 to F4 follicles for each Egg line hen and the F1 to F10 follicles for each Growth line hens as well as the SYF and LWF from both genetic lines were utilized for subsequent RNA isolation. The granulosa cell layer was manually separated from the theca cell layers of each of the large hierarchical follicles and saved (Huang and Nalbandov, 1979). The connective tissue was removed from the SY and LW follicles, and then the yolk material was expelled from each follicle. Next, the combined theca and granulosa tissue layers from the SYF and LWF were pooled by size and placed in RNALater (Ambion, Austin, TX). Granulosa samples from the large hierarchical follicles were frozen and stored at –80°C in 1 mL of guanidinium thiocyanate solution for subsequent RNA extraction, whereas the theca/granulosa samples from the SYF and LWF were maintained in RNALater solution at 4°C for subsequent RNA extraction.

RNA Extraction and Northern Analysis

Total RNA was extracted from the individual granulosa layers of each of the hierarchical follicles collected from each hen and from the combined theca and granulosa layers of the SYF and LWF follicles of each hen using a guanidinium thiocyanate-phenol-chloroform method (Chomcynski and Sacchi, 1987). For each sample, 40 μg of total RNA was electrophoresed on a 1.5% agarose/formaldehyde gel and then transferred to a nylon membrane as previously described (Davis and Johnson, 1998). A total of 14 Northern blots were produced in this manner. Six of the replicate blots each contained F1 to F4 granulosa samples obtained from a different Egg line and Growth line hen for each replicate blot. Six other replicate blots each contained the F1 to F10 granulosa samples

Table 1. Total egg production and average egg weight for Egg line and Growth line turkey hens through 54 wk of age[1]

Genetic line	Eggs/hen[2]	Egg weight[2] (g)
Egg	103 ± 2.0[a]	63 ± 0.5[a]
Growth	45 ± 2.0[b]	91 ± 0.5[b]

[a,b]Values within a column without a common superscript differ, $P < 0.05$.

[1]The hens were photostimulated at 37 wk of age.

[2]Values are means ± SEM, n = 37 (Egg line), n = 36 (Growth line).

obtained from a different Growth line hen for each replicate blot. The final 2 replicate blots each contained 3 SYF and 3 LWF samples from 3 different hens of each of the genetic lines for each replicate blot.

The cDNA clones were prepared for the chicken inhibin α-subunit, inhibin/activin β_A- and β_B-subunits, follistatin, and glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) and labeled with ^{32}P for Northern blot analyses as previously described (Davis and Johnson, 1998). The hybridization and densitometry procedures were also performed as described previously (Davis and Johnson, 1998). After each hybridization, the blots were subjected to a stringent wash and exposed to x-ray film as described previously (Davis and Johnson, 1998). The order of the hybridizations for each blot was follistatin, inhibin/activin β-subunit, inhibin/activin β_A-subunit, and inhibin α-subunit. To verify and correct for equality of RNA loading and transfer, a final hybridization of each blot was done with GAPDH. The relative mRNA expression of the inhibin α-, β_A-, and β_B-subunits was determined for the samples of each blot by calculating the signal intensity for each sample relative to the strongest signal which was assigned a value of 1. Before calculating the relative inhibin α-, β_A-, and β_B-subunit mRNA expression levels, GAPDH mRNA expression was used to correct the inhibin α-, β_A-, and β_B-subunit values for equality of RNA loading and transfer for each blot.

Statistics

Data were subjected to ANOVA using the GLM procedure with replicate and follicle size or genetic line as factors. Tukey's multiple-comparison procedure (Neter et al., 1990) was used to detect significant differences in follicular expression of the inhibin subunits among the differently sized follicles. All statistical procedures were done with the Minitab Statistical Software package (Release 13, State College, PA). Differences were considered significant when P-values were <0.05.

RESULTS

Characterization of Follicular Hierarchies

Egg line hens produced a greater number of eggs, but egg weight was lower than Growth line hens (Table 1). At 45 and 58 wk of age, the total follicular weight relative to BW was significantly greater in the Egg line hens compared with the Growth line hens, even though the Growth line hens possessed a significantly greater number of hierarchical follicles (Table 2). The Growth line hens had more LWF than the Egg line hens at 45 and 58 wk of age (Table 2). The number of SYF was greater in Growth line hens than in Egg line hens at 45 wk of age but not at 58 wk of age (Table 2). The average size of an individual SYF relative to body size from the Egg line hens was significantly greater at 45 wk of age (3.95×10^{-5} vs. 2.04×10^{-5} g/g) and at 58 wk of age (6.18×10^{-5} vs. 3.03×10^{-5} g/g) than from the Growth line hens. Similarly, the average size of an individual LWF relative to body size from the Egg line hens was significantly greater at 45 wk of age (2.46×10^{-5} vs. 7.20×10^{-6} g/g) and at 58 wk of age (1.76×10^{-5} vs. 7.38×10^{-6} g/g) than from the Growth line hens. The F1 to F5 follicles of the Egg line hens were significantly larger relative to body size and displayed a distinct size hierarchy compared with the F1 to F5 follicles of the Growth line hens at 45 (Figure 1) and 58 (Figure 2) wk of age. In contrast, the F8 to F10 follicles at 45 wk of age and F8 to F9 follicles at 58 wk of age of Growth line hens were significantly larger relative to body size than in the Egg line hens. At 45 and 58 wk of age, the absolute follicular weight of all the follicles from the Growth line hens was significantly larger than those from the Egg line hens (Figures 1 and 2).

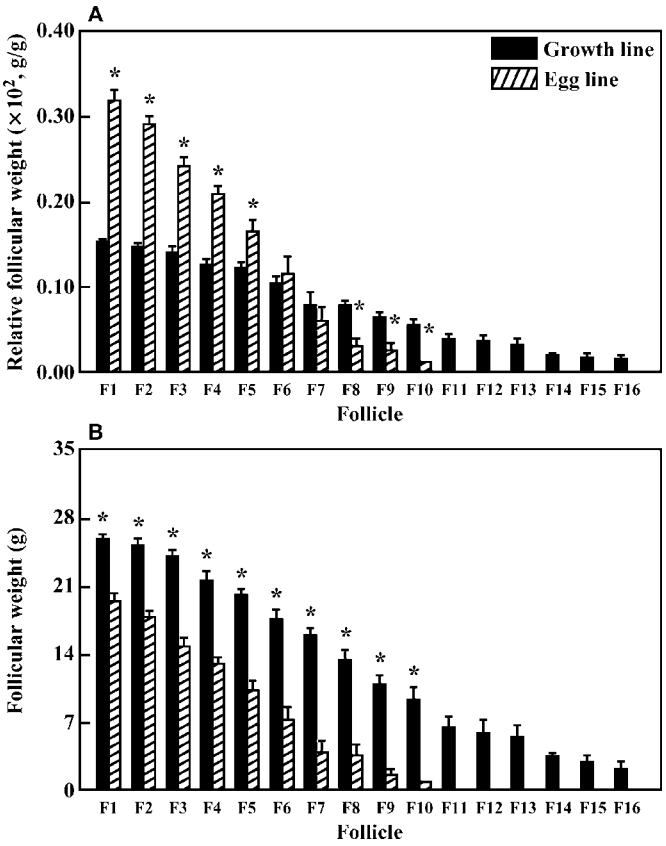

Figure 1. Relative follicular weights (A) and absolute follicular weights (B) of hierarchical follicles in Growth line and Egg line hens at 45 wk of age. Values are means ± SEM, n = 6 replicate hens. *Means differ ($P < 0.05$) from the corresponding mean for the other genetic line.

Table 2. Characterization of follicular number and weight in 2 genetic lines of turkey hens in early and late lay[1]

Genetic line	Age (wk)	BW[2] (g)	Number of follicles[2,3]			Follicular weight[2,4] (g)	Relative follicular weight[2,5] (g/g)
			>12 mm	>5 to ≤12 mm	2 to 5 mm		
Egg	45	6,179 ± 155[b]	9.14 ± 0.46[b]	6.43 ± 1.56[b]	15.00 ± 2.83[b]	94.10 ± 5.80[b]	0.015 ± 0.0006[a]
Growth	45	17,120 ± 526[a]	15.60 ± 1.03[a]	20.60 ± 5.71[a]	28.40 ± 3.66[a]	218.20 ± 13.3[a]	0.012 ± 0.0006[b]
Egg	58	5,662 ± 143[b]	9.00 ± 0.26[b]	9.3 ± 1.93	12.60 ± 1.09[b]	79.58 ± 6.47[b]	0.014 ± 0.0008[a]
Growth	58	15,727 ± 397[a]	13.50 ± 0.56[a]	12.2 ± 1.25	26.80 ± 4.73[a]	174.60 ± 7.50[a]	0.011 ± 0.0008[b]

[a,b]Values within a column for a given age without a common superscript differ, $P < 0.05$.

[1]The hens were photostimulated at 37 wk of age.

[2]Values are means ± SEM, n = 6 replicate hens for each genetic line at both 45 and 58 wk of age.

[3]Follicles were counted and classified as hierarchical (>than 12 mm in diameter), small yellow follicles (>than 5 mm but ≤12 mm in diameter) or large white follicles (2 to 5 mm in diameter).

[4]The total weight of all follicles ≥2 mm in diameter.

[5]Total follicular weight divided by BW.

The relative and absolute follicular weights and the size of the hierarchy obtained the following year utilizing the subsequent generation of Egg line and Growth line hens at 45 and 58 wk of age mirrored those previously obtained the year before (data not shown). The significant differences observed between the 2 genetic lines of hens during the previous year of the project remained for this generation of hens.

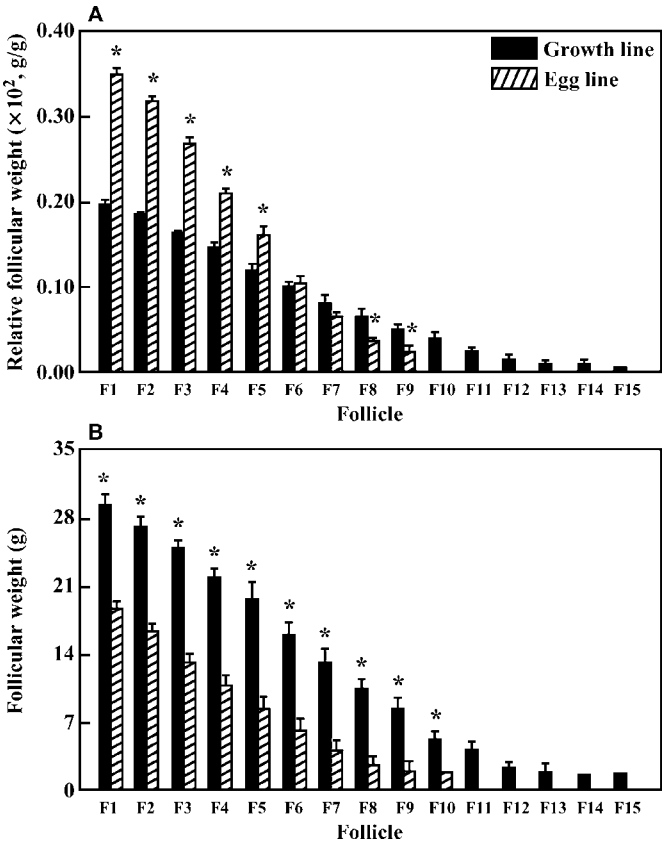

Figure 2. Relative follicular weights (A) and absolute follicular weights (B) of hierarchical follicles in Growth line and Egg line hens at 58 wk of age. Values are means ± SEM, n = 6 replicate hens. *Means differ ($P < 0.05$) from the corresponding mean for the other genetic line.

Expression of the Inhibin α-Subunit

In Growth line hens, the granulosa cells of the F4 follicle expressed a significantly lower amount of inhibin α-subunit mRNA compared with the granulosa cells of the F1 and F2 follicles (Figure 3). There was no significant difference in the mRNA expression of the inhibin α-subunit between the granulosa cells of the F1 to F4 follicles in the Egg line hens (Figure 3). Overall, granulosa cell expression of the inhibin α-subunit was significantly greater in the Egg line F1 to F4 hierarchical follicles (0.80 ± 0.04) when compared with the Growth line hens (0.60 ± 0.04). The relative mRNA expression of the inhibin α-subunit between the individual F1, F2, F3, and F4 follicles of Egg and Growth line hens was (mean ± SEM) 0.73 ± 0.09 vs. 0.70 ± 0.04; 0.73 ± 0.08 vs. 0.72 ± 0.06; 0.84 ± 0.05 vs. 0.54 ± 0.10; and 0.91 ± 0.05 vs. 0.37 ± 0.04, respectively. Thus, the higher overall granulosa cell expression of the inhibin α-subunit in the 4 largest follicles in the Egg line hens compared with the Growth line hens was primarily based on the significantly higher expression of the inhibin α-subunit mRNA in the granulosa cells from the F3 and F4 follicles of the Egg line hens compared with the Growth line hens.

In the extended follicular hierarchy of the Growth line hens, the mRNA expression of the inhibin α-subunit was greater in the granulosa cells from the largest and smallest follicles compared with granulosa cells from the midsized follicles (Figure 4).

Expression of the Inhibin/Activin β_A-Subunit

In the Growth line hens, the granulosa cells from the F4 follicle had significantly less inhibin/activin β_A-subunit mRNA expression than the granulosa cells from the F1, F2, and F3 follicles; however, there was no difference in β_A-subunit mRNA expression between the granulosa cells from the F1, F2, and F3 follicles (Figure 5 and 6). In Egg line hens, the granulosa cells from the F2 and F3 follicles had significantly less inhibin/activin β_A-subunit mRNA

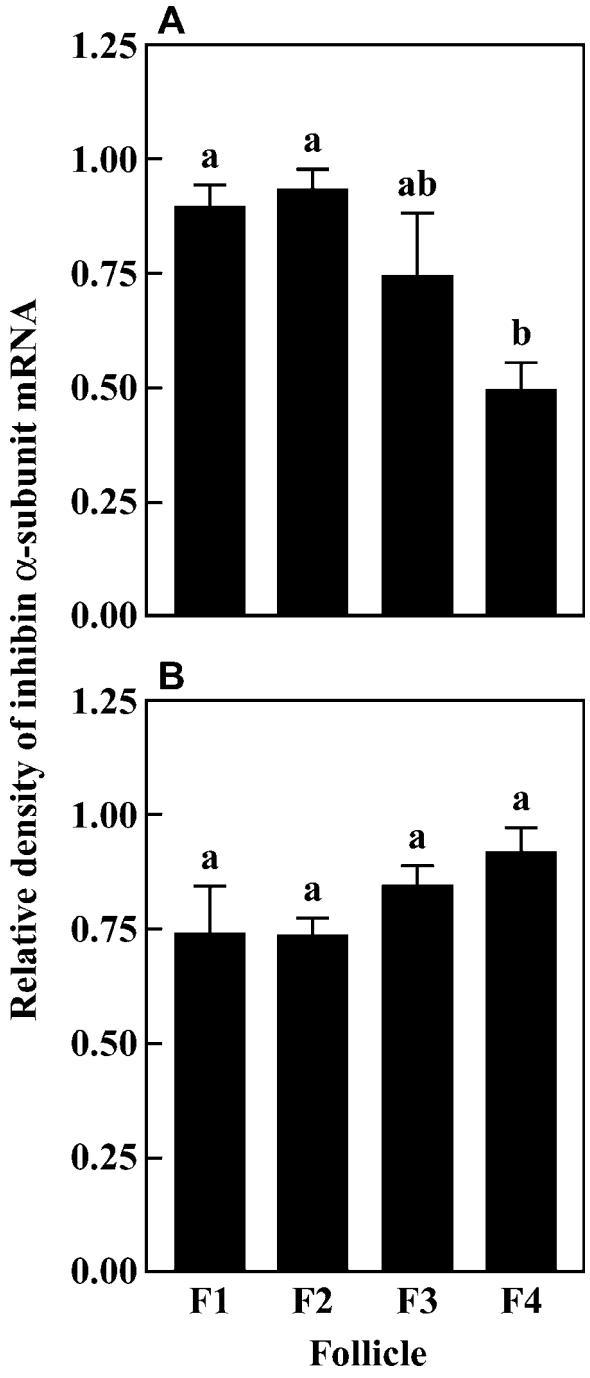

Figure 3. The relative density of the inhibin α-subunit mRNA in the individual F1-F4 hierarchical follicles of the Growth line hens (A) and Egg line hens (B). Values are means ± SEM, n = 6 replicate hens. [a,b]Means within each genetic line with different letters differ, $P < 0.05$. Note that the relative densities of inhibin α-subunit mRNA to one another are specific for each genetic line of turkey hen.

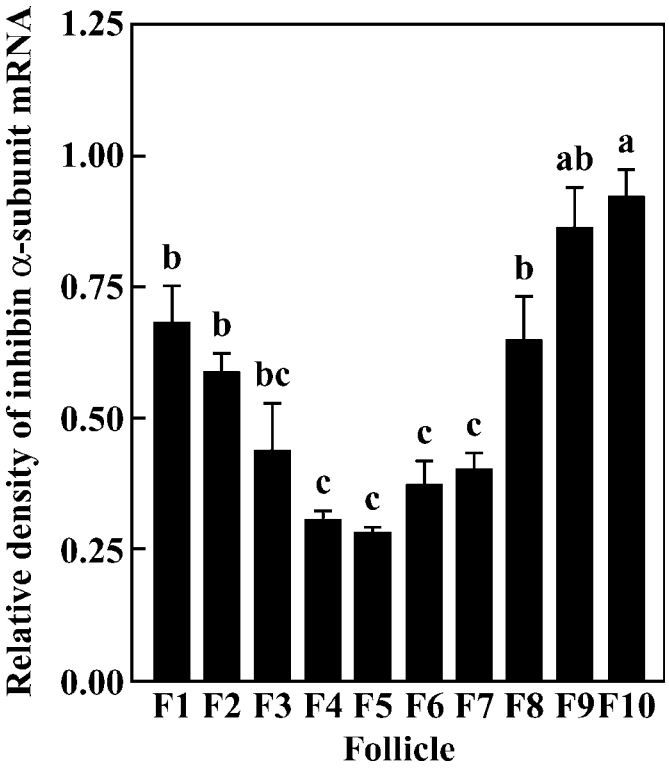

Figure 4. The relative density of the inhibin α-subunit mRNA in the individual F1 to F10 hierarchical follicles of the Growth line hens. Values are means ± SEM, n = 6 replicate hens. [a–c]Means with different letters differ, $P < 0.05$.

expression than the granulosa cells from the F1 follicle, but there was no difference in inhibin/activin β_A-subunit mRNA expression between the granulosa cells from the F2 and F3 follicles (Figure 6). The overall mRNA expression of the inhibin/activin β_A-subunit was significantly greater in the F1 to F4 hierarchical follicles from the Growth line hens than the Egg line hens due to the significantly higher relative mRNA expression of the inhibin/activin β_A-subunit in the granulosa cells from the F2 and F3 follicles of the Growth line hens than the Egg line hens. The relative mRNA expression of the inhibin/activin β_A-subunit between the individual F1, F2, and F3 follicles of Egg and Growth line hens was (mean ± SEM) 0.60 ± 0.20 vs. 0.84 ± 0.09; 0.04 ± 0.03 vs. 0.69 ± 0.15; and 0.03 ± 0.01 vs. 0.61 ± 0.2, respectively.

Expression of Follistatin and the Inhibin/Activin β_B-Subunit

Follistatin and the inhibin/activin β_B-subunit mRNA were detected in the combined theca and granulosa samples of the SYF and LWF of Egg and Growth line hens (data not shown). Despite extended film exposure times, no mRNA expression for the inhibin/activin β_B-subunit and follistatin were detected in the hierarchical granulosa samples (data not shown).

DISCUSSION

The correlation between selection for rapid growth rate and a decline in reproductive fitness is well established in poultry breeding. In particular, the relationship between egg production and body weight when selecting for either one of these traits is well documented for the

Egg and Growth lines of turkeys used in the present research (Nestor et al., 1996, 2000). The negative impact of genetic selection for rapid growth rate on reproductive fitness has been observed in the form of decreased egg production and increased numbers of hierarchical follicles in meat strain turkeys as compared with egg strain turkeys (Nestor et al., 1970, 1980; Liu et al., 2001). Jaap (1969) proposed that the inheritance for rapid growth favors rapid protein anabolism and may also favor rapid formation of lipoprotein in the liver and therefore increased yolk production for ovarian follicular development. In the present study, at 45 and 58 wk of age, Growth line hens selected for rapid growth rates had a significantly greater number of hierarchical follicles and nonhierarchical follicles (with the exception of SY follicles at 58 wk of age) than Egg line hens. These findings are in accordance with those of Nestor et al. (1970) and Nestor and Bacon (1972) and support the theory that selection for growth has led to increased yolk production (Jaap, 1969) when total follicular weight is considered.

In contrast to previous studies, the present study considered follicular weights relative to BW, which revealed that Egg line hens actually had significantly greater relative total follicular weights and thus total yolk mass at 45 and 58 wk of age when compared with Growth line hens. Individual relative follicular weights for each of the 5 largest hierarchical follicles, SYF, and LWF of the Egg line hens were significantly greater at 45 and 58 wk than in the Growth line hens. If yolk production is considered relative to BW, Egg line hens are, in fact, producing more yolk than Growth line hens on a gram per gram basis, which contradicts the theory of increased yolk synthesis capacity in hens selected for growth.

The F6 and F7 follicles had equivalent follicular weights between the 2 lines at 45 and 58 wk of age. As follicular maturation proceeded from this point forward, the deposition of yolk in the Egg line hens occurred in a very orderly fashion such that a distinct size hierarchy based on total yolk deposition was established. This was not the case for the Growth line hens. Schuster et al. (2004) reported that high levels of occludin expression in the tight junctions located between granulosa cells of white follicles prevents the paracellular transport of lipid [very low density lipoproteins (**VLDL**) particles] and thus yellow yolk deposition. Occludin levels decrease drastically just prior to the initiation of yellow yolk deposition (Schuster et al., 2004). Once the paracellular pathway was open for lipid transport, Schuster et al. (2004) indicated that lipoprotein uptake by follicles would depend on the oocyte's receptor-mediated uptake capacity and the delivery of VLDL particles to the plasma membrane of the oocyte. The reasons for the failure of the F1 to F5 follicles of the Growth line hens to take up yolk at differential rates are unclear. It is possible that, with the greater number of developing follicles, there is actually a deficiency in available VLDL from liver synthesis to meet the lipid demands of all the follicles. It is also possible that, as the F6 follicle matures into an F5 follicle and beyond in the Growth line hens vs. the Egg line hens, there is a severe depression in the receptor-mediated uptake capacity of lipid so that the follicle takes up little additional lipid before ovulation. For example, at 45 wk of age, the F5 follicle in the Growth line hens only gained 30% more weight as it developed for several days into an F1 follicle. In contrast, there was a 102% increase in follicular weight as the F5 follicle transitioned into the F1 follicle in the Egg line hens.

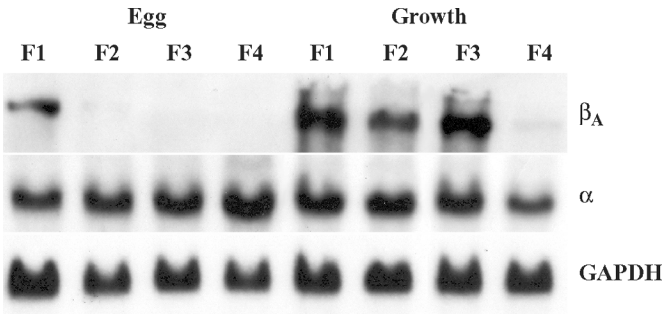

Figure 5. Autoradiograms from the Northern analysis of chicken inhibin α- and β_A-subunits from one of the replicate blots. Total RNA (40 μg) was loaded for each F1 to F4 follicle sample from Egg line and Growth line hens. GAPDH = glyceraldehyde-3-phosphate dehydrogenase; α = inhibin α-subunit; β_A = inhibin β_A-subunit.

The relative follicular weights obtained for the F1 follicles of both genetic lines of hens in the current research are indicative of a previous report that examined egg size in these 2 genetic lines of hens. Selection for increased egg production in the Egg line of turkeys has resulted in a reduction in total egg weight that has been proportional across all components of the egg (Nestor and Noble, 1995). However, selection for rapid BW gains in the Growth line of turkeys has resulted in an increase in total egg weight that is primarily the result of a disproportional increase in the amount of albumen (Nestor and Noble, 1995).

Why the capacity for follicular lipid uptake would be downregulated in the large follicles of the Growth line hens is unclear. However, we would note that the production of hormones capable of paracrine and autocrine actions within the largest hierarchical follicles might be different. Specifically, the current research indicates that the production of inhibin and activin may be altered between the 2 genetic lines of turkey hens because the mRNA expression patterns for the inhibin α-subunit and the inhibin/activin β_A-subunit were significantly different between Egg and Growth lines of hens.

The pattern of mRNA expression of the inhibin/activin subunits in the granulosa layer during follicular development in the White Leghorn laying hen has been well characterized (Davis and Johnson, 1998). Specifically, the mRNA for the inhibin α-subunit was greatest in the F5 follicle, and it decreased significantly with each advance in the hierarchy to the F3 follicle stage at which point it remained constant through ovulation (Davis and Johnson, 1998). The mRNA expression of the inhibin α-subunit decreased significantly in the hierarchical folli-

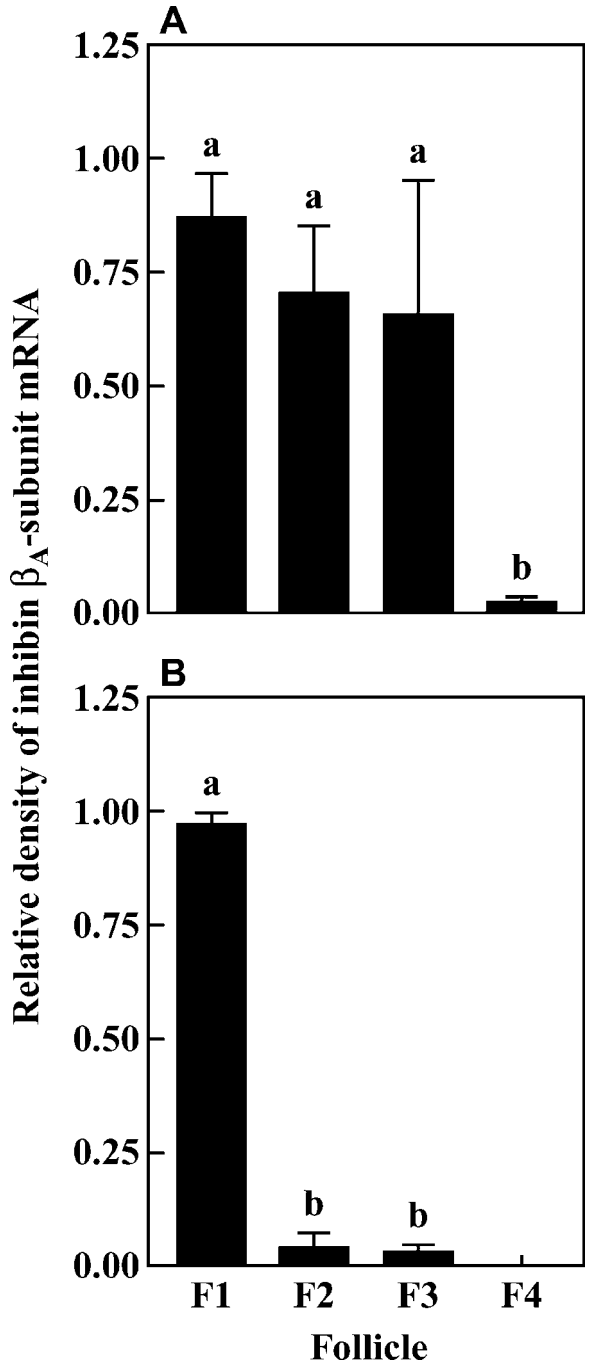

Figure 6. The relative density of the inhibin β_A-subunit mRNA in the individual F1 to F4 hierarchical follicles of the Growth line hens (A) and Egg line hens (B). Values are means ± SEM, n = 6 replicate hens. [a,b]Means for each genetic line with different letters differ, $P < 0.05$. Note that the relative densities of inhibin β_A-subunit mRNA to one another are specific for each genetic line of turkey hen and that the expression of the mRNA for the inhibin β_A-subunit was not detectable in the F4 follicle samples obtained from the Egg line hens.

cles smaller than F5 and was undetectable in LWF (Davis and Johnson, 1998). In contrast, expression of the inhibin/activin β_A-subunit is greatest in the F1 follicle with considerably lower amounts detected in the other hierarchical follicles (Davis and Johnson, 1998). Results from protein expression studies are consistent with the mRNA expression patterns discerned for the inhibin/activin subunits in the ovarian follicles of the hen. Specifically, the F1 follicle granulosa cells produce by far the most inhibin-A and very little activin-A (Lovell et al., 1998, 2003).

The expression of the inhibin α-subunit mRNA in Growth line turkey hens observed in the present study was not characteristic of the pattern observed in chicken laying hens. Specifically, this pattern was reversed in the Growth line hens with lower levels of inhibin α-subunit mRNA being expressed by the F4 or F5 follicles than the F1 or F2 follicles and the F9 or F10 follicles. Egg line turkey hen follicles exhibited an inhibin α-subunit mRNA expression pattern more similar to laying hens whereby decreasing inhibin α-subunit expression was observed as follicles matured from F4 to F1. Although there was no significant difference in inhibin α-subunit expression between the individual Egg line follicles, a regression analysis indicated that inhibin α-subunit expression significantly decreased ($P < 0.05$) as follicles matured from an F4 to an F1.

Expression of the inhibin/activin β_A-subunit in Growth line hens was also atypical compared with the pattern observed in laying hens and in Egg line hens. In Egg line hens, mRNA expression followed the pattern of expression observed previously in laying hens (Davis and Johnson, 1998) with significantly greater inhibin β_A-subunit mRNA expression detected in the F1 follicle as compared with the F2 and F3 follicles. In Growth line hens, however, no significant differences in mRNA expression of the inhibin β_A-subunit were observed in the F1 to F3 follicles. The abnormal expression patterns observed for the inhibin α-subunit and the inhibin/activin β_A-subunit may be responsible for the absence of a distinct follicular hierarchy observed in the Growth line hens. Previous studies in laying hens indicate that the production of inhibin-A is vastly greater in the granulosa cells of the F1 follicle than in the granulosa cells of other hierarchical or nonhierarchical follicles (Akashiba et al., 1988; Lovell et al., 1998, 2003). However, the abnormal expression patterns of the inhibin α-subunit and the inhibin/activin β_A-subunit in the Growth line turkey hens suggest that the F1 follicle may not be producing a greater amount of bioactive inhibin-A than the F2 and F3 preovulatory follicles. Furthermore, total inhibin-A production may exceed normal production levels if all 3 (F1 to F3) follicles are secreting inhibin in the Growth line hens. The abundant production of inhibin-A may be disrupting normal follicular development and ovulation in the Growth line hens. Further research is needed to determine if production of inhibin-A is higher in the Growth line hens than the Egg line hens.

Even though the Growth line hens have a greater number of preovulatory follicles in rapid development (hierarchical follicles) than the Egg line hens, on a per bird basis the Growth line hens produce less than half the total number of eggs than the Egg line hens. Although the current research focused on differences in the follicular hierarchy of these 2 lines of turkey hens, pertinent

observations were made that affect total egg production. Over a 2-yr period, we collected follicular tissue samples from 57 Growth line and 50 Egg line hens at 45 or 58 wk of age for the current research and other research. The incidence of hierarchical follicular atresia was equally rare between the 2 genetic lines of hens (data not shown). The incidence of internal ovulations, however, between the 2 lines of hens differed greatly. No signs of internal ovulation were detected in any of the Egg line hens, whereas 27 of the 57 Growth line hens had free-floating yolk material in their abdominal cavity. Furthermore, 5 of the Growth line hens had eggs (membrane, hard-shelled, or both) in their abdominal cavity. The number of eggs in the abdominal cavity of these hens ranged from 1 to 8. The biological reasons for the internal ovulations and the reverse peristalsis that allowed fully formed eggs to be deposited in the abdominal cavity in hens that were laying normal eggs is unknown and warrants further investigation.

In summary, selection for egg production in the Egg line of turkey hens and for increased 16-wk BW in the Growth line of turkey hens has resulted in the hens of these 2 lines having distinct differences in their follicular hierarchies. Although the Growth line hens have a larger follicular hierarchy, total follicular weight relative to BW is significantly smaller than the relative weight of the Egg line follicular hierarchy. The deposition of yolk material in the 5 largest follicles of the Growth line follicular hierarchy does not proceed at the rate of deposition found in the Egg line hens, and this results in the ovulation of a relatively smaller follicle in the Growth line hens than in the Egg line hens. Finally, the lack of size definition in the follicular hierarchy of Growth line hens compared with Egg line hens may result from abnormally high inhibin-A production in the largest follicles of the Growth line hens based on the mRNA expression of the inhibin-A subunits in these hens.

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