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Is the infertility in hypothyroidism mainly due to ovarian or pituitary functional changes?

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Abstract

The objective of the present study was to examine whether hypothyroidism affects the reproductive system of adult female rats by evaluating ovarian morphology, uterus weight and the changes in serum and pituitary concentrations of prolactin and gonadotropins. Three-month-old female rats were divided into three groups: control (N = 10), hypothyroid (N = 10), treated with 0.05% 6-propyl-2-thiouracil (PTU) in drinking water for 60 days, and T_4-treated group (N = 10), receiving daily *sc* injections of L-thyroxine (0.8 µg/100 g body weight) during the last 10 days of the experiment. At the end of 50 days of hypothyroidism no hypothyroid animal showed a regular cycle, while 71% of controls as well as the T_4-treated rats showed regular cycles. Corpora lutea, growing follicles and mature Graafian follicles were found in all ovaries studied. The corpora lutea were smaller in both the hypothyroid and T_4-replaced rats. Graafian follicles were found in 72% of controls and only in 34% of hypothyroid and 43% of T_4-treated animals. Serum LH, FSH, progesterone and estradiol concentrations did not differ among the three groups. Serum prolactin concentration and the pituitary content of the three hormones studied were higher in the hypothyroid animals compared to control. T_4 treatment restored serum prolactin concentration to the level found in controls, but only partially normalized the pituitary content of gonadotropins and prolactin. In conclusion, the morphological changes caused by hypothyroidism can be a consequence of higher prolactin production that can block the secretion and action of gonadotropins, being the main cause of the changes observed.

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Key words
- Hypothyroidism
- Prolactin
- Gonadotropins
- Folliculogenesis
- Estrous cycle

Introduction

It is well known that hypothyroidism impairs reproductive function both in humans and experimental animals. However, the mechanism of this dysfunction has not been completely established. In several species irregular estrous cycles were also detected (1,2). Ovary atrophy was reported when hypothyroidism was induced in adult female rats (3). Chan and Ng (4) failed to observe morphological changes in the uterus and fal-

lopian tubes when hypothyroidism was induced in rats on postnatal day one; however, they showed a decrease in the number of primordial, antral and Graafian follicles, with no considerable consequences for reproduction in these animals. Dijstra et al. (5) reported disturbed folliculogenesis and absence of corpora lutea when hypothyroidism was induced since birth. In women, hypothyroidism is associated with delay in the onset of puberty (6), anovulation (7), amenorrhea or hypermenorrhea, menstrual irregularity, infertility and increased frequency of spontaneous abortions (8-10). It was suggested that these alterations may be caused by a decrease in LH secretion. An increase in the incidence of galactorrhea caused by hyperprolactinemia has been observed in hypothyroid women. Prolactin decreases GnRH secretion (11), LH frequency and pulsatility (12), counteracts the morphological effects of LH in culture of granulosa cells (13), having a luteolytic effect (14) and causing inhibition of folliculogenesis (15), estrogen synthesis (16), and ovulation (17). This could explain the decrease in gonadotropin stimulation in the ovaries of hypothyroid women. However, there is a scarcity of data associating the increase in prolactin production in hypothyroidism under experimental conditions and the changes in ovary cycle, morphology and gonadotropin production.

In the present study our aim was to examine whether hypothyroidism affects the reproductive system of adult female rats by evaluating several aspects of the hormonal regulation and morphological analysis of the ovary, particularly the changes in serum and pituitary concentrations of prolactin and gonadotropins.

The objective of the present investigation was to study simultaneously several parameters for the evaluation of reproductive function in hypothyroid states that are usually not present together in other reports.

Material and Methods

Animals

Thirty 3-month-old female Wistar rats presenting regular estrous cycles were selected and divided into three experimental groups: control (N = 10), hypothyroid (N = 10), treated with 0.05% 6-propyl-2-thiouracil (PTU, Sigma, St. Louis, MO, USA) in drinking water for 60 days (18), and T_4-treated group (N = 10), receiving daily *sc* injections of L-thyroxine (0.8 µg/100 g body weight) during the last 10 days of the experiment. The other two groups received *sc* injections of saline for the same period of time as T_4 treatment. Vaginal smears were evaluated during the last 20 days of the experiment. The rats were killed by decapitation and pituitary, uterus and ovaries were dissected and weighed. Trunk blood was collected for the determination of serum concentrations of progesterone, estradiol, TSH, FSH, LH and prolactin.

Histological analysis

The ovaries were dissected out and fixed directly in Bouin's fixative overnight for histological examination. The ovaries were cut into 5-7-µm thick sections and stained with hematoxylin and eosin. For each ovary, at least five sections were selected, and the total number of corpora lutea and Graafian follicles was counted under the light microscope (19,20). The follicles were classified as secondary when they presented two or more layers of granulosa cells and as antral when they contained fluid. For an antral follicle to be considered mature or Graafian, the oocyte has to occupy an excentric position and the antral cavity must be completely filled with fluid, with the presence of cumulus oophorus, corona radiata and cellular peduncle (21,22). The corpora lutea were measured using a computer-assisted morphometric program

(KS400/Zeiss Vision).

Radioimmunoassays

Pituitaries were homogenized in 500 µl of 1% PBS/BSA buffer and centrifuged at 3,000 rpm at 4°C for 15 min, and LH, FSH and prolactin were measured in the supernatant. TSH, LH, FSH and prolactin were measured with kits supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD, USA) and are reported in terms of the reference preparation (RP3). Within-assay variation and the coefficient of variation between assays and minimum assay detection were 7.9%, 6.7% and 0.52 ng/ml for TSH, 3.5%, 16.8% and 0.04 ng/ml for LH, 2.5%, 10.2% and 0.19 ng/ml for FSH, and 2.3%, 12.2% and 0.19 ng/ml for prolactin, respectively.

Progesterone and estradiol concentrations in serum were determined using kits from DPC (Diagnostic Products Co., Los Angeles, CA, USA) corrected for rat serum.

Statistical analysis

Data are reported as means ± SEM, with the level of significance set at $P<0.05$. One-way ANOVA followed by the Student-Newman-Keuls multiple comparison test was used to assess significance for all data, except morphological analysis.

Results

Hypothyroid animals showed lower body weight than controls, and T_4 treatment was insufficient to normalize body weight. Ovarian weight was slightly lower in the hypothyroid group and treatment did not reverse these values. Uterine weight was significantly lower in the hypothyroid rats, and the T_4-treated animals showed intermediate values between controls and hypothyroid animals. Pituitary weight did not change with hypothyroidism or treatment (Table 1).

Estrous cycle

At the end of 50 days of hypothyroidism no animal showed a regular cycle in the PTU-treated group, while 5 out of 7 animals showed regular cycles in the control group. After T_4 treatment the cycle showed no difference between control and PTU + T_4 group (Table 2).

Morphology

Corpora lutea, growing follicles and mature Graafian follicles were found in all ovaries studied. However, the corpora lutea were smaller in number and diameter in hypothyroid rats. T_4 treatment did not reverse this alteration (diameter: control, 0.42 ± 0.06; hypothyroid animals, 0.33 ± 0.02; T_4-treated group, 0.36 ± 0.03 µm^2, and number: con-

Table 1. Body, pituitary, uterus and ovarian weights of control, PTU-treated and T_4-treated hypothyroid rats.

Groups	Body weight	Uterus		Ovary		Pituitary	
	(g)	mg	mg/mg x 10^{-2} bw	mg	mg/mg x 10^{-2} bw	mg	mg/mg x 10^{-2} bw
Control	184 ± 6.6	585 ± 0.03	320 ± 0.4	74 ± 8.4	40.2 ± 0.12	10.4 ± 0.6	5.6 ± 0.3
PTU	142 ± 1.5$^+$	367 ± 0.02$^+$	260 ± 1.3$^+$	53 ± 9.2	37.3 ± 0.61$^+$	8.8 ± 0.5	6.1 ± 0.3
PTU + T_4	153 ± 5.8*	410 ± 0.03	270 ± 0.5$^+$	50 ± 1.7	32.6 ± 1.7$^+$	8.5 ± 0.7	5.4 ± 0.3

Data are reported as means ± SEM. bw = body weight. PTU = 6-propyl-2-thiouracil-induced hypothyroid rats; PTU + T_4 = PTU-induced hypothyroid rats treated with thyroxine.
$^+P<0.001$ *vs* control; *$P<0.005$ *vs* control (Student-Newman-Keuls multiple comparison test).

Table 2. Percentage of estrous cycle irregularities in control rats, hypothyroid rats and rats submitted to T_4 replacement.

Groups	50 days of PTU (%)			60 days of PTU and 10 days of T_4 (%)		
	NC	IC	DIIa	NC	IC	DIIa
Control	71 (5/7)	29 (2/7)	0 (0/7)	86 (6/7)	14 (1/7)	0 (0/7)
PTU	0 (0/13)	38 (5/13)	62 (8/13)	0 (0/6)	67 (4/6)	33 (2/6)
PTU + T_4	-	-	-	71 (5/7)	29 (2/7)	0 (0/7)

The number of animals affected/total animals in the group is shown in parentheses. PTU = 6-propyl-2-thiouracil-treated hypothyroid rats; PTU + T_4 = PTU-induced hypothyroid rats treated with thyroxine; NC = normal cycle; IC = irregular cycle; DIIa = diestrus II arrest.

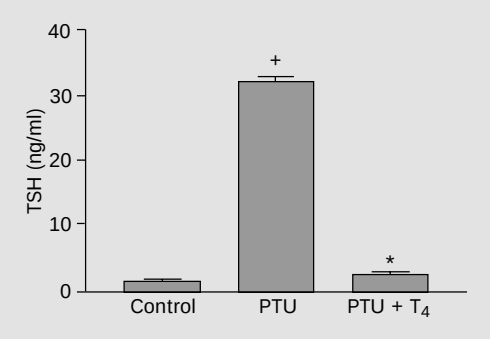

Figure 1. Serum TSH concentration in controls, 6-propyl-2-thiouracil-induced hypothyroid rats (PTU) and thyroxine-replaced hypothyroid rats (PTU + T_4). Data are reported as means ± SEM. *P<0.05 compared with control animals and $^{+}$P<0.05 compared with hypothyroid animals (univariate ANOVA and Newman-Keuls multiple comparison test).

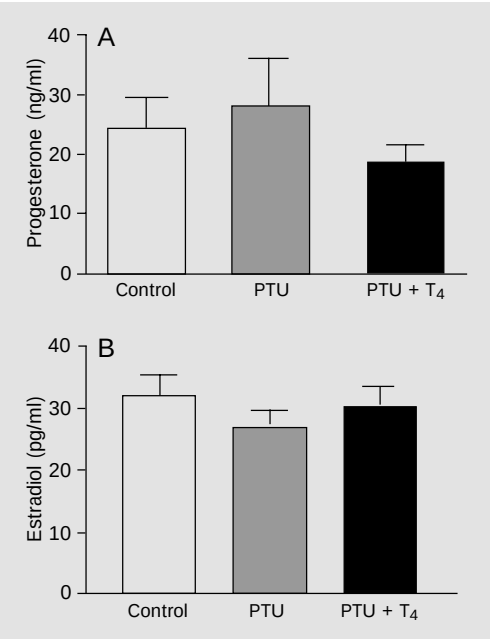

Figure 2. Serum progesterone (A) and estradiol (B) concentrations in controls (N = 9), 6-propyl-2-thiouracil-induced hypothyroid rats (PTU, N = 8), and thyroxine-replaced hypothyroid rats (PTU + T_4, N = 9). Data are reported as means ± SEM (univariate ANOVA and Newman-Keuls multiple comparison test).

trol, N = 4; hypothyroid animals, N = 2; T_4-treated group, N = 3). The presence of Graafian follicles was found in 5 of 7 (72%) control animals, but only in 2 of 6 (34%) hypothyroid animals and a discrete recovery was observed in the T_4-treated group (3 of 7, 43%).

Hormone levels

Serum TSH was 10 times higher in hypothyroid animals than in controls. T_4 treatment restored these values close to control (Figure 1).

Serum progesterone and estradiol concentrations did not differ among the three groups (Figure 2).

Serum LH and FSH concentrations did not differ among the three groups. However, serum prolactin concentration and the pituitary content of the three hormones studied were higher than control in the hypothyroid animals. T_4 treatment restored serum prolactin concentration to the level found in the control, but failed to normalize the pituitary content of LH, FSH and prolactin (Figure 3).

Discussion

Increased serum TSH levels in PTU-treated rats and the almost normal TSH serum levels in T_4-treated hypothyroid rats confirmed the effectiveness of the treatment.

Hypothyroidism caused a significant impairment in the weight of uterus and ovary, but only a slight decrease in pituitary weight. T_4-replaced hypothyroid rats showed a heavier uterus than hypothyroid ones, but the relative weight was still lower than in the controls. T_4 treatment was not sufficient to normalize ovarian weight, a fact probably explained by the short time of T_4 replacement or a more permanent effect of severe hypothyroidism on uterine weight and ovarian morphology. These data are in agreement with a previous report by Leathern (23) studying a model of congenital hypothyroid-

ism and by Dijstra et al. (5) who treated prepubertal rats with PTU. Our data show that, even after puberty, hypothyroidism may produce an involution of uterus and ovary.

The small changes verified in pituitary weight may reflect some maintenance of pituitary function in hypothyroidism. The data about gonadotropin secretion in hypothyroidism are still controversial (24-28), with reports of increased, decreased and normal serum levels. Ortega et al. (3) suggested a reversal to prepubertal reproductive function in adult rats turned hypothyroid based on the secretion of FSH, LH and estradiol under basal and GnRH-stimulated conditions. In our study, despite the small changes in serum LH and FSH, we found an important change in prolactin secretion. Since we found higher pituitary LH, FSH and prolactin content in hypothyroid rats in absolute terms or relative to the pituitary weight, we suggest that the synthesis of these hormones in hypothyroidism is relatively preserved and the defect is in the mechanism of secretion.

The fact that the ovarian follicles were not well developed in the hypothyroid animals despite normal serum LH, FSH, estradiol and progesterone suggests that thyroid hormones could have a direct effect on the growth of ovarian follicles, without a significant effect on sex steroid production by the ovary. In fact, T_3 receptors were found in the granulosa cells of porcine (29) and human (30) ovaries. Thyroid hormones increase the

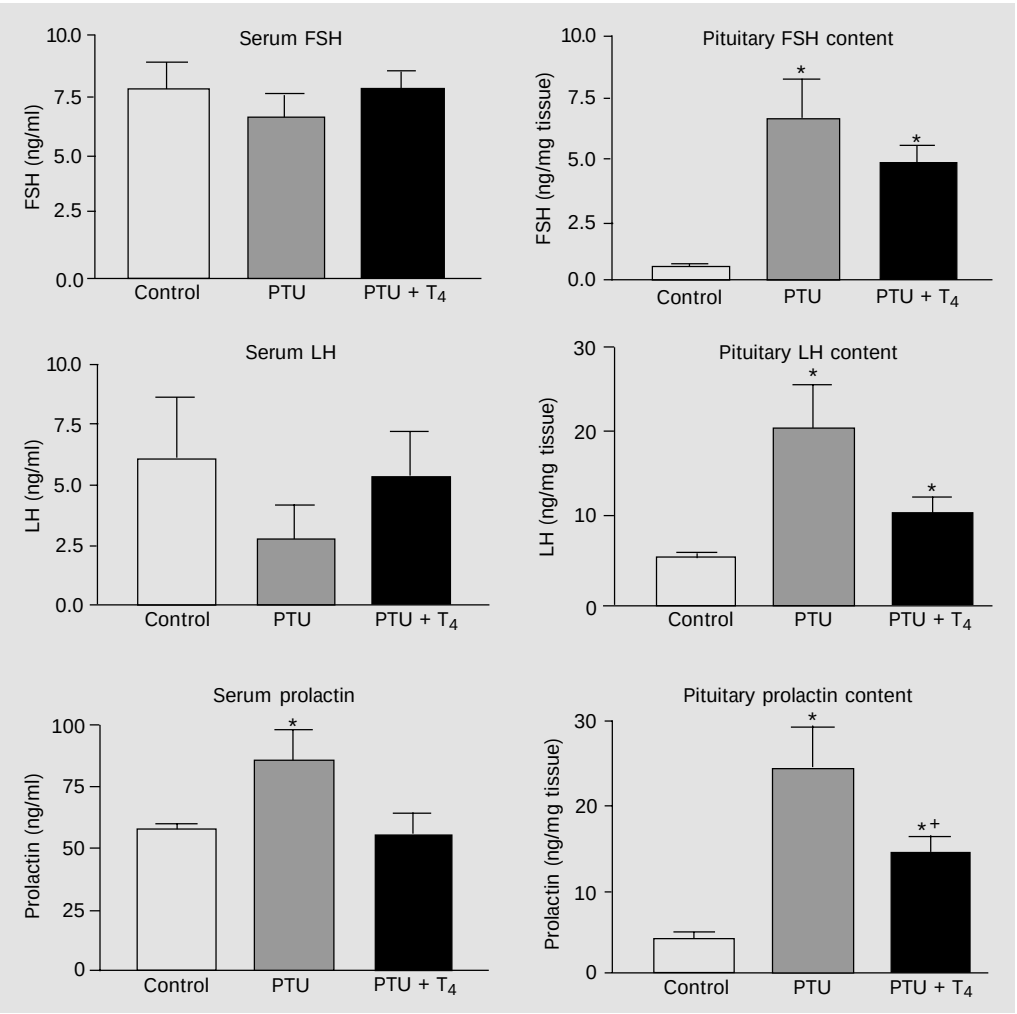

Figure 3. Serum LH, FSH and prolactin concentrations (on the left), and pituitary content (on the right) of controls, 6-propyl-2-thiouracil-induced hypothyroid rats (PTU) and thyroxine-replaced hypothyroid rats (PTU + T_4). Data are reported as means ± SEM. *P<0.05 compared with control animals and $^{+}$P<0.05 compared with hypothyroid animals (univariate ANOVA and Newman-Keuls multiple comparison test).

action of FSH in cultures of porcine granulosa cells (31), suggesting a direct effect of T_3 on the ovaries. Other investigators (32-34) have suggested an indirect effect caused by low gonadotropic stimuli of the ovaries. We did not find any significant difference in serum gonadotropin concentrations. However, LH and FSH, despite being immunologically normal, can be biologically inactive (27). This was observed in the case of central hypothyroidism in which biological TSH abnormality was detected but with preservation of its immunoactivity (35). The same could happen as a result of lower GnRH stimuli in hypothyroid rats.

The cycle arrest in diestrus II is considered by several authors (1-3) as a signal of anovulatory cycle and usually is preceded by irregular cycles. Hypothyroid T_4-replaced rats presented a partial recovery of the cycle pattern as well as follicular growth, showing that the changes observed in hypothyroid animals were not due to a toxic effect of PTU, but rather to the lack of thyroid hormones, and possibly to prolactin normalization. It is well known that T_4 decreases both TRH synthesis and secretion. Thus, the decrease in prolactin observed in the T_4-treated hypothyroid rats could be due to lower serum TRH concentration in this group since TRH stimulates prolactin.

The presence of corpora lutea and mature follicles, even though in smaller numbers, in hypothyroid rats could be explained by the presence of these structures before PTU treatment, since the animals were postpubertal. When the animals were treated before puberty (5), the absence of corpora lutea and a decrease of antral follicles were found. The smaller corpora lutea could explain a lower estrogen and progesterone production in the secretory phase of the estrous cycle. Since both sex steroids inhibit the synthesis of gonadotropins, the lower inhibitory action of these hormones despite normal serum concentration could explain the higher pituitary FSH and LH content in hypothyroid animals. On the other hand, higher serum prolactin concentration could be the cause of both inhibition of gonadotropin secretion (12), delay in folliculogenesis (15) and poor corpus luteum development (5).

In conclusion, hypothyroid rats had a dysfunction in the pituitary-ovarian axis that impaired follicular maturation and development of corpora lutea. The higher prolactin production could block gonadotropin secretion and action, although maintaining sex steroid production by the corpora lutea. Other studies blocking prolactin secretion in this situation are necessary to test this hypothesis.

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