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EFFECTS OF LEVOTHYROXINE ADMINISTRATION ON OVULATION RATE AND SEX HORMONE LEVELS IN PREPUBERTAL AND ADULT RATS

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ABSTRACT

Thyroid gland dysfunction is associated with disorders of female reproductive functions. The aim of this study was to examine the effects of hyperthyroidism on ovulation rate and peripheral sex steroid levels in prepubertal and adult rats.

Two groups of female rats aging 30 days (prepubertal rats) and 60 days (adult rats) were made hyperthyroid by oral administration of levothyroxine daily. In the two control groups (n=10) rats with the same ages received the same volume of normal saline. After 10 days the serum levels of T3, T4, LH, FSH, estradiol and progesterone were measured by RIA technique and also sections of ovaries were prepared for histological studies. All ovarian follicles and corpora lutea were counted to estimate ovulation rate.

The results indicated that serum estradiol and progesterone levels of adult hyperthyroid rats were significantly lower than those of the control group ($p<0.05$). However, there was no significant difference between estradiol levels in the prepubertal hyperthyroid group and its control group. In the hyperthyroid groups the mean number of primary, secondary and antral follicles and corpora lutea was significantly less than those of control groups.

It was concluded that high levels of plasma thyroid hormones resulted in lowered body weight and disturbed ovarian follicle development and differentiation leading to reduction in ovarian steroidogenesis and ovulation.

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Keywords: Ovulation, Levothyroxine, Hyperthyroidism, Prepubertal rats

INTRODUCTION

Thyroid hormones have many target tissues in the body with a wide physiological role in the control of growth, development and metabolic activities of organs. Certain plasma levels of these hormones are required for normal gonadal functions. Inadequate thyroid hormone supply causes abnormal folliculogenesis and anovulation in female rats and mice.[1-3] Early hypothyroidism in male rats causes increased adult testis and reproductive organ size and increase in sperm production.[4,5] Amenorrhea, in hyperthyroidism, was described as early as 1840 by von Basedow. Thereafter, a broad spectrum of reproductive disorders ranging from abnormal sexual development to infertility were reported.[6-10] It has been shown that in hyperthyroid women, plasma estradiol levels are increased due to an increase of peripheral conversion of androstenedione to estrone.[11] Some investigations have shown that administration of low doses of thyroid hormones activate the hypothalamic-pituitary-gonadal axis, but the

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excess of T3 and T4 interfere with ovarian functions.[12]

Information about the effects of high plasma levels of thyroid hormones on ovarian follicle development and ovulation in premature and adult mammals are conflicting.[13-15] It has been shown that thyroid hormone receptors in granulosa cells increase in early follicular maturation and thyroid hormone acts as a biological amplifier of gonadotropin effects on granulosa cells.[1,16,17] These cells develop and produce more estrogen and progesterone if gonadotropins and different doses of thyroid hormones are used in culture or in the body.[18-20] Therefore, to elucidate the effects of high plasma levels of thyroid hormones on female gonadal function, in the current study we evaluated ovarian steroidogenesis and ovulation rate in prepubertal and adult hyperthyroid female rats.

MATERIAL AND METHODS

Forty Charles River female rats at two different ages were obtained from the Animal House of Shiraz Medical School and categorized in four groups (n=10); two prepubertal groups (30 days old rats weighing 50-60g) and two adult groups (60 days old rats weighing 140-160g).[21] One group of prepubertal rats and one group of adult female rats were made hyperthyroid by daily administering 100μg/100g body weight of levothyroxine orally for 10 days.[22] In control groups rats received the same volume of normal saline for the same period of time. Vaginal smears were taken from rats to determine the phases of estrous cycle and the first time of drug administrations were started at the proestrous expected day. Rats were kept in a controlled temperature of 22-25°C with a light period from 06.00-18.00 h daily. Standard dry pellets and tap water was available ad libitum.

At the 11th or 12th day, during the diestrous phase, under ether anesthesia the serum was collected from each animal's heart to estimate serum levels of T3, T4, LH, FSH, estradiol and progesterone by RIA technique. Ovaries were removed, weighed and fixed in formalin. Serial sections of ovaries (10μm) were prepared and stained with hematoxylin and eosin. In all sections of both ovaries, based on micromorphological criteria, all follicles (primary, secondary and antral follicles) and corpora lutea were inspected and counted differentially.[23] The number of fresh corpora lutea was taken into account as an indicator of ovulation rate. In each group of rats mean number of follicles and corpora lutea was calculated and analysed statistically using ANOVA following Student's t-test and Mann Whitney U-test. Differences were considered significant when $p<0.05$. The values expressed in the figures and tables are Mean±SD.

RESULTS

Serum hormone levels

The statistical analysis of data showed that in levothyroxine-treated rats, serum levels of T3 and T4 were significantly higher than those of control groups ($p<0.001$) indicating that rats were made hyperthyroid. There were no significant differences between levels of LH and FSH in hyperthyroid and control groups (Table I).

The serum levels of progesterone in both hyperthyroid groups (prepubertal and adult rats) were significantly ($p<0.01$) lower than those of their control groups (4.69±0.79 vs 8.43±2.47 in prepubertal and 22.42±7.27 vs 38.37±5.38 ng/mL in adult rats) (Fig. 1). The serum estradiol levels of adult hyperthyroid rats were significantly lower than those of the control group (68.90±12.71 vs 98.90± 21.06pg/mL; $p<0.05$), while there was no significant difference (92.40±44.90 vs 78.00±19.67pg/mL;

Table I: Serum hormone levels and body weights of hyperthyroid rats compared to those of the control groups. Values are Mean ±SD.

Groups (n=10)		Body weight (g)	T3 (ng/100mL)	T4 (μg/100mL)	LH (mIU/mL)	FSH (mIU/mL)
Prepubertal	Control	96.9 ±11.1	132.0 ± 13.6	5.61 ± 0.73	1.23± 0.37	1.11± 0.54
	Hyperthyroid	83.4 ± 3.5*	215.0 ±45.2**	7.18 ± 1.71*	0.97± 0.54	1.42± 0.89
Adult	Control	168.4 ±16.1	138.5± 13.3	5.36 ± 1.61	1.75± 0.35	1.30± 0.28
	Hyperthyroid	152.8 ±10.8*	180.7 ± 48.4*	8.88± 2.37*	1.61± 0.25	1.54± 0.44

*= $p<0.05$ statistically significant.

**= $p<0.001$ statistically significant.

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Table II: The mean number of follicles in the two ovaries and ovarian weights of hyperthyroid rats compared to those of the control groups. Values are Mean± SD.

Groups(n=10)		Ovarian weight(mg)	Primary follicles	Secondary follicles	Vesicular follicles
Prepubertal	Control	31.4 ± 5.1	26.0± 7.9	14.5 ± 4.4	8.0 ±2.5
	Hyperthyroid	35.0 ± 7.0	19.0± 1.9*	10.3 ± 2.5*	3.5 ± 1.6*
Adult	Control	51.2 ± 4.7	21.9± 5.7	11.5 ± 2.2	6.5± 2.2
	Hyperthyroid	45.1 ± 10.4	16.0± 2.8*	7.5 ± 2.4*	2.0 ± 1.3*

*= $p<0.05$ statistically significant.

$p>0.05$) between serum estradiol levels of prepubertal hyperthyroid and control animals (Fig. 2).

Body and ovarian weights

Treatment with levothyroxine resulted in a remarkable ($p<0.05$) decrease in the body weights of the prepubertal and adult rats compared to control animals (Table I). However, there was no significant difference between paired ovarian weights of hyperthyroid and those of their control groups (Table II).

Ovary histological results

The mean number of primary, secondary and antral follicles counted from two ovaries of the hyperthyroid rats was considerably less than those of control rats (Table II). In both hyperthyroid groups the mean number of corpora lutea were significantly ($p<0.05$) less than those of their control groups (5.40±1.39 vs 9.50±2.43 in prepubertal and 6.60±3.07 vs 10.60±4.14 in adult rats) (Fig. 3).

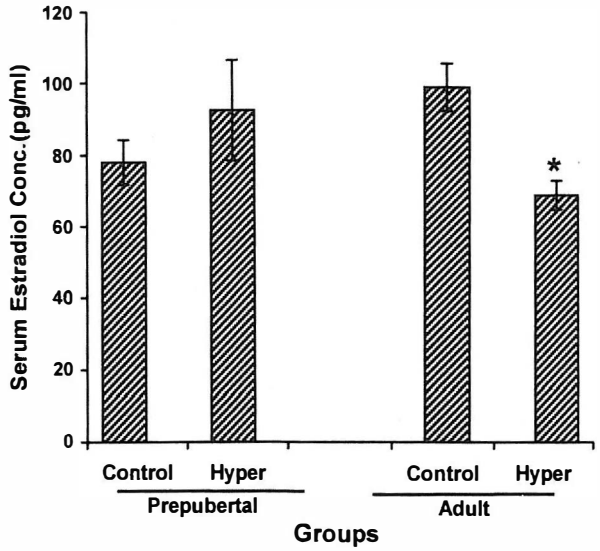

Fig. 2. Serum estradiol levels of hyperthyroid rats compared to those of controls (Mean±SD). *$p<0.05$.

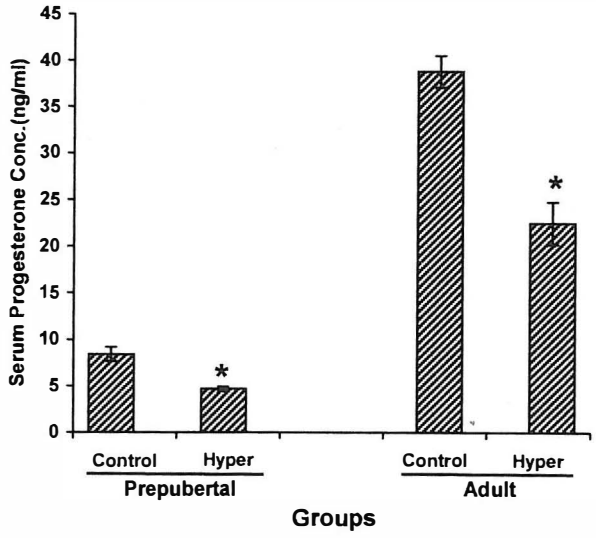

Fig. 1. Serum progesterone levels of hyperthyroid rats compared to those of controls (Mean±SD). *$p<0.05$.

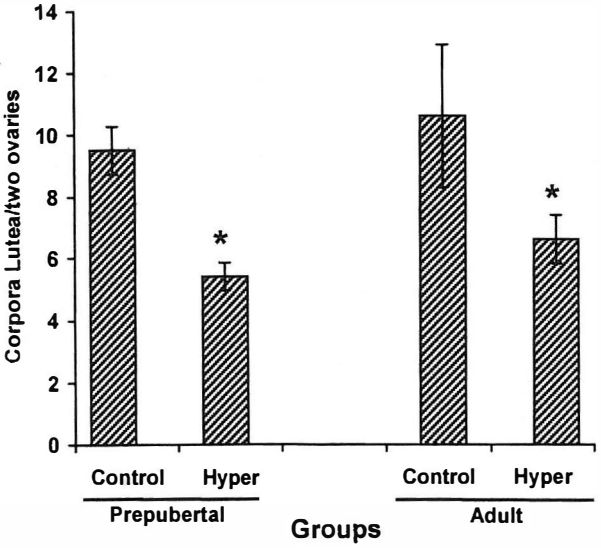

Fig. 3. Mean numbers of corpora lutea in the two ovaries of hyperthyroid rats compared to those of controls (Mean±SD). *$p<0.05$.

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DISCUSSION

According to available data, thyroid diseases influence female reproductive performance, including menstrual irregularities, abnormal sexual development, hirsutism and infertility.[6,8,9,24,25] In the present study, hyperthyroidism was successfully induced by levothyroxine administration in sexually premature and adult female rats, as low body weights of levothyroxine treated animals was a confirming sign of hyperthyroidism.

Our results demonstrated that in hyperthyroid rats the number of follicles and corpora lutea were reduced, indicating that during hyperthyroidism folliculogenesis and ovulation were impaired. It seems that low body weight (less than 90g) of our prepubertal hyperthyroid rats was the main cause of delay in puberty onset and initiation of ovulation.[2] The results showed lower serum estradiol and progesterone levels in the adult hyperthyroid rats than in the control animals. These studies confirm previous results indicating that thyroid hormones inhibit FSH-induced aromatase activity in granulosa cells and suppress ovulation.[14,26] The results are not in agreement with the data reported by Thomas and Reid indicating high levels of LH, FSH and estrogen in women with thyrotoxicosis,[24] and by Jiang and colleagues indicating lower progesterone levels in female hypothyroid mice.[3] Although thyroxine treatment in hypothyroid women,[25] rats[1,21] and mice[3] recently has been reported to improve follicular development and increase ovulation rate, in the present experiments excess T3 and T4 in adult rats caused disturbance in steroidogenesis and ovulation.

In our prepubertal animals, serum estradiol level was not affected by high thyroid hormone levels. It seems that at prepubertal ages, the adrenal cortex is the main source of estradiol production . Although physiological concentrations of thyroid hormones (T3, T4) *in vitro* stimulate progesterone production and augment gonadotropin-induced steroidogenesis in granulosa cells,[19,20,27] the high concentrations of T3 and T4 attenuate this effect. However, *in vivo*, feedback effects of sex steroids on gonadotropin secretion modulate estrogen and progesterone production in the body.[12,26] On the other hand, modulation of sex hormone-binding globulin concentration by excess thyroid hormones result in an increase in estradiol and progesterone clearance.[28] It has been shown that high levels of circulating thyroid hormones augment gonadotropin response to gonadotropin-releasing hormone (LHRH).[29] Hence, in addition to its peripheral effects on sex steroid levels, thyroid hormone may affect sex steroid secretion through the hypothalamic-hypophysial-gonadal axis.[12,26] Our findings in this study are inconsistent with the study of Tanaka et al. who demonstrated that high levels of thyroid hormones have considerable effect on gonadotropin levels.[29] Whether hyperthyroidism exerts its effects primarily at the level of the hypothalamo-pituitary system or at the ovarian level remains to be investigated.

In conclusion, hyperthyroidism, induced by levothyroxine treatment of prepubertal and adult female rats, resulted in lowered body weight and abnormal folliculogenesis leading to disturbed ovulation. On the basis of present data, we conclude that high levels of thyroid hormones cause a reduction in ovarian steroidogenesis and ovulation rate, although thyroid hormone replacement therapy is of great value for ovulation induction in patients with subclinical hypothyroxinemia.[18] Further work with isolated and *in vitro* cultured follicles is necessary to investigate the direct effects of high concentrations of thyroid hormones on follicular development and ovulation.

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