Gonadotropin profile in experimentally induced hypothyroid and hyperthyroid cyclic female rats

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Article information

Abstract

The current study examined the association of thyroid disorders with reproductive dysfunction by determining its effect on gonadotropin secretion in cyclic female rats. Sixty cyclic females were assigned to three groups (20 each) and supplemented, for 30 days plus two consequent estrous cycles, with drinking water (control), methimazole in drinking water (0.02% w/v) (hypothyroid group), and thyroxine in drinking water (0.002% w/v) and gastric gavage of 200 μg/kg body weight (hyperthyroid group). At late proestrus, ten females from each group (for each cycle) were anesthetized and dissected. Blood samples were obtained to assess thyroid-stimulating hormone, free and total triiodothyronine, free and total thyroxin, follicle-stimulating hormone, luteinizing hormone, and prolactin concentrations. Ovarian and pituitary tissue samples were obtained for molecular analysis of ovarian thyroid receptor genes and pituitary TSH, FSHβ, and LHβ genes.

In comparison with control, the Hypo group revealed increased serum concentrations of TSH and PRL and the expression levels of pituitary TSH and ovarian TRαTRα genes and significant decrease of FT3, TT3, FT4, TT4, FSH, and LH concentrations and the expression levels of pituitary FSHβ and LHβ genes. In contrast, the Hyper group showed increased serum FT3, TT3, FT4, TT4, and LH concentrations and the expression levels of pituitary LHβ and ovarian TRαTRα genes and decreased serum TSH FSH and PRL concentrations and pituitary FSHβ and TSH gene expression levels. It is concluded that thyroid dysfunction is associated with altered serum gonadotropin secretion and reproductive failure.

Keywords: Thyroid dysfunction, Hypothyroidism, Hyperthyroidism, Gonadotropin, Reproduction

Introduction

Thyroid hormones (THs) have a vital role in the proper functional status of the female reproductive system since they control the metabolism, growth, and development of female reproductive organs, including ovaries (1). THs and THs perform their functional role on the ovarian tissues directly and indirectly. They act directly through specific nuclear receptors which control the development and metabolism of the ovary (2) and act indirectly throughout multiple interactions with many other hormones or growth factors, such as estradiol (E2), prolactin (PRL), and insulin-like growth factors (IGFs), or via the hypothalamic-pituitary-gonadal axis by its role in the release of gonadotrophin-releasing hormone (GnRH) from the hypothalamus (3). Therefore, TH disturbances such as hypo- and hyperthyroidism could be led to low fertility or infertility in different animals (4). Hypothyroidism in animals may be associated with impaired reproductive functions, such as delayed puberty, anovulation, the occurrence of ovarian cysts, and irregularity estrus cycle (3), which mostly lead to infertility (5). On the other hand, some evidenced findings suggest that infertility is sometimes associated with hyperthyroidism, where some studies find a relationship between hyperthyroidism and primary and secondary female infertility (4). Changed TH concentrations are linked to altered folliculogenesis, decreased fertilization rates, and poor embryo quality (6) and can potentially lead to ovarian...
failure in extreme situations (7). Variations in thyroid hormone levels in the blood, such as hypothyroidism and hyperthyroidism, can cause subfertility or infertility in humans and animals (1,4,6).

Because thyroid dysfunction is associated with several physiological alterations, including reproductive disorders in animals, the objective of this study was to determine the role of THs/THs in gonadotropin secretion and their role on ovarian physiology and reproductive fecundity in female rats.

Materials and methods

Experimental animals

Adult female rats aged 75-80 days and weighted 150-160 g, with regular estrous cycles, were accommodated in the animal house of the College of Veterinary Medicine, the University of Al-Qadisiyah under controlled conditions of temperature 22-23°C, dark and light 12:12 and feeding ad libitum. Female rats were housed in groups of 5 females in each cage for 3-4 estrous cycles, and the cyclic female rats were used in the experiment. Hypothyroidism was induced in 20 adult female rats by administration of methimazole in drinking water 0.02% w/v for 30 days (8). Hypothyroid females were examined by assessing thyroid hormones (T3 and T4) to ensure hypothyroidism.

Induction of hyperthyroidism

Hyperthyroidism was induced in 20 adult female rats by administration of exogenous thyroxin (T4) in drinking water 0.002% w/v and intragastric gavage of 200 T4 μg/kg bodyweight for 30 days (8). To ensure hyperthyroidism, hyperthyroid females were examined by assessing thyroid hormones (T3 and T4).

Experimental protocol

According to the ethics and policies guidelines of the University of Al-Qadisiyah, this study was carried out on sixty adult female rats. The females were assigned to three equal groups (20 each) and treated as follow: Control group (C) 20 euthyroid (intact) cyclic female rats were administered daily with drinking water for 30 days and then continued for two consecutive estrus cycles. Hypothyroidism group 20 hypothyroid cyclic female rats were maintained in the oral administration with methimazole in drinking water (0.02% w/v) for two further consecutive estrous cycles. Hyperthyroidism group 20 hyperthyroid cyclic female rats were continued in the oral administration with exogenous thyroxine (T4) in drinking water (0.002% w/v) and intragastric gavage of T4 (200 μg/kg body weight) for two further consecutive estrous cycles.

At late proestrus of the third estrous cycle, after treatment for two estrus cycles, ten females from each group (for each cycle) were anesthetized, dissected, and venous blood samples were obtained from abdominal veins in non-heparinized tubes for assessment of TSH, FT3, TT3, FT4, TT4, FSH, LHLH, and PRL concentrations. Also, ovarian and pituitary tissue samples were obtained for molecular analysis of ovarian TRsTRs genes and pituitary TSH, FSHβ, and LHβ genes.

Blood sampling and sera preparation

After collecting blood samples, the serum was isolated by centrifugation of the samples at 5000 rpm for 10 minutes, then aspirated into an Eppendorf tube and reserved at -20°C until hormonal assay (9).

A hormonal assay using ELISA Technique

Serum TSH, FT3, TT3, FT4, TT4, FSH, LHLH, and PRL concentrations were assessed according to the manufacturer’s instructions (Lid. Com, China and Shanghai Biological Co. Ltd, China).

Total RNA extraction

TRizol® reagent kit was used for total RNA extraction from female rats’ pituitary and ovarian tissues. The extraction procedure was performed according to the instruction of the manufacturer company (Bioneer, Korea).

Estimation of quantity and purity of extracted total RNA yield

Nanodrop spectrophotometer (THERMO, USA) was used to measure the quantity of extracted RNA. To control the quality of the extracted RNA, the concentration (ng/μL) and the purity of RNA were measured, as described by the manufacturer (Promega Company, USA).

DNase I treatment

Using DNase I enzyme kit, the trace amounts of genomic DNA were removed from the eluted total RNA (accordingly to the procedure described by Promega Company, USA).

cDNA synthesis

DNase-I treated total RNA samples were utilized in the cDNA synthesis stage by employing the AccuPower® RocktScript RT PreMix kit (Bioneer company, Korea).

Quantitative Real-Time PCR (qRT-PCR) master mix preparation

qRT-PCR master mix was done using AccuPower™ Green Star Real-Time PCR kit depending on SYBER Green dye determination of gene amplification Real-Time PCR system (Bioneer company, Korea).

The analysis of the data of qRT-PCR

The levels of the relative quantification gene (fold change) ΔΔCT Livak approach was used to test the obtained data of qRT-PCR for studied and housekeeping genes. This approach was described according to Livak and Schmittgen (10). The amount gained from the q RT-PCR experiment and the relative quantification procedure was normalized to meaningful biologic data. In this approach, the calibrator is
one of the studied samples like control samples, where the relative expression levels were produced by dividing every value of the normalized target (CTCT values) using the normalized target value of the calibrator.

**Statistical analysis**

Statistical analysis of data was performed using the GraphPad Prism-Version 5 (SAS Institute, Inc., USA). Results were expressed as mean ± standard deviation. One-way ANOVA was used with Newman-Keuls (11) to calculate the significant differences among means. *P* < 0.05 is considered significant.

**Results**

**The serum concentration of TSH and THs**

Hypothyroid female rats (Table 1) recorded a significant decrease (*P* < 0.05) of serum concentration of TSH and the ratio of T3/T4, and a significant increase (*P* < 0.05) of serum TT4, FT4, TT3, and FT3 concentrations in comparison with control females. In contrast, hyperthyroid female rats recorded a significant increase (*P* < 0.05) of serum concentration of TSH and the ratio of T3/T4, and a significant decrease (*P* < 0.05) of serum TT4, FT4, TT3, and FT3 concentrations in comparison with control females. The results showed insignificantly (*P* > 0.05) changes when comparing the two estrous cycles for each group.

**Serum concentrations of FSH, LHLH, and PRL**

The results of hypothyroid female rats (Table 2) revealed lower (*P* < 0.05) concentrations of serum FSH and LHLH and higher (*P* < 0.05) concentrations of serum PRL than female control rats, whereas hyperthyroid females recorded lower than (*P* < 0.05) concentrations of serum FSH and PRL and higher concentration of LHLH in comparison with control. On the other hand, the results of the two estrous cycles showed no significant (*P* > 0.05) changes between each other.

### Table 1: Serum concentrations of TSH and THs in hypothyroid and hyperthyroid cyclic female rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period</th>
<th>C</th>
<th>Hypo</th>
<th>Hyper</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH conc. (x 0.01) (n.mole/L)</td>
<td>1st estrus cycle</td>
<td>198.2±18.33 bA</td>
<td>254.4±22.14 aA</td>
<td>148.1±11.22 cA</td>
</tr>
<tr>
<td></td>
<td>2nd estrus cycle</td>
<td>195.8±19.19 bA</td>
<td>248.6±18.66 aA</td>
<td>152.5±14.29 cA</td>
</tr>
<tr>
<td>TT4 conc. (n.mole/L)</td>
<td>1st estrus cycle</td>
<td>65.3±5.38 bA</td>
<td>36.3±3.98 cA</td>
<td>85.8±6.08 aA</td>
</tr>
<tr>
<td></td>
<td>2nd estrus cycle</td>
<td>68.5±7.45 bA</td>
<td>38.8±4.24 cA</td>
<td>88.4±5.22 aA</td>
</tr>
<tr>
<td>FT4 conc. (n.mole/L)</td>
<td>1st estrus cycle</td>
<td>36.6±4.79 bA</td>
<td>20.4±2.68 cA</td>
<td>51.1±4.88 aA</td>
</tr>
<tr>
<td></td>
<td>2nd estrus cycle</td>
<td>33.5±4.91 bA</td>
<td>22.9±3.45 cA</td>
<td>53.6±4.29 aA</td>
</tr>
<tr>
<td>TT3 conc. (x 0.1) (n.mole/L)</td>
<td>1st estrus cycle</td>
<td>23.8±2.62 bA</td>
<td>9.6±0.94 cA</td>
<td>42.6±3.53 aA</td>
</tr>
<tr>
<td></td>
<td>2nd estrus cycle</td>
<td>24.3±3.18 bA</td>
<td>9.1±0.88 cA</td>
<td>44.3±4.02 aA</td>
</tr>
<tr>
<td>FT3 conc. (x 0.1) (n.mole/L)</td>
<td>1st estrus cycle</td>
<td>9.5±0.8 bA</td>
<td>5.9±0.84 cA</td>
<td>26.5±1.86 aA</td>
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<tr>
<td></td>
<td>2nd estrus cycle</td>
<td>9.9±0.97 bA</td>
<td>6.6±0.95 cA</td>
<td>28.3±1.91 aA</td>
</tr>
<tr>
<td>T3/T4 ratio</td>
<td>1st estrus cycle</td>
<td>2.74±0.28 bA</td>
<td>3.78±0.41 aA</td>
<td>2.01±0.29 cA</td>
</tr>
<tr>
<td></td>
<td>2nd estrus cycle</td>
<td>2.82±0.27 bA</td>
<td>4.26±0.39 aA</td>
<td>2.00±0.22 cA</td>
</tr>
</tbody>
</table>

Values denote Mean ± SDSD. Cyclic female rats were supplemented for 30 days plus two consequent estrous cycles with drinking water (control), methimazole in drinking water (0.02% w/v) (Hypo group), and thyroxine (T4) in drinking water (0.002% w/v) and intragastric gavage of 200 T4 μg/kg b.w. (Hyper group). Different small letters denote significant differences (*P* < 0.05) between groups for each estrous cycle. Different capital letters denote a significant difference (*P* < 0.05) between the estrous cycles for each group.

### Table 2: Serum concentrations of Gn and PRL in hypothyroid and hyperthyroid cyclic female rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period</th>
<th>C</th>
<th>Hypo</th>
<th>Hyper</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH conc. (IU/L)</td>
<td>1st estrus cycle</td>
<td>24.03±2.24 aA</td>
<td>8.23±1.06 bA</td>
<td>9.91±1.62 bA</td>
</tr>
<tr>
<td></td>
<td>2nd estrus cycle</td>
<td>23.12±2.07 aA</td>
<td>10.35±2.06 bA</td>
<td>9.82±1.66 bA</td>
</tr>
<tr>
<td>LHLH conc. (IU/L)</td>
<td>1st estrus cycle</td>
<td>14.69±2.71 bA</td>
<td>6.02±1.79 cA</td>
<td>23.55±3.70 aA</td>
</tr>
<tr>
<td></td>
<td>2nd estrus cycle</td>
<td>14.82±2.63 bA</td>
<td>7.13±1.74 cA</td>
<td>24.66±4.67 aA</td>
</tr>
<tr>
<td>PRL conc. (ng/L)</td>
<td>1st estrus cycle</td>
<td>205.8±15.86 bA</td>
<td>429.6±35.94 aA</td>
<td>142.6±13.53 cA</td>
</tr>
<tr>
<td></td>
<td>2nd estrus cycle</td>
<td>214.3±23.18 bA</td>
<td>449.4±40.88 aA</td>
<td>144.3±14.02 cA</td>
</tr>
</tbody>
</table>

Values denote Mean ± SDSD. Cyclic female rats were supplemented for 30 days plus two consequent estrous cycles with drinking water (control), methimazole in drinking water (0.02% w/v) (Hypo group), and thyroxine (T4) in drinking water (0.002% w/v) and intragastric gavage of 200 T4 μg/kg b.w. (Hyper group). Different small letters denote significant differences (*P* < 0.05) between groups for each estrous cycle. Different capital letters denote a significant difference (*P* < 0.05) between the estrous cycles for each group.
The expression level of the pituitary TSH gene recorded a significant increase (P<0.05) in hypothyroid female rats and a significant decrease (P<0.05) in hyperthyroid female’s rats in comparison with control (Figure 1). In comparison between the two estrous cycles for each group, the results showed no significant (P>0.05) changes between each other.

Figure 1: The expression levels of pituitary TSH gene in the hypothyroid and hyperthyroid cyclic female rat. Values denote Mean ± SDSD. Cyclic female rats were supplemented for 30 days plus two consequent estrous cycles with drinking water (control), methimazole in drinking water (0.02% w/v) (Hypo group), and thyroxine (T4) in drinking water (0.002% w/v) and intragastric gavage of 200 T4 μg/kg b.w. (Hyper group). Different small letters denote significant differences (P<0.05) between groups for each estrous cycle. Different capital letters denote a significant difference (P<0.05) between the estrous cycles for each group.

The expression level of the pituitary FSHβ gene showed a significant decrease (P<0.05) of hypothyroid and hyperthyroid female rats in comparison with control (Figure 2). On the other hand, the comparison between the two estrous cycles for each group showed no significant (P>0.05). Differences of ovarian TRs gene (Figure 4) showed a significant decrease (P<0.05) of hyperthyroid female rats in comparison with control and hyperthyroid female rats, which showed no significant differences (P>0.05) between each other. On the other hand, the two estrous cycles for each group showed no significant (P>0.05) changes between each other.

Figure 2: The expression levels of pituitary FSHβ gene in the hypothyroid and hyperthyroid cyclic female rat. Values denote Mean ± SDSD. Cyclic female rats were supplemented for 30 days plus two consequent estrous cycles with drinking water (control), methimazole in drinking water (0.02% w/v) (Hypo group), and thyroxine (T4) in drinking water (0.002% w/v) and intragastric gavage of 200 T4 μg/kg b.w. (Hyper group). Different small letters denote significant differences (P<0.05) between groups for each estrous cycle. Different capital letters denote a significant difference (P<0.05) between the estrous cycles for each group.

The expression level of the pituitary LHβ gene decreased significantly (P<0.05) and of hyperthyroid female rats increased significantly (P<0.05) compared with control females (Figure 3). No significant (P>0.05) changes have been shown in comparing the two estrous cycles for each group.

Figure 3: The expression levels of pituitary LHβ gene in the hypothyroid and hyperthyroid cyclic female rat. Values denote Mean ± SDSD. Cyclic female rats were supplemented for 30 days plus two consequent estrous cycles with drinking water (control), methimazole in drinking water (0.02% w/v) (Hypo group), and thyroxine (T4) in drinking water (0.002% w/v) and intragastric gavage of 200 T4 μg/kg b.w. (Hyper group). Different small letters denote significant differences (P<0.05) between groups for each estrous cycle. Different capital letters denote a significant difference (P<0.05) between the estrous cycles for each group.
The expression levels of ovarian TH receptor genes in the hypothyroid and hyperthyroid cyclic female rat. Values denote Mean ± SDSD. Cyclic female rats were supplemented for 30 days plus two consequent estrous cycles with drinking water (control), methimazole in drinking water (0.02% w/v) (Hypo group), and thyroxine (T4) in drinking water (0.002% w/v) and intragastric gavage of 200 T4 µg/kg b.w. (Hyper group). Different small letters denote significant differences (P<0.05) between groups for each estrous cycle. Different capital letters denote a significant difference (P<0.05) between the estrous cycles for each group.

Discussion

The decline of serum TT4, FT4, TT3, and FT3 levels accompanied by an elevation of serum TSH level might indicate that methimazole is a good choice as an anti-thyroid agent for induction of hypothyroidism in female rats. In hypothyroid female rats, an increased level of TSH results from declined T3 and T4, which is due to the secretion of pituitary TSH due to the negative feedback role (12). Homeostasis of THHTH is usually regulated by the hypothalamic-pituitary-thyroid axis, as hypothalamic thyroid releasing hormone (TRH) controls TSH secretion from the anterior pituitary, which in turn regulates the secretion of THsTHs from the thyroid gland. On the other hand, this axis is negatively controlled by T3 and T4 action on both the hypothalamus and the anterior pituitary (13).

On the other hand, the elevation of serum TT4, FT4, TT3, and FT3 levels accompanied by the decline of serum TSH level in hyperthyroid female rats is attributed to the exogenous administration of thyroxin, whereas the decline of serum TSH level results from the decreased T3 and T4 levels through the exact negative feedback mechanism (12), by which pituitary TSH secretion will be declined.

Although T4 is the primary hormone produced by the thyroid gland's follicular cells with a long half-life, it is less biologically active than T3, primarily synthesized outside of the thyroid gland with a short half-life (14). Therefore, the deficiency in thyroid hormones is due to the administration of methimazole and the increased demand of body cells for energy. It seems that most of T4 will be converted to T3, so the ratio between T3 and T4 was significantly high among the current experimental groups. The ratio of hyperthyroid female rats is lower than the ratio in control because the high level of T4 from the administration of exogenous thyroxin led to a decrease in T3 to T4. On the other hand, oral administration of thyroxine for 30 days indeed leads to a significant increase in FT3 concentration, as indicated by Elbandrawy et al. (15). However, this increase did not match the increase in T4 concentration due to exogenous administration. Therefore, the ratio between T3 and T4 remains low in female rats of this group.

The current resulted in significant changes in the levels of reproductive hormones in both hypothyroid and hyperthyroid female rats were at the levels of the pituitary glands and ovaries. Among these changes, in both groups of female rats, the results revealed a decrease in FSH and LHLH in the blood serum, and this was also confirmed by the decrease in the expression level of pituitary FSHβ and LHβ genes. This decline negatively affected the growth and development of the ovaries, as well as a decrease in their functions in oogenesis and folliculogenesis and a decrease in their ability to manufacture reproductive hormones (estrogen and progesterone). It has been mentioned that estradiol and progesterone have vital regulatory roles in the estrous cycle (16,17). Zarifkar et al. (18) found decreased estradiol and progesterone levels in female rats daily orally administered with levothyroxine (100µg/100g b.w) for ten days. These results could result from the suppression effect of thyroid hormones by preventing FSH from stimulating aromatase activity in granulosa cells (19) and therefore inhibiting estrogen synthesis and ovulation. Moreover, Hapon et al. (20) reported alterations of reproductive hormones to profile in short-term hypothyroid cycling female rats associated with increasing estrogen receptors' expression level and cyp19A1 aromatase during the estrus phase.

Thyroid hormone encourages all stages of female reproductive development and function, including regulating the estrous cycle and maintaining fertility (21). It is known that Thyroid dysfunction is one of the endocrine disorders that can be acquired at any time of life (22). Due to the changes in reproductive hormones recorded in the present study, it can be confirmed that hypothyroidism has an essential association with retard ovarian function and female fecundity via affecting the reproductive axis (23). Various studies have confirmed that hypothyroidism results in an elevation in the hypothalamic secretion of TRH, which in turn stimulates pituitary gland secretion of TSH and PRL. PRL is considered a gonadotropin inhibitor at the pituitary gland level and is associated with different types of infertility (24,25).

Furthermore, the ovary and pituitary glands are the target tissues for thyroid hormones. Thyroid hormones contribute to regulating the growth, development, and metabolic activities of the ovaries, in addition to stimulating the secretory activity of the anterior lobe of the pituitary gland.
(18), and that any imbalance in the concentration of thyroid hormones leads to a defect in the follicular growth and development and anovulation (26).

In hyperthyroid female rats, it has been demonstrated reduction of the ovarian follicle and corpus luteum number, meaning. In contrast, impairing ovarian follicular development and ovulation is associated with hyperthyroidism, accompanied by decreased serum estradiol and progesterone levels (18). On the other hand, confirmed results indicated the role of excess thyroid hormones inhibition of granulosa cell's aromatase activity by FSH and suppression of ovulation (19). In contrast to the stimulatory role of thyroid hormones in physiological concentrations, which augments the action of gonadotropins to stimulate steroidogenesis in ovarian granulosa cells, high concentrations of thyroid hormones cause this role to be attenuated (27).

The ovarian follicle is the fundamental unit of the ovary that provides the essential microenvironment for oogenesis and steroid hormones production. Follicular growth undergoes three phases. In the first phase, follicular growth is independent of gonadotropin; in the second phase, the follicles respond to gonadotropin, and in the third phase, folliculogenesis depends on gonadotropin (28). Down-regulation of pituitary FSHβ and LHβ in hypothyroid female rats coincided with the decreased levels of serum FSH, LHLH, and estradiol, as well as ovarian folliculogenesis and increased serum PRL level, reported in the present study. On the other hand, down-regulated FSHβ is also reported in hyperthyroid female rats coinciding with decreased serum FSH levels. This finding was unexplained and needs more research to determine the reason for this decrease, which may be related to a decrease in GnRH secretion from the hypothalamus and a decrease in the sensitivity of pituitary gonadotropins to GnRH factor or thyroid hormones.

The actions of thyroid hormones are regulated by a group of intracellular enzymes called deiodinases, one of which (deiodinase II) converts T4 to T3 in both the hypothalamus and pituitary and thus plays a central role at the level of the hypothalamus-pituitary-thyroid axis (29). Thyroid receptors facilitate thyroid hormones action (TRα and TRβ) (29). Thyroid receptors are distributed in different body tissues, including ovarian follicular cells, where any down-regulation of thyroid receptors decreases follicle numbers and lowers fertility (29). The changes occurring in the ovarian TRTR gene expression levels in female rats. The current study is related to the changes in the levels of thyroid hormones, as the level recorded a significant decrease in hyperthyroid female rats and a significant increase in hyperthyroid female rats, since the function of thyroid hormones, particularly T3, either through genomic action inside the nucleus, by increasing the level of protein synthesis, or nongenomic action outside the nucleus through the plasma membrane, cytoplasm, or mitochondria, by increasing the number of mitochondria and energy production (29).

Conclusion

In conclusion, thyroid disorders (hypothyroidism and hyperthyroidism) negatively affect the secretion of gonadotropin from the pituitary gland and affect its role in ovarian function, which affects the female reproductive function.

Acknowledgments

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Conflict of interest

No conflict.

References


