TGF-β4 and HSP70 responses in breeder hens treated with thyroxine

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ABSTRACT

A hypothesis was tested that long-term administration of thyroxine (T4) in broiler breeder hens would affect fertility, sperm penetration rate, and the duration of fertility. Relative abundance of oviductal TGF-β4 and HSP70 mRNA was determined to ascertain whether T4 treatment affected these genes, and modulated the sustained storage of spermatozoa within the uterovaginal sperm storage tubules of hens. A total of 70, 47-week-old Cobb 500 breeder hens was randomly allotted to two treatment groups (T4 treatment (ET) and control). The T4 was orally administered to the ET group (0.3 mg T4/bird/day) for 100 consecutive days; whereas the control group was not administered T4 during the experimental period. Breeder hens were artificially inseminated to evaluate specific reproductive variables. On the last day of the treatment period two hens / replicate were randomly killed to estimate oviductal gene expression. The T4 treatment resulted in an increase in plasma concentration of T4; however, the T3 concentration was not affected. The long term administration of T4 had no effect on fertility; however, it resulted in a decreased sperm penetration rate and decreased the duration of fertility compared with the control group. The relative abundance of TGF-β4 and HSP70 mRNA in the SST was not influenced by T4 supplementation. The correlation coefficients between fertility and sperm penetration rate with relative abundance of TGF-β4 and HSP70 were not significant. Overall, among the diverse reproductive variable assessed in the current study, the sperm penetration rate and the duration of fertility were most responsive to long-term treatment with T4.

1. Introduction

Thyroid hormones have an important role in regulating time of puberty and reproductive function in birds (Kirby et al., 1996). In birds, the role of thyroid hormones in reproduction and gonadal development was investigated by McNabb (2007) and there have been effects of this hormone on various bodily functions in other species (Hulbert, 2000). In poultry, thyroidectomy caused gonadal aplasia and a reduction in egg production in hens (Falconer, 1971). Thyroid hormones affect the initiation and maintenance of the ovarian performance in turkey hens (Lien and Siopes, 1989). Thus thyroid hormones contribute to different physiological systems, such as reproductive function (Pérez et al., 2018).

The biology of reproduction in birds differs from that of mammals. In contrast to mammals, birds do not have estrous cycles, thus, the time of mating and ovulation is not synchronized in birds and the reproductive success is dependent on sperm storage in the...
oviduct (Bakst, 2011). Sperm storage occurs in the female reproductive tract of poultry species (Bakst, 2011). In avian species, spermatozoa are stored in sperm storage tubules (SST) which allows for sustaining fertility for an extended period of time. The SSTs are located in the lamina propria of mucosal folds in the utero-vaginal junction (UVJ) of the oviduct (Etches, 1996; Bakst, 2011). Both the number of sperm-containing SSTs, and SST function, directly affect fertility rate (Das et al., 2008) and the duration of fertility when defined as the number of successive days in which there was oviposition of fertile eggs after insemination until there was oviposition of the first infertile egg (Pierson et al., 1988). The mechanism involved in sperm storage and survivability within the avian oviduct, however, remains poorly understood. The factors which influence the SST microenvironment have an important role in sustaining spermatozoa viability in the SST for prolonged periods (Das et al., 2005). Likewise, some factors contributing to duration of sperm viability in the oviduct have been suggested. These are spermatozoa in the SST should be in a quiescent state, with lesser motility and metabolism than when sperm are located at the oviductal site of fertilization (Bakst, 1985; Ito et al., 2011). Previous researchers have elucidated other factors such as localization of carbonic anhydrase (Holm et al., 1996), calcium (Holm et al., 2000), avidin (Long et al., 2003), aquaporin (Zaniboni and Bakst, 2004), and alkaline phosphatase (Bakst and Akuffo, 2007) in the UVJ which have a facilitating role for sperm storage. Furthermore, anti-sperm reactions have been detected in the UVJ, and, therefore, the suppression of immuno-responses in the bird oviduct is important for prolonged sperm storage in SSTs (Das et al., 2007). Apart from a role in cell growth and differentiation, transforming growth factor β (TGF-β) is one of the plausible proteins which might reduce local immunity in the UVJ to preserve sperm viability and associated improvements of fertility (Das et al., 2008). In the bird oviduct, three growth factor β isoforms (TGF β2, β3 and β4) and the receptors for these proteins have been recognized (Chowdhury et al., 2004). The presence of heat shock protein 70 (HSP70) has also been ascertained to occur in the Japanese quail UVJ SST, thus, augmenting sperm motility and facilitating sperm release for transport to the site of fertilization (Hiyama et al., 2014). Furthermore, the contribution of thyroid hormones to the expression of these genes has been reported with a recent study indicating triiodothyronine hormone (T3) enhanced the TGF-β promoter activity by six- to eight-fold in the human liver (Yen et al., 2006). Furthermore, the expression of heat shock protein in the human thyroid gland was affected by thyroid hormones (Wallin et al., 1992). Apart from the fundamental functions of these genes in the SSTs of hens, TGF-β1 mRNA was present in the mouse preimplantation embryos and modulates reproductive tract function and embryonic development (Chow et al., 2001). The functional importance of transforming growth factor β receptor in the human female reproductive tract has been reported (Li et al., 2011a). The expression of the TGF-β1 gene in obstructed fallopian tubes was negatively associated with postsurgical pregnancy of infertile women (Li et al., 2011c). One form of oviducal heat shock protein 70 extended the viability of boar, ram and bull sperm at body temperatures in vitro (Lloyd et al., 2012). There have been results from some studies that indicate heat shock proteins are important in early embryonic development. Colony-stimulating factor 2 increases the development of rodent and ruminant preimplantation embryos through heat shock protein 70 (Wen et al., 2017). Also results of some studies indicate transforming growth factor β signaling in rat, human, monkey, and mink is regulated by heat shock proteins (Li et al., 2011b; Shang et al., 2014; Ikezaki et al., 2016).

There are few reports about the comparative effects of hyper- and hypothyroidism on reproductive function in broiler breeder hens. The present research, therefore, focused on studying the effects of long-term thyroxine (T4) administration, as a model for hyperthyroidism, on reproductive attributes of broiler breeder hens with special attention to functional aspects such as fertility, sperm penetration rate, and the duration of fertility. Furthermore, this study was conducted to determine whether there is any association between the relative mRNA abundance of oviductal TGF-β and HSP70 of the T4 treatment (ET) of hens and the associated fertility.

2. Materials and methods

2.1. Animal ethics

All procedures in the present study were approved by the Animal Care and Welfare Committee of Department of Animal Science, College of Agriculture, Shiraz University (Shiraz, Iran).

2.2. Birds and experimental treatments

A total of 50, 47-week-old Cobb 500 breeder hens was randomly allotted to two treatment groups (five replicates of seven hens each), (control and T4 treatment (ET)). The T4 (Iran Hormone Pharmaceutical Company, Tehran) was orally administered by gavage to the ET group (0.3 mg T4/bird/day) for 100 days consecutively; whereas the control group was orally administered the drinking water by gavage. The birds were maintained with the same management in an environmentally controlled facility, and fed a corn-soybean based diet (Table 1).

2.3. Blood sampling and analyses

Every 2 weeks blood samples were collected from the wing vein of the birds, using a 5 mL syringe, placed in EDTA-coated tubes. The blood samples were centrifuged (1800 × g for 12 min) and plasma was separated and stored at −20 °C until analyzed for T3 and T4 assays, using commercially kits (Padtan Elm, Iran). The intra- and inter-assay coefficients of variation were 12.6 and 13.2 for T3, and 7.6 and 2.2 for T4, respectively (Akhlaghi et al., 2012).
2.4. Artificial insemination

A total of twenty 47-wk-old Cobb 500 breeder roosters were habituated by abdominal massage for semen collection and artificial insemination. The semen samples were pooled and diluted 1:5 with pasteurized low-fat milk prior to insemination. This was undertaken to reduce the effects of sperm quality and concentration on relative abundance of TGF-β and HSP70 mRNA.

2.5. Reproductive performance

To assess fertility, eggs were collected from the second day following insemination for seven consecutive days and visual examinations of blastodiscs were performed (Etches, 1996). Assessment of sperm penetrating the perivitelline layer was conducted using the technique of Al-Daraji (Al-Daraji, 2001). To evaluate the duration of fertility after insemination, the number of successive days which there was ovipositon of fertile eggs until the first infertile egg was observed.

2.6. Oviductal mRNA

On the last day of the treatment period (64 wk), two hens/replicate (20 hens total) were randomly selected and killed by cervical dislocation. Utero-vaginal junction sampling was performed according to the procedure described by Foye-Jackson et al. (2011). The UVJ mucosa, which contains SST, was confirmed microscopically by the presence of SST and isolated. Tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C for RNA isolation. The RNA isolation and complementary DNA (cDNA) synthesis were assessed as previously described (Zuccotti et al., 2002). Among the three types of TGF-βs, the mRNA for the TGF-β4 isoforms was in greatest relative abundance for longest period in the bird oviduct (Das et al., 2006); therefore, in the present study the TGF-β4 isoform was selected for assessment. The relative abundance of TGF-β4 and HSP70 mRNA in the UVJ region was assessed, by using the real-time PCR technique. Real-time PCR reactions were performed in a total volume of 14 μL for DNA Master SYBR Green I Mix. Details about the primers used in the current study are included in Table 3 and the reference gene used was β-actin (Li et al., 2010) as an endogenous control to normalize the relative abundance of TGF-β4 and HSP70 mRNA. Relative quantification of the mRNA was measured for each sample by using the 2(−ΔΔCt) method (Livak and Schmittgen, 2001).

2.7. Ovarian follicle diameter

On the day of slaughter the ovaries and oviducts were dissected, removed, and weighed, and the diameter of hierarchical (F₁ to F₆) and nonhierarchical ovarian follicles were measured.
2.8. Statistical analysis

The experiment was conducted as a completely randomized design. The data were tested for normality, and transformations of data were used when appropriate. Data were subjected to the GLM procedure, but repeated measures data were analyzed by the PROC MIXED (Institute, 2004). The Pearson correlation coefficients were determined using the correlation procedure (Institute, 2004). Body weight was included in the model as a covariate for analysis of variance. The means were compared by the least squares means and significance being considered at \( P \leq 0.05 \).

3. Results

3.1. Plasma concentrations of thyroid hormones

The long term administration of T4 resulted in an increase in plasma concentration of T4 with no apparent effect on plasma T3 concentration compared to the control group. Administration of T4 in breeder hens had an effect on the T3:T4 ratio; however, there were no significant thyroid hormones × week (age) interactions (Table 2).

3.2. Fertility, sperm penetration rate and duration of fertility

Non-significant effects of treatment (T4), week (age), and treatment × week interaction on fertility occurred in control and ET groups (Table 2). The effect of long-term administration of T4 was significant for sperm penetration rate (Table 2) with the sperm penetration rate (expressed as mean ± SE) being less in hens treated with T4 (101.93 ± 2.79) compared to the control group (116.20 ± 2.79). Also, the T4 × week interaction effect was significant for sperm penetration rate (Fig. 1) with a decreasing trend in sperm penetration rate in week 62 for the ET group compared to the control group. Hens treated with T4 had a lesser duration of fertility \( (P < 0.001; \text{Table 2}) \). There was also a shorter duration of fertility for ET group (8.55 ± 0.31 d) compared with the control group (12.01 ± 0.31 d).

3.3. Oviductal relative abundance of TGF-β and HSP70 mRNA

The results from analyses of the relative abundances of TGF-β4 and HSP70 mRNA in SSTs are depicted in Figs. 2 and 3. Oviductal abundances of TGF-β4 and HSP70 mRNA in the UVJ region were not affected by treatment with T4. Data regarding correlation of fertility and relative abundances of TGF-β4 and HSP70 mRNA indicated there were no differences between the treatments (Table 4).

### Table 2

<table>
<thead>
<tr>
<th>Trait</th>
<th>Variable</th>
<th>Control</th>
<th>ET</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_3 ) (ng/mL)</td>
<td>1.59 ± 0.26</td>
<td>1.64 ± 0.24</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>( T_4 ) (ng/mL)</td>
<td>10.24 ± 0.36(^b)</td>
<td>27.08 ± 0.89(^a)</td>
<td>0.003</td>
<td>NS</td>
</tr>
<tr>
<td>( T_3:T_4 ) ratio</td>
<td>0.152 ± 0.007(^a)</td>
<td>0.059 ± 0.007(^b)</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td>Fertility (%)</td>
<td>95.72 ± 2.75</td>
<td>87.84 ± 2.75</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Sperm penetration rate</td>
<td>116.20 ± 2.79(^a)</td>
<td>101.93 ± 2.79(^b)</td>
<td>0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Duration of Fertility (day)(^d)</td>
<td>12.01 ± 0.31(^a)</td>
<td>8.55 ± 0.31(^b)</td>
<td>&lt; 0.001</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^a,b\)Within rows, values with different superscripts differ \( (P \leq 0.05) \).  
\(^1\)Thyroxine (T4) was orally administered to the extra T4 treatment (ET) group (0.3 mg/bird/day) and the control group was not treated with thyroxine \( (\text{wk 47–64}; \ n = 35 \text{ hens/treatment}) \).  
\(^2\)Duration of fertility data were single measurement.  
NS: Non-significant.

### Table 3

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5'-3')</th>
<th>Size (bp)</th>
<th>Accession number</th>
</tr>
</thead>
</table>
| TGF-β4 | Forward: CGGCCGACGATGAGTGCGTC  
Reverse: CGGGGCCCATCTCACAGGGGA | 100 | M31160.1 |
| HSP70 | Forward: AGCGTAACACCACCATTCC  
Reverse: TGCTTCCACACCCTATC | 372 | AY288298 |
| \( \beta \)-actin\(^a\) | Forward: TGCCGGGTACATTGTTGTA  
Reverse: TGGGTGGGTACATTGTTGTA | 300 | NM_205518 |

\(^a\) Beta-actin considered as a housekeeping gene.
Also the correlation coefficients of sperm penetration rate and TGF-β4 and HSP70 in control and ET groups were not different (Table 4).

### 3.4. Follicular diameters

Overall means (± SE) of ovarian weights in the control (64.9 ± 3.80 g) and ET (56.1 ± 4.25 g) groups were not significantly different. Additionally, oviductal weight in control (96.3 ± 6.98 g) and ET (93.2 ± 9.01 g) groups was not affected by treatment (Table 5; \( P > 0.05 \)). The diameter of hierarchical (F2 to F6) and nonhierarchical (small white) ovarian follicles was not affected by T4 administration; however, the diameter of F1 follicles in the control group was larger than that of the ET group (Table 5).

### 4. Discussion

In this study, T4 treatment increased plasma T4 concentrations in the ET group compared to the control group, without any significant changes in plasma T3 concentrations. Considering the involvement of thyroid hormones in regulating the onset of puberty.
and reproductive performance in poultry (Kirby et al., 1996), it was hypothesized that reproductive performance, might be influenced by long-term administration of T4 in broiler breeder hens. To our knowledge, the present study is the first where the effects of long-term administration of T4 on reproductive function in birds were assessed. Das et al. (2008) reported that the involvement of SSTs in storage of sperm is related with fertility rate in laying hens. The long-term administration of T4 did not, however, affect fertility rate in the present study. Also, Akhlaghi et al. (2012) reported that transient hyperthyroidism did not affect fertility in breeder hens. Hen fertility rate is determined by sperm penetration of perivitelline layer (Bramwell et al., 1995). The numbers of sperm-containing SST is associated with the duration of fertility rate in domestic hens and there is a relationship between egg production and the duration of fertility (Brillard, 1993). Considering that enhancement or maintenance of oviductal sperm number a

### Table 4
Correlation coefficients of fertility and sperm penetration rate with relative abundance of oviductal mRNA for TGF-B4 and HSP70 in Cobb 500 broiler breeder hens

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative Abundance of TGF-B4&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Relative Abundance of HSP70&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertility</td>
<td>0.02&lt;sup&gt;+&lt;/sup&gt; (NS)</td>
<td>0.21 (NS)</td>
</tr>
<tr>
<td>Sperm penetration rate</td>
<td>0.34&lt;sup&gt;+&lt;/sup&gt; (NS)</td>
<td>0.30 (NS)</td>
</tr>
</tbody>
</table>

NS: Non-significant.

<sup>a</sup> Thyroxine (T<sub>4</sub>) treatment group was administered 0.3 mg/bird/day of this hormone and the control group was not treated with thyroxine (wk 47–64; n = 35 hens/treatment).

<sup>b</sup> Beta-actin considered as a housekeeping gene.

<sup>*</sup> Within rows, values for the correlation coefficient of fertility and sperm penetration rate as well as relative abundances of TGF-B4 and HSP70 mRNA; TGF-β: Transforming growth factor beta; HSP: Heat shock protein.

<sup>**</sup> Values with asterisks represent P value.

### Table 5
Effect of long-term T<sub>4</sub> administration on oviduct and ovary weight (g) and the diameter of ovarian follicles in Cobb 500 broiler breeder hens (LS means ± SE).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Control</th>
<th>ET</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oviduct weight (g)</td>
<td>96.3 ± 6.98</td>
<td>93.2 ± 9.01</td>
<td>NS</td>
</tr>
<tr>
<td>Ovary weight (g)</td>
<td>64.9 ± 3.80</td>
<td>56.1 ± 4.25</td>
<td>NS</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; follicle diameter (mm)</td>
<td>26.1 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.2 ± 0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.03</td>
</tr>
<tr>
<td>F&lt;sub&gt;2&lt;/sub&gt; follicle diameter (mm)</td>
<td>23.0 ± 0.68</td>
<td>21.3 ± 0.68</td>
<td>NS</td>
</tr>
<tr>
<td>F&lt;sub&gt;3&lt;/sub&gt; follicle diameter (mm)</td>
<td>18.5 ± 0.98</td>
<td>17.1 ± 0.98</td>
<td>NS</td>
</tr>
<tr>
<td>F&lt;sub&gt;4&lt;/sub&gt; follicle diameter (mm)</td>
<td>15.4 ± 1.30</td>
<td>14.0 ± 1.30</td>
<td>NS</td>
</tr>
<tr>
<td>F&lt;sub&gt;5&lt;/sub&gt; follicle diameter (mm)</td>
<td>8.2 ± 0.75</td>
<td>8.1 ± 0.75</td>
<td>NS</td>
</tr>
<tr>
<td>F&lt;sub&gt;6&lt;/sub&gt; follicle diameter (mm)</td>
<td>1.6 ± 0.43</td>
<td>1.2 ± 0.37</td>
<td>NS</td>
</tr>
<tr>
<td>SWF&lt;sup&gt;2&lt;/sup&gt; diameter (mm)</td>
<td>0.68 ± 0.06</td>
<td>0.67 ± 0.06</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>1</sup> Thyroxine (T<sub>4</sub>) was orally administered 0.3 mg/bird/day and the control group was not treated with thyroxine (wk 47–64; n = 35 hens/treatment).

<sup>2</sup> Small white follicle.

<sup>a,b</sup> Within rows, values with different superscripts differ (P ≤ 0.05).

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The definition of the duration of fertility in the present study is the number of days from the day after the second insemination to the day before there is oviposition of two successive interfile eggs (Goerzen et al., 1996). Pierson et al. (1988) reported that the number of sperm-containing SST is associated with the duration of fertility rate in domestic hens and there is a relationship between the duration of fertility and sperm storage function in the SST of broiler breeder hens. In poultry only 1% to 2% of inseminated sperm remain in the SST (Bakst et al., 1994) and the number of sperm which reach the SST is correlated with the duration of fertility (Brillard, 1993). Considering that enhancement or maintenance of oviductal sperm number affects the duration of fertility (Goerzen et al., 1996) it is plausible that the decreased duration of fertility in hens of the ET group in the present study was due to the reduced number of sperm transported to SST in the UVJ in the hens treated with T<sub>4</sub>. Beaumont et al. (1992) reported that there is a significant association between egg production and the duration of fertility in both layer and broiler hens. It was thought that the egg production of the layer chickens was correlated with the duration of fertility, so that the layer chickens with a greater rate of oviposition had a
longer duration of fertility. In the present experiment the long-term administration of T₄ reduced the duration of fertility whereas, egg production was not influenced by the treatment. It, therefore, is likely that the reduction in sperm penetration rate and the duration of fertility in hens of the ET group may be due to failure of sperm storage in female oviduct. The presumption is that spermatozoa are released from SSTs in hens of the ET group in greater numbers than in hens of the control group, and the sperm stored in the ET do not viable for as long as those in hens of the control group. Also, it is possible that the sperm receptors on the ovum surface might be less as a result of T₄ administration; however, this possibility has not been verified. In the present study, T₄ treatment reduced sperm penetration rate and the duration of fertility in hens of the ET compared with the control group. The shorter sperm penetration rate and duration of fertility in hens of the ET group might affect the fertility rate, hatchability and chick quality. Although in the present study T₄ treatment for a long-term period did not affect fertility rate and the hatching capacity of eggs and chick viability were not determined in the current study.

The expression of thyroid hormone receptor genes in granulosa and theca cells of chicken ovarian follicles has been reported and there are effects of thyroid hormones in genomic and non-genomic functions of the bird ovary (Sechman, 2013). The largest hierarchy preovulatory follicle (F₁) is the follicle from which ovulation would have initially occurred within 24 h if the ovarian tissues had not been collected (Sechman, 2013). In a previous study, there was an association of follicle diameters with egg size and clutch size (Williams, 2012). Additionally, egg-size affected off-spring size within the first week after hatching and there was a positive correlation between the egg and chick size (Christians, 2002). In the current study, administration of T₄ to breeder hens for a long-term resulted in a decrease in the diameter of F₁ follicles, leading to possible effects on chick viability; however, the chick quality was not evaluated in the current study.

Additionally, the association of thyroid hormones and regulation of TGF-β and HSP70 gene expression led us to hypothesize that T₄ administration for a long-term may increase oviductal expression of TGF-β and HSP70 genes and consequently fertility rate in broiler breeder hens. Das et al. (2007) conducted a study to confirm whether expression of TGF-β isoform genes and the receptors for this protein are associated with sperm survival in the SST in UVJ and fertility rate in chickens. It was confirmed that there is a positive relationship between transforming growth factor β gene expression in the oviduct and fertility of chickens. Furthermore, it has been reported that transforming growth factor β suppresses the immuno-response to sperm during sperm storage in SSTs for prolonged periods (Das et al., 2008). Transforming growth factor β isoforms prevent propagation of T- and B-lymphocytes and also reduce local immunity in the UVJ of the hen oviduct (Das et al., 2006). Furthermore, Hiyama et al. (2014) reported that HSP70 has an important role in sperm transport in the oviduct of birds and the fertility rate. It was reported that both the HSP70 protein and HSP70 mRNA are present in the quail UVJ and infundibulum which are the primary and secondary sites for sperm storage, respectively. Also, it was observed that HSP70 induced Japanese quail sperm motility in vitro, and that HSP70 activates sperm movement from the SST to the fertilization site in the infundibulum. As reported by Hiyama et al. (2014) HSP70 stimulates sperm flagellar motility and migration of sperm from SST toward the fertilization site by affecting glycolysis and stimulating ATP production. Furthermore, it was reported that HSP70 affects voltage-dependent anion channel protein 2 (VDAC2) and increases intracellular Ca²⁺ concentrations which are essential for sperm motility.

Because of the contribution of the expression of TGF-β4 and HSP70 genes in the UVJ to fertility, it was hypothesized that there would be an association between relative abundance of oviductal TGF-β and HSP70 mRNA in the treated hens with fertility. Inconsistent with the hypothesis in the present study, neither the expression of the TGF-β4 and HSP70 gene in the UVJ nor fertility rate was affected by long-term T₄treatment. Furthermore, the correlation coefficients of oviductal TGF-β4 and HSP70 gene expression with fertility and sperm penetration rate were not significant.

5. Conclusion

The T₄ treatment had no undesirable effects on fertility rate and abundances of mRNA for TGF-β and heat shock protein 70 in the oviduct; however, sperm penetration rate and the duration of fertility were the most sensitive variables to long-term administration of T₄. Further studies, however, will be needed to elucidate the effects of long-term administration of T₄ on female reproductive function, especially egg production, egg quality, hatchability, and chick viability.

Conflict of interest

The authors declare that they have no conflict of interest.

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