Characterization of hypothalamo–pituitary–thyroid axis gene expression in the hypothalamus, pituitary gland, and ovarian follicles of turkey hens during the preovulatory surge and in hens with low and high egg production

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ABSTRACT Dysregulation of the preovulatory surge (**PS**) leads to lowered egg production. The hypothalamo–pituitary–thyroid (**HPT**) axis has been shown to influence plasma progesterone levels and follicle ovulation. The presence of thyroid hormone receptors (**THR**) in the reproductive axis suggests possible effects of thyroid hormone. To further understand the potential role of thyroid hormone on the PS, HPT axis plasma hormone concentrations and gene expression were characterized surrounding the PS in average egg producing hens (**AEPH**), low egg producing hens (**LEPH**), and high egg producing hens (**HEPH**) (n = 3 hens/group). Data were analyzed using the mixed models procedure of SAS, with significance indicated at $P < 0.05$. Average egg producing hens and HEPH displayed lower levels of triiodothyronine (**T3**) and higher levels of thyroxine (**T4**) inside of the PS, whereas LEPH showed inverse T3 and T4 levels relative to the PS. Expression of mRNA for hypothalamic thyrotropin-releasing hormone (***TRH***), pituitary thyrotropin (***TSHB***), and the main thyroid hormone metabolism enzyme (***DIO2***) were downregulated during the PS in AEPH and HEPH. Low egg producing hens displayed higher expression of mRNA for hypothalamic *TRH* as well as pituitary *TSHB* and *DIO2* compared with HEPH. Average egg producing hens expression of THR mRNAs was upregulated during the PS in the hypothalamus but downregulated in the pituitary. High egg producing hens showed decreased expression of THR mRNAs in both the hypothalamus and pituitary when compared with LEPH. In ovarian follicles, THR mRNAs were more prevalent in the thecal layer of the follicle wall compared with the granulosa layer, and expression tended to decrease with follicle maturity. Minimal differences in follicular THR expression were seen between LEPH and HEPH, indicating that THR expression is unlikely to be responsible for steroid hormone production differences occurring between LEPH and HEPH. Generally, downregulation of the HPT axis was seen during the PS in AEPH and HEPH, whereas upregulation of the HPT axis was seen in LEPH. Further studies will be required to clarify the role of the HPT axis in the regulation of ovulation and egg production rates in turkey hens.

Key words: turkey, egg production, HPT axis, HPG axis, preovulatory surge

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INTRODUCTION

Two neuroendocrine axes have been shown to regulate reproductive activity in avian species, the hypothalamo–pituitary–gonadal (**HPG**) axis and the hypothalamo–pituitary–thyroidal (**HPT**) axis (Follett and Nicholls, 1985). The HPT axis is comprised of the hypothalamus, the anterior pituitary gland, and the thyroid gland and predominantly regulates metabolism (Paster, 1991); however, proper HPT axis function is necessary for egg production to occur (Lien and Siopes, 1989a). Previous studies in avian species have defined a role for the HPT axis in the initiation and cessation of egg production (Proudman and Siopes, 2006) as well as in the regulation of seasonal rhythms (Nakayama and Yoshimura, 2017). The HPT axis has not been characterized in commercial lines of turkey hens during peak egg production,

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and the influences of hen egg production level and of the reproductive preovulatory hormone surge (**PS**) on HPT axis function have yet to be investigated.

Within the HPT axis, hypothalamic thyrotropin-releasing hormone (**TRH**) is released and binds to TRH receptors on pituitary thyrotrophs, leading to release of thyroid-stimulating hormone (**TSH**) and upregulation of both TSH subunits (***CGA*** and ***TSHB***) (Fekete and Lechan, 2014). The TSH acts on the thyroid to induce production of the thyroid hormones, triiodothyronine (**T3**), and thyroxine (**T4**). The T3 and T4 production occurs by the iodination of the tyrosine residues on thyroglobulin followed by protease digestion to release T3 and T4 (McNabb and Darras, 2014). Circulating T4 is less active than T3 but can be converted to T3 by deiodinases (Decuypere et al., 2005). The enzyme type II deiodinase is responsible for the conversion of T4 to T3 in target tissues and is encoded by *DIO2* (Nakane and Yoshimura, 2014).

Thyroid hormones elicit their actions through the binding of thyroid hormone receptors (**THR**). Thyroid hormone receptors are present in the each tissue of the HPG axis in avian species, namely the hypothalamus, pituitary, and ovary (Sechman, 2012). Two types of receptors are capable of thyroid hormone binding, the nuclear receptors THRs α and β, and the integrin cell membrane receptors. Thyroid hormone receptors α and β are encoded by *THRA* and *THRB*, respectively, whereas, the 2 genes *ITGAV* and *ITGB3* encode the 2 subunits of the integrin receptor (Hammes and Davis, 2015). Nuclear THR elicits genomic effects by recruiting coactivators to thyroid response elements in target genes to regulate transcription (Cheng et al., 2010). The integrin receptor regulates nongenomic effects such as protein translocation and phosphorylation events (Davis et al., 2008). Both types of THR have been shown to be present in the hypothalamus, the pituitary, and the ovarian follicles, indicating that thyroid hormone is capable of eliciting both genomic and nongenomic effects in the tissues of the avian reproductive axis (McNabb, 2007).

Commercial line turkey hens show a large variation in egg production levels that we have classified as groups of low egg producing hens (**LEPH**), average egg producing hens (**AEPH**), and high egg producing hens (**HEPH**). Hypothalamic and pituitary transcriptome analysis in LEPH and HEPH inferred upregulation of thyroid hormone production and metabolism in LEPH when compared with HEPH (Brady et al., 2020c). The goals of this study were to define normal HPT axis function in AEPH and to characterize how HPT axis function might be perturbed in LEPH and HEPH. In addition, the impact of fluctuations in reproductive hormones during the ovulatory cycle on HPT function was assessed in the 3 egg production level groups. This study examined AEPH, LEPH, and HEPH turkey hens sampled outside and inside of the PS to (1) define the plasma concentrations of T3 and T4 and (2) characterize the expression of key HPT axis genes in the reproductive tissues, namely the hypothalamus, pituitary, and the preovulatory follicle layers responsible for progesterone and estradiol production, the F1 granulosa, and the F5 theca externa, respectively.

MATERIALS AND METHODS

Hen Selection and Cell Isolation

Hens (200 hens total) from a commercial line (Hendrix Genetics, Kitchener, Ontario, Canada) were housed in individual cages at the Beltsville Agricultural Research Center. Hen maintenance, lighting schedule, and diet have been described previously (Brady et al., 2019). Hens were classified as AEPH, LEPH, and HEPH based on eggs per day calculation cutoffs as previously described (Brady et al., 2019, 2020a). In brief, eggs per day cutoffs were as follows: LEPH (<0.6 eggs per day), AEPH (0.68–0.72 eggs per day), and HEPH (>0.8 eggs per day). All hens were sampled at 37 wk of age and with the second egg of the hen's laying sequence in the reproductive tract. Estimation of the timing of the PS was performed based on egg lay timing, as previously described (Brady et al., 2019). The hypothalamus, pituitary, F1 follicle, and F5 follicle were isolated from 6 AEPH, 6 LEPH, and 6 HEPH, with half of each group sampled outside of the PS and half during the PS (n = 3 per group). Hens were confirmed to be outside or during the PS by plasma progesterone levels from blood samples taken immediately before tissue sampling as previously described (Brady et al., 2019). The hypothalamus and pituitary were snap frozen as whole tissues, whereas the granulosa and theca externa layers were isolated from the F1 and F5 follicles as previously described (Porter et al., 1989; Brady et al., 2019). All animal procedures were approved by the Institutional Animal Care and Use Committee at Beltsville Agricultural Research Center and at the University of Maryland.

Radioimmunoassay

The radioimmunoassay used for progesterone, T3, and T4 were coated tube kits (MP Biomedicals, Solon, OH), with all protocols performed as directed by the manufacturer. Technical replicates and validation for each radioimmunoassay were performed as previously described (Brady et al., 2019). The intraassay coefficients of variation determined by pools run every 30 samples were 4.26% for progesterone, 2.37% for T3, and 2.06% for T4. The ratio of T3 to T4 plasma concentrations was calculated by dividing the T3 concentration by the T4 concentration for each hen.

RT-qPCR

Total RNA was isolated from the hypothalamus, pituitary, and ovarian granulosa and theca externa cell from the F1 and F5 follicles, respectively, with RNeasy Mini kits (Qiagen, Valencia, CA), including on-column deoxyribonuclease digestion. Quantification of RNA, reverse transcription reactions, and RT-qPCR were performed as previously described (Brady et al., 2019).

Reactions were diluted by tissue type as previously described before PCR analysis (Brady et al., 2019). Primers (IDT, Skokie, IL) were designed and used with cycling parameters described previously (Brady et al., 2019). Data normalization and analysis were performed as previously described (Brady et al., 2019).

Statistics

The SAS software (SAS Institute, Cary, NC) was used to analyze log2 transformed RT-qPCR data. A one-way ANOVA using the mixed models procedure (PROC MIXED) was used to compare plasma hormone concentrations and log2 transformed gene expression data from AEPH outside and inside of the PS. A two-way ANOVA using the mixed models procedure (PROC MIXED) was conducted to compare plasma hormone concentrations and log2 transformed gene expression data from LEPH and HEPH outside and inside of the PS. The least squares means for each group were compared using the test of least significant difference (PDIFF statement) when this indicated an overall significance level of $P < 0.05$. The AEPH data are presented relative to basal steroid hormone concentration or gene expression, whereas, data from LEPH and HEPH are presented relative to LEPH basal steroid hormone concentration or gene expression.

RESULTS

In AEPH and HEPH, plasma T3 concentrations were significantly higher outside of the PS, whereas, LEPH showed no change in plasma T3 concentrations during the ovulatory cycle (Figures 1A and 1B). Additionally, HEPH displayed plasma T3 levels outside of the PS that were 1.4-fold higher than levels seen in LEPH. No differences in plasma T3 levels were seen between LEPH and HEPH during the PS. Plasma T4 concentrations showed a 2-fold increase during the PS in AEPH and HEPH and a 3-fold decrease during the PS in LEPH (Figures 1C and 1D). While T4 plasma levels did not differ between LEPH and HEPH outside of the

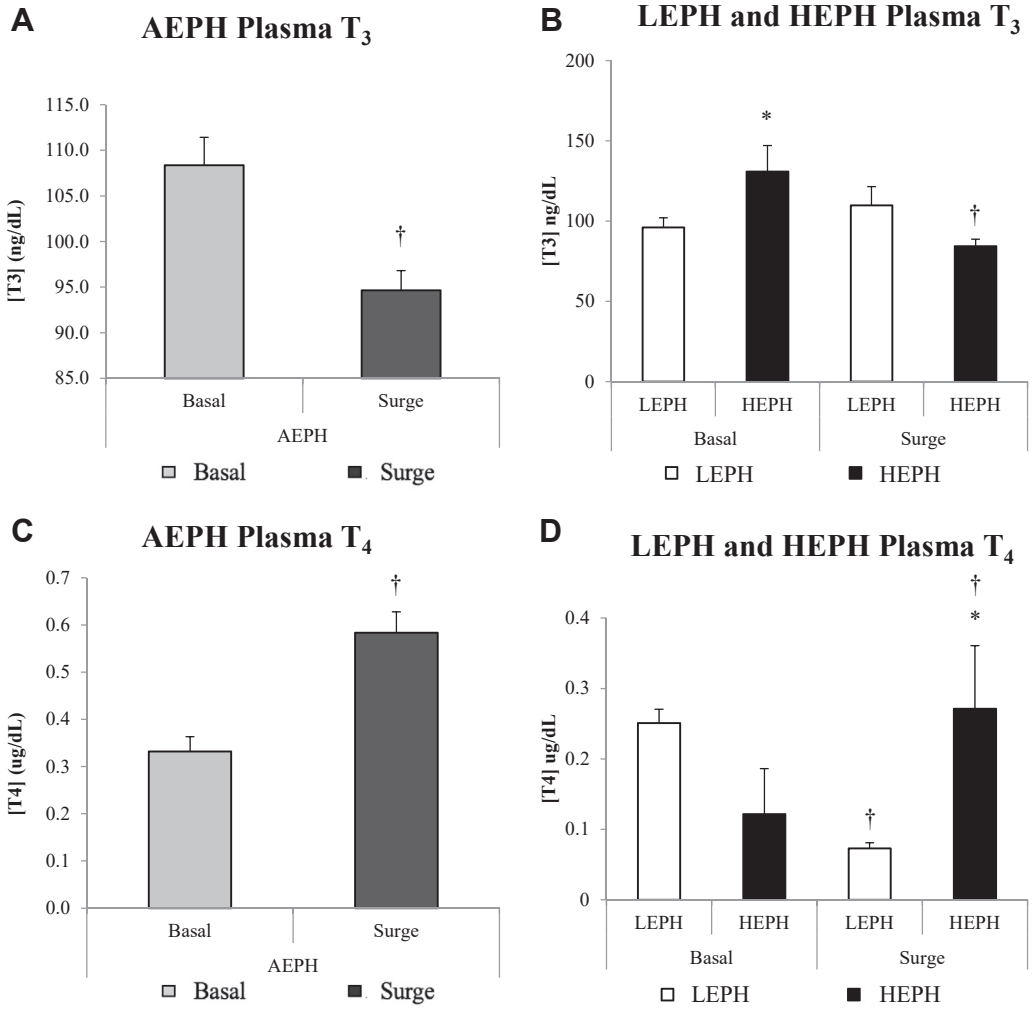

Figure 1. Plasma triiodothyronine (T3) and thyroxine (T4) hormone profiles in average. egg producing hens (AEPH) (Figures 1A and 1C) as well as low egg producing hens (LEPH) and high egg producing hens (HEPH) (Figures 1B and 1D). All 3 groups of hens were sampled outside (basal) and inside (surge) of the preovulatory surge (PS). Significant plasma thyroid hormone concentration differences between LEPH and HEPH for a given condition are denoted with an asterisk, whereas significant differences between basal and surge plasma thyroid hormone concentrations for a given egg production group are denoted with a dagger.

PS, T4 plasma levels during the PS were 3.6-fold higher in HEPH when compared with LEPH.

The T3:T4 ratio in AEPH decreased significantly during the PS, dropping from 0.3 to 0.2 (Figure 2A). This same trend was seen in the T3:T4 ratio of HEPH with the T3:T4 ratio decreasing over 10-fold during the PS, whereas in LEPH, the T3:T4 ratio increased nearly 4-fold during the PS (Figure 2B). In addition, HEPH displayed a 6.3-fold higher T3:T4 ratio outside of the PS and a 6.5-fold lower T3:T4 ratio during the PS when compared with LEPH. Combined T3 and T4 plasma concentrations were 1.5- and 2.4-fold higher during the PS in AEPH and HEPH, respectively, compared with basal levels but 1.9-fold higher outside of the PS in LEPH compared with levels during the PS (Figures 2C and 2D). Moreover, LEPH displayed an 84% higher combined T3 and T4 plasma concentration outside of the PS, but a 60.5% lower combined T3 and T4 plasma concentration during the PS when compared with HEPH.

Hypothalamic expression of the main stimulatory releasing factor as well as 2 THR differed significantly in AEPH outside and during the PS (Figure 3A). Thyrotropin-releasing hormone mRNA levels decreased by 2-fold in AEPH during the PS, in contrast to levels outside of the PS. Expression of 2 THR, *THRB* and *ITGB3*, exhibited 1.7- and 2.9-fold increases in AEPH during the PS. In LEPH and HEPH, significant hypothalamic gene expression differences were seen in *TRH* and in THR *THRA*, *THRB*, and *ITGB3* (Figure 3B). Similar to AEPH, HEPH showed a 2-fold reduction in mRNA levels for *TRH* during the PS, whereas LEPH showed a 1.65-fold increase in mRNA levels for *TRH* during the PS. The HEPH also exhibited a 1.3-fold increase in mRNA levels for *THRB* during the PS as was seen in AEPH, whereas LEPH showed a 1.4-fold decrease in *THRB* mRNA levels during the PS. On the other hand, LEPH showed a nearly 2-fold increase in mRNA levels for *ITGB3* during the PS as was also seen in AEPH, whereas HEPH exhibits a 2.5-fold

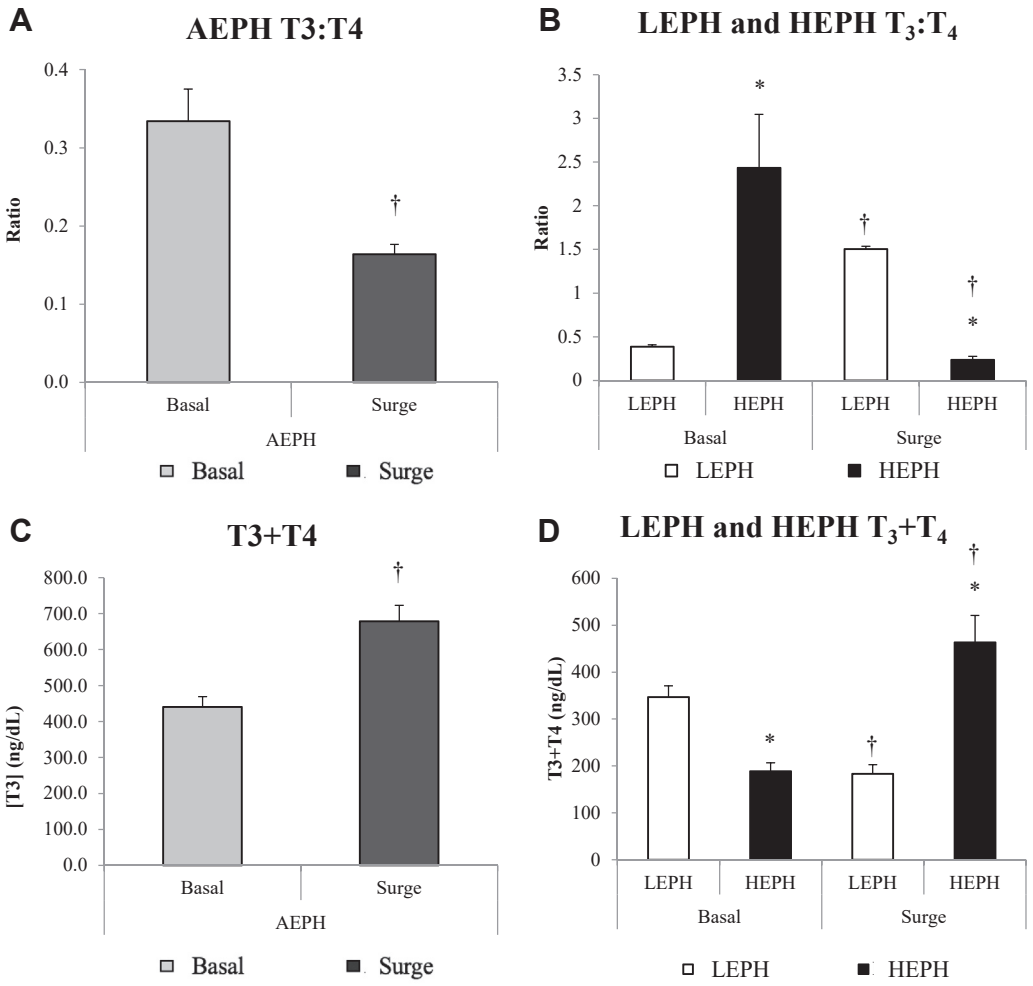

Figure 2. Triiodothyronine (T3) to thyroxine (T4) ratio in average egg producing hens. (AEPH) (A) as well as low egg producing hens (LEPH) and high egg producing hens (HEPH) (B). All 3 groups of hens were sampled outside (basal) and inside (surge) of the preovulatory surge (PS). Combined T3 and T4 plasma concentrations of AEPH under basal and surge conditions as well as of LEPH and HEPH, taking the PS into account, are shown in Figures 2C and 2D, respectively. Significant thyroid hormone ratio or combined plasma concentration differences between LEPH and HEPH for a given condition are denoted with an asterisk, whereas significant differences between basal and surge plasma thyroid hormone concentrations for a given egg production group are denoted with a dagger.

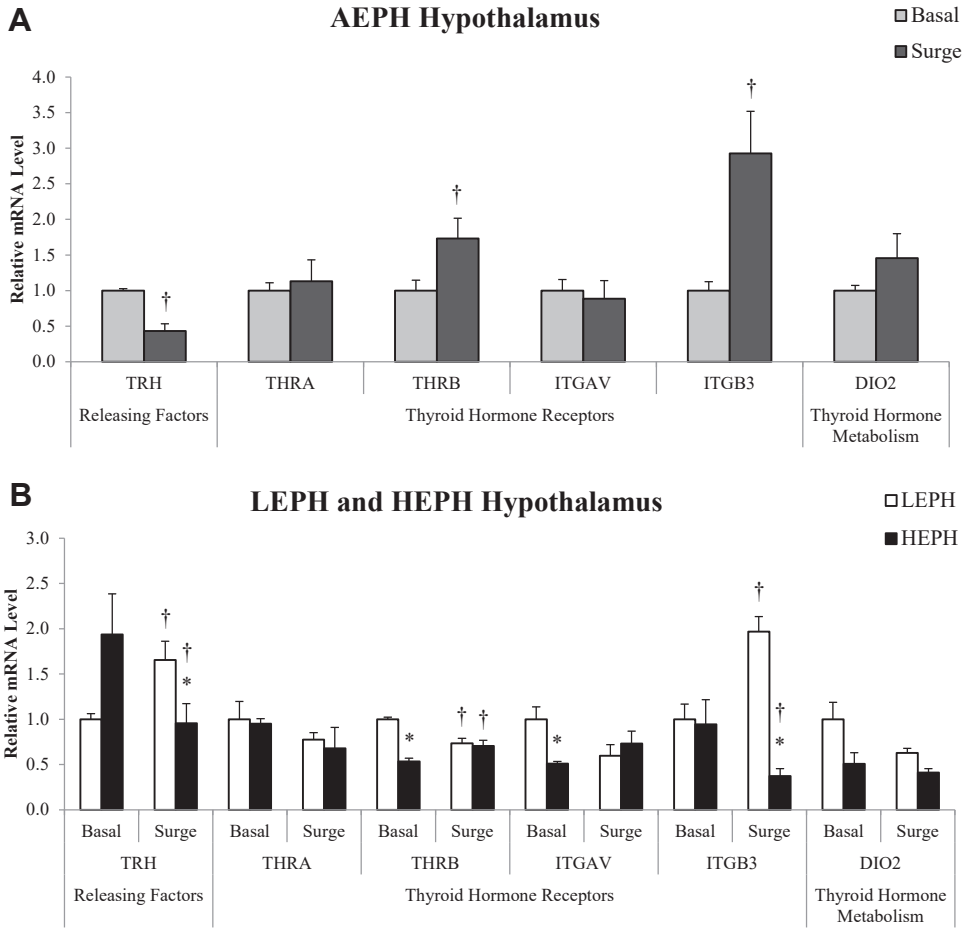

Figure 3. Hypothalamic gene expression of hypothalamo–pituitary–thyroid axis releasing factors, thyroid hormone receptors, and thyroid hormone metabolism enzymes in average egg producing hens (AEPH), low egg producing hens (LEPH), and high egg producing hens (HEPH) sampled outside (basal) and inside (surge) of the preovulatory surge (PS). The AEPH expression under basal and surge conditions is presented in Figure 3A, whereas LEPH and HEPH expression, taking the PS into account, is presented in Figure 3B. Normalized data are presented relative to basal expression for each gene. Significant expression differences between LEPH and HEPH for a given condition are denoted with an asterisk, whereas significant differences between basal and surge expression for a given egg production group are denoted with a dagger.

decrease in *ITGB3* mRNA levels during the PS. Additionally, LEPH showed higher mRNA levels for *TRH* and for 3 of the 4 THR than those seen in HEPH. The LEPH displayed upregulation of *THRB* and *ITGAV* outside of the PS as well as *TRH* and *ITGB3* during the PS when compared with HEPH.

Pituitary expression of 2 THR, the main thyrotropin subunit, and the deiodinase involved in T4 metabolism to T3 differed in AEPH outside and during the PS (Figure 4A). Thyroid hormone receptors *THRB* and *ITGB3* both exhibited a 1.7-fold reduction in mRNA levels in AEPH during the PS. Moreover, a 1.5- and 1.9-fold reduction in expression of *TSHB* and *DIO2* was seen in AEPH during the PS when compared with mRNA levels outside of the PS. Significant expression differences between LEPH and HEPH were seen for *THRB*, *ITGB3*, as well as for *TSHB* and *DIO2* (Figure 4B). The HEPH exhibited 2-, 1.6-, and 2.2-fold decreases in mRNA levels for *THRB*, *TSHB*, and *DIO2*, respectively, during the PS, similar to expression trends seen in AEPH. The LEPH also showed 1.5- and 2.5-fold decreases in mRNA levels for *THRB* and *TSHB*, respectively, during the PS but did not show decreased expression of *DIO2* during the PS. The LEPH also showed increased expression of *ITGB3* under basal and surge conditions, of *TSHB* outside of the PS, and of *THRB* during the PS, when compared with HEPH. The HEPH displayed increased mRNA levels for *DIO2* outside of the PS but decreased mRNA levels for *DIO2* during the PS in relation to LEPH expression.

Thyroid axis gene expression in the F1 granulosa layer was not impacted by the preovulatory surge or by egg production level (Figures 5A and 5B). In the F5 theca externa, expression of 3 of the 4 THR genes was significantly different between AEPH outside and during the PS (Figure 6A). In AEPH, *THRA* mRNA levels were reduced by 2.8-fold during the PS, whereas expression of integrin subunits, *ITGAV* and *ITGB3*, was upregulated by 1.9- and 2.4-fold, respectively, during the PS. The LEPH and HEPH only showed differential mRNA levels for *ITGAV* (Figure 6B). Diverging from AEPH, LEPH showed a 3.8-fold decrease in expression of *ITGAV* during the PS, whereas HEPH showed no change in *ITGAV* mRNA levels during the PS.

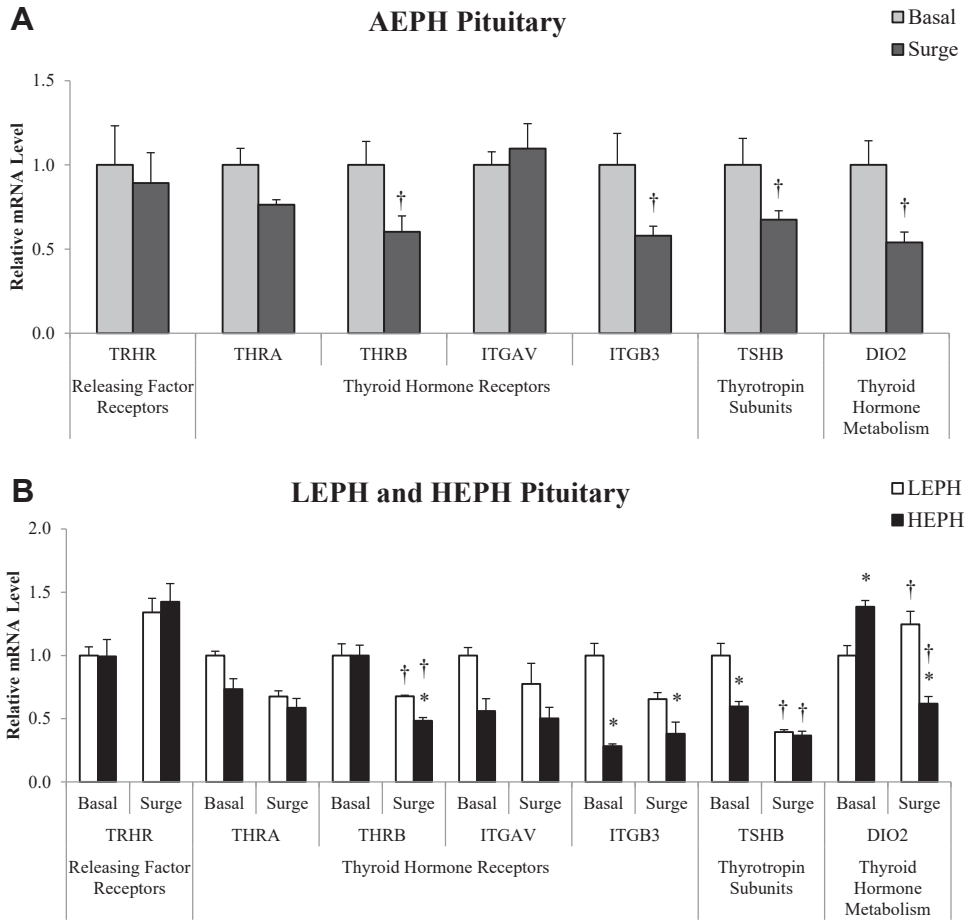

Figure 4. Pituitary gene expression for hypothalamo–pituitary–thyroid axis. releasing factor receptors, thyrotropin subunits, thyroid hormone receptors, and thyroid hormone metabolism enzymes in average egg producing hens (AEPH), low egg producing hens (LEPH), and high egg producing hens (HEPH) sampled outside (basal) and inside (surge) of the preovulatory surge (PS). The AEPH expression under basal and surge conditions is presented in Figure 4A, whereas LEPH and HEPH expression, taking the PS into account, is presented in Figure 4B. Normalized data are presented relative to basal expression for each gene. Significant expression differences between LEPH and HEPH for a given condition are denoted with an asterisk, whereas significant differences between basal and surge expression for a given egg production group are denoted with a dagger.

Additionally, LEPH showed decreased mRNA levels for *ITGA V* both outside and during the PS when compared with HEPH.

DISCUSSION

The HPT axis circulating thyroid hormone levels and expression of genes related to thyroid hormone production and metabolism differed during the ovulatory cycle and with egg production level. In general, HEPH displayed thyroid axis hormone profiles and gene expression consistent with those seen in AEPH, whereas LEPH tended to display opposite trends. In addition, LEPH tended to exhibit higher basal thyroid axis expression when compared with HEPH. This investigation is the first study to quantify T3 and T4 plasma concentrations surrounding the PS in commercial turkey hens with varied egg production levels. Furthermore, this study provides novel insights into the mRNA expression of key HPT axis genes, also taking the expression changes with regard to egg production levels and position in the ovulatory cycle into account. Based on the results from this study, HPT axis function is not consistent during the hen ovulatory cycle or in hens with differential egg production, reinforcing the intertwining roles of the thyroid and reproductive axes in the regulation of ovulation.

Thyroid hormone concentrations had not been previously examined in avian species with regard to the ovulatory cycle or to egg production level. Prior studies focused on plasma concentrations of the thyroid hormones during initiation of egg lay and during periods of ovarian regression, mainly focusing on photoresponsiveness and photorefractoriness (Lien and Siopes, 1989b; Siopes et al., 2010). Earlier studies found that suppression of T3 plasma levels is necessary for initiation of egg lay (Decuypere et al., 2005). In the current study, AEPH and HEPH plasma levels of T3 declined 12.7% and 35.5%, respectively, during the PS, whereas, no changes in plasma T3 levels during the PS in LEPH were exhibited. Decreased circulating T3 following PS could play a role in initiating the next ovulation of a hen's laying sequence. Earlier studies showed that increased T4 plasma concentrations are associated

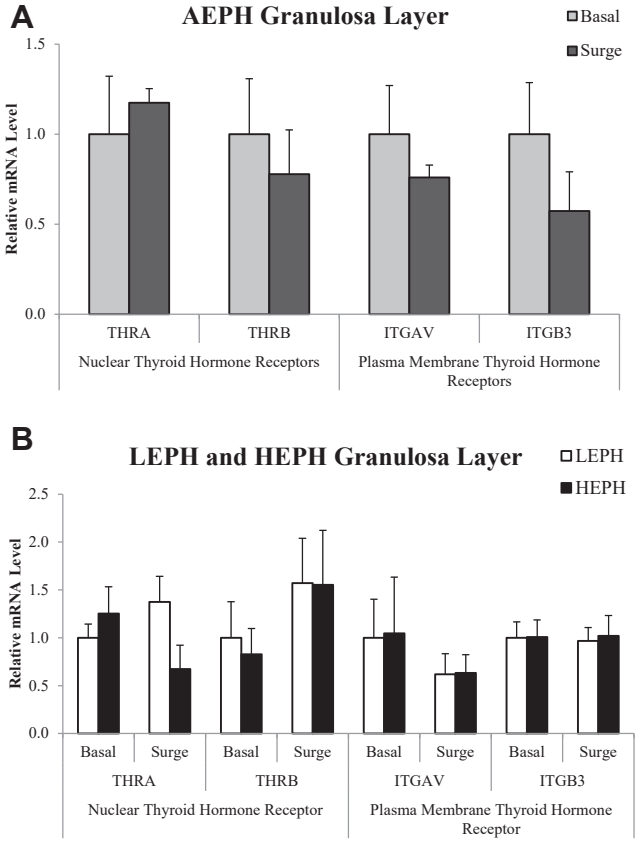

Figure 5. F1 follicle granulosa layer gene expression of hypothalamo–pituitary–thyroid. axis thyroid hormone receptors in average egg producing hens (AEPH), low egg producing hens (LEPH), and high egg producing hens (HEPH) sampled outside (basal) and inside (surge) of the preovulatory surge (PS). The AEPH expression under basal and surge conditions is presented in Figure 5A, whereas LEPH and HEPH expression, taking the PS into account, is presented in Figure 5B. Normalized data are presented relative to basal expression for each gene. Significant expression differences between LEPH and HEPH for a given condition are denoted with an asterisk, whereas significant differences between basal and surge expression for a given egg production group are denoted with a dagger.

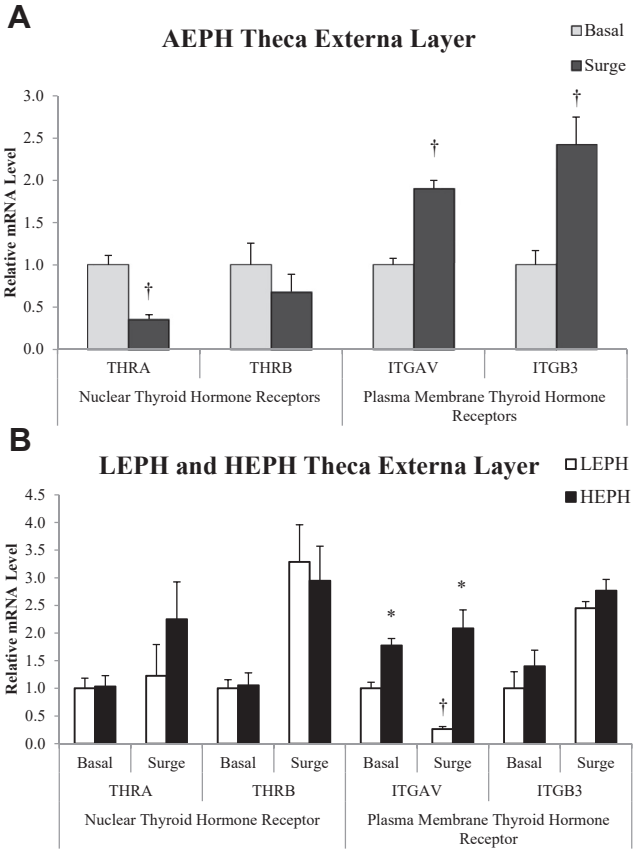

Figure 6. F5 follicle theca externa layer gene expression of hypothalamo–pituitary–thyroid axis thyroid hormone receptors in average egg producing hens (AEPH), low egg producing hens (LEPH), and high egg producing hens (HEPH) sampled outside (basal) and inside (surge) of the preovulatory surge (PS). The AEPH expression under basal and surge conditions is presented in Figure 6A, whereas LEPH and HEPH expression, taking the PS into account, is presented in Figure 6B. Normalized data are presented relative to basal expression for each gene. Significant expression differences between LEPH and HEPH for a given condition are denoted with an asterisk, whereas significant differences between basal and surge expression for a given egg production group are denoted with a dagger.

with molt, or gonadal regression, in both chicken and turkey hens (Sekimoto et al., 1987; Siopes, 1993). Our data broadened this concept to include ovulatory cycling throughout egg production, where both AEPH and HEPH, exhibited roughly a 2-fold increase in T4 plasma concentrations during the PS, but LEPH T4 plasma concentrations decreased over 3-fold during the PS. Increased circulating T4 during the PS may aid in returning the reproductive hormones involved in the PS to basal levels to prepare for the next ovulation. Finally, previous studies showed that the T3:T4 ratio in hens does not significantly change throughout the laying cycle (Proudman and Siopes, 2005); however, this is the first study to examine the ratio during the ovulatory cycle and among hens with varied egg production traits. The T3:T4 ratio differed during the ovulatory cycle in each group of hens examined, with AEPH and HEPH showing a similar reduction in the ratio and LEPH showing an increase in the ratio during the PS. These results indicate that not only individual plasma concentrations of T3 and T4 but also the ratio of T3:T4 may play a role in the rate of egg production.

Thyroid-related gene expression had not been previously examined in relation to the ovulatory cycle. The AEPH expression of the main hypothalamic releasing factor as well as pituitary thyrotropin and thyroid hormone metabolism enzymes were downregulated during the PS. Additionally, AEPH expression of THR was upregulated during the PS in the hypothalamus but downregulated in the pituitary, indicating possible differential regulation in the hypothalamus and pituitary. The HPT axis gene expression in the hypothalamus and pituitary of HEPH was more consistent with expression profiles seen in AEPH than LEPH, including similar expression profiles of hypothalamic *TRH* and *THRB* as well as pituitary *THRB*, *TSHB*, and *DIO2*. The LEPH, in contrast, only shared similar expression profiles of hypothalamic *ITGB3* and pituitary *THRB* and *TSHB* with AEPH. Earlier studies in mammalian models found that higher T3 and T4 concentrations,

both individually and combined, had an inhibitory effect on *TRH* mRNA levels (Kakucska et al., 1992). In the present study, though decreased expression of *TRH* during the PS in AEPH and HEPH coincided with a decrease in plasma T3 concentrations, combined plasma concentrations of T3 and T4 were significantly increased in AEPH and HEPH during the PS. In contrast, LEPH displayed a decrease in combined plasma concentrations of T3 and T4 during the PS, which coincided with increased expression of TRH. The negative correlation of TRH mRNA levels with combined plasma levels of T3 and T4 most likely results from thyroid hormone negative feedback on the HPT axis; however, the differences seen during the ovulatory cycle and in hens with differing egg production levels could indicate that thyroid hormone feedback may play a role in ovulatory timing and/or ovulation rates. Moreover, the increased mRNA levels for critical THR and hormone genes in the hypothalamus and pituitary in LEPH when compared with HEPH suggest that LEPH may be more capable of HPT axis stimulation and feedback in the hypothalamus and pituitary. Interestingly, HEPH displayed higher mRNA levels for pituitary *DIO2* outside of the PS compared with LEPH. Transcriptome analysis of red-feather Taiwan country chickens revealed that *DIO2* was upregulated in the pituitary of hens with high egg production under basal conditions (Chen et al., 2007).

In regard to ovarian follicle gene expression, THR was more prevalent in the thecal layer of the follicle wall than in the granulosa layer, and the expression of THR tended to decrease with follicle maturity. This is consistent with previous studies examining the effects of thyroid hormones on steroidogenesis (Sechman, 2012). However, not previously reported, THR in the F5 theca externa layer showed expression changes during the PS, further solidifying the potential role of thyroid hormones in the regulation of steroidogenesis. Triiodothyronine inclusion in F1 granulosa cell cultures in chickens increased progesterone synthesis as well as expression of genes related to progesterone production (Sechman et al., 2009). Previous work determined that HEPH displayed increased expression of progesterone production genes outside of the PS and increased progesterone synthesis in response to luteinizing hormone treatment in vitro when compared with LEPH (Brady et al., 2020a, 2020b). Coupled with the increased plasma T3 levels in HEPH outside of the PS in the present study, it is possible that differential levels of circulating thyroid hormones may partially regulate the differences seen in LEPH and HEPH progesterone gene expression and in vitro progesterone production by the F1 granulosa layer. The DIO2 was not found to be expressed in the F1 granulosa layer, which may prevent T4 from impacting the F1 granulosa layer because of minimal T4 to T3 conversion. Expression of THR did not change during the ovulatory cycle and did not show differential expression between LEPH and HEPH, indicating that possible thyroid hormone regulation of F1 granulosa progesterone production is most likely because of the differences in circulating thyroid hormones rather than THR expression. Only *ITGA V* in the F5 theca externa layer showed differential expression between LEPH and HEPH. Previous studies have not examined the effects of T3 or T4 treatment on estradiol production in the F5 theca externa layer; however, T3 treatment did suppress gonadotropin-stimulated estradiol production in small white follicles from chicken hens (Sechman et al., 2009). Previous work found that HEPH showed increased expression of estradiol production genes during the PS in the F5 theca externa (Brady et al., 2020a), which coincides with decreased plasma T3 concentrations in the current study. Further studies will be necessary to assess the effects of in vitro thyroid hormone treatment on estradiol production in the F5 theca externa layer. However, because of the minimal THR expression differences between LEPH and HEPH, it is more likely that effects of thyroid hormone on estradiol production occurs through differential circulating thyroid hormone levels rather than differential THR expression.

Timing in the ovulatory cycle and egg production level both impacted plasma thyroid hormone concentrations and HPT axis gene expression in the hypothalamus and pituitary. While influences of the timing in the ovulatory cycle and egg production level on HPG axis gene expression and ovarian steroid hormone production in the F1 granulosa and F5 theca externa layers have been established (Brady et al., 2020a), the influence of thyroid hormone on these aspects appears to be because of circulating thyroid hormone levels rather than THR expression. In general, HPT axis expression tended to be downregulated during the PS and tended to be upregulated in LEPH. Further studies will be required to elicit the full influence of the thyroid axis on reproductive function and to determine what impact the thyroid axis has on follicle development and ovulation rates to ultimately impact egg production levels.

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DISCLOSURES

All authors confirm that they have no conflicts of interest regarding the research described in this manuscript.

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