

# HISTOPATHOLOGICAL CHANGES IN TESTICLES AND UTERUS OF RATS WITH HYPERTHYROIDISM AND HYPOTHYROIDISM

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## ABSTRACT

**PURPOSE:** The aim of this article was to determine the features of pathomorphological changes in testicles and uterus of rats associated with thyroid hormone (TH) levels.

**MATERIAL AND METHODS:** Experimental dysfunction of thyroid gland was induced in rats by daily administration of thyroxin in a dose of 50 ug/100 g (hyperthyroidism) and mercazolil in a dose of 5 ug/100g (hypothyroidism). Thyroid and sex hormone profiles were determined. Microscopy and morphometry of testicles in males and uterus in females were performed. The quantity of spermatids and spermatozoa as well as total number of spermatogenic cells was calculated.

**RESULTS:** Hypothyroidism reduced TH concentrations in female rats and increased sex hormones while hyperthyroidism augmented TH and sex hormone levels. In hypothyroid male rats, a decreased TH and unchanged testosterone levels were found out while in hyperthyroid ones there were increased TH and decreased testosterone levels. There was a gender difference in terms of TH changes in hyperthyroidism, i.e. T3 and T4 elevation was more outlined in male than in female rats. The most significant histopathological changes were established in the uterus of hyperthyroid female rats and presented with signs of an acute vascular insufficiency, inflammation, hypertension and fibrosis. Hypothyroidism exerted a crucial effect on pathomorphological changes in rat testicles consisting in atrophy of spermatogenic epithelium, vascular insufficiency, hypertension and fibrosis as well.

**CONCLUSION:** TH level significantly influences on the pathomorphological changes in the male and female reproductive system.

**Key words:** *thyroid hormones, sex hormones, hyperthyroidism, hypothyroidism, rats*

## INTRODUCTION

Thyroid gland dysfunction is a common disease in Ukraine. During the recent decade, there is a considerably increased incidence rate of thyroid dysfunctions by more than three times (8,9). The thyroid dysfunction is recognized as one of the primary

reasons along with diabetes mellitus for disability in adults in Ukraine (9). Disturbed thyroid status can cause changes in various systems including the male and female reproductive systems. Despite the fact that the effect of thyroid hormone (TH) activity on the development of the reproductive system was previously described (1,3,5), the mechanism by which TH affect the tissues and the various functions of the gonads remain not clarified yet. It is known that triiodothyronine (T3) regulates the growth of gonads by controlling the proliferation and differentiation of Leydig cells and Sertoli cells in humans and rodents during the testicular development (1,3,5). The presence of TH receptors in human and rat gonads dur-

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ing the entire period of development suggests that T3 may influence on the reproductive system by a classical genetic mechanism.

The objective of this article was to determine the features of pathomorphological changes in the testes and uterus of rats associated with TH levels.

## MATERIAL AND METHODS

This experimental study covered 232 mature white rats which were kept on a normal diet under standard vivarium conditions in Odessa National Medical University, Odessa, Ukraine. Rodents were divided into three groups: first - experimental hyperthyroidism was induced by daily administration of thyroxin (50 ug/100 g) to 41 female and 41 male rats; second - experimental hypothyroidism was caused by daily administration of mercazolil (5 ug/100g) to 45 female and 44 male rats, and third - control (intact) group consisting of 31 female and 30 male rats. Duration of the experiment was two-week long. Thyroid (T3 tetraiodothyronine, T4) and sex hormone profiles were determined. Microscopy and morphometry of testicles in male and of uterus in female rats were performed. The quantity of spermatids and spermatozoa as well as the total number of spermatogenic cells was calculated. ELISA hormone assay of Xema-Medica firm was used. Statistical software was applied for data analyses by means of group t-test. Results were presented as mean±standard error of the mean (SEM). P-values less than 0,05 were considered statistically significant.

## RESULTS

### Hormones

Concentrations of T4, T3 and sex hormones such as estradiol and progesterone in female and testoster-

one in male rats in hyperthyroid, hypothyroid and control groups are presented in Table 1 and Table 2.

Experimental hypothyroidism leads to significant reduction of the concentrations of T4 (by 1,3 times), T3 (by 2,6 times) in female rats and reduces the concentrations of T4 (by 3,6 times) and T3 (by 2 times) in male rats compared with those in the control group. T3 level in hypothyroid male rats decreases by 1,2 times more than that in hypothyroid female rats while T4 concentration decreases by 1,5 times more than that in hypothyroid female rats. There are considerable changes of TH concentrations and, especially of T4, in hyperthyroidism. Estradiol and progesterone concentrations in hypothyroid female rats are by twofold higher than in those in the control group. Testosterone level in hypothyroid male rats remains almost unchanged ( $p<0,05$ ).

In female rats with experimental hyperthyroidism, there is an increased T4 level (by 2,8 times) and T3 level (by 2 times). In male rats with hyperthyroidism, there is T4 elevation (by 3,8 times) and T3 one (by 2,5 times) than the values in the control group. In rodents with hyperthyroidism, T3 concentration is by 1,4 times and T4 one is by in 23 times in male than in female rats. Estradiol concentration is by 1,5 times higher and progesterone one is by 1,7 times higher in hyperthyroid female rats than that in the control group. Testosterone concentration is by 2,6 times lower in hyperthyroid male rats when compared with that in the control rats.

### Histology

The histological study of the testicles shows that both experimental hyperthyroidism and hypothyroidism lead to structural changes in the testicular parenchyma. There are some initial signs of atrophy consisting in reduced spermatogenic epithelium, un-

Table 1. Hormone concentrations in female rats

Hormones	control rats (n=31)	hyperthyroid rats (n=41)	hypothyroid rats (n=45)
T4 (pmol/L)	17,86±0,84	33,82±8,43§	6,07±0,85
T3 (nmol/L)	2,280±0,008	4,730±0,002*,§	0.870±0.006*
estradiol (nmol/L)	0,28000±0,00014	0,4200±0,0002	0,51000±0,00035
progesterone (nmol/L)	54,92±4,15	98,18±20,69	117,42±29,44

Data are presented as mean±SEM; \* $p<0,001$  versus control rats; § $p<0,001$  versus hypothyroid rats

Table 2. Hormone concentrations in male rats

Hormones	control rats (n=30)	hyperthyroid rats (n=41)	hypothyroid rats (n=44)
T4 (pmol/L)	21,30±0,84	78,38±7,60*,§	5,90±0,08#
T3 (nmol/L)	2,790±0,013	7,02±0,17*,§	1,380±0,008*
testosterone (nmol/L)	19,00±0,77	7,14±0,14*,§	16,40±1,57#

Data are presented as mean±SEM; \* $p<0,001$  versus control rats; # $p<0,05$  versus control rats; § $p<0,001$  versus hypothyroid rats

even diminution of the number of the spermatozoa in seminiferous tubules revealed by two times more frequently in hypothyroid male rats than in hyperthyroid ones. The following initial signs of atrophy of spermatogenesis are found out in hypothyroid male rats only: formation stage in several seminiferous tubules only; formation of spermatozoa on sustentocytic surface not only in the middle but also in the lower level of adluminal layer; dark cells dominate among spermatogonia, while the number of light cells reduces; in some seminiferous tubules (no more than  $\frac{1}{4}$  of the total number), there are neither spermatozoa, nor spermatogenic epithelium. There are only first-order spermatocytes as spermatids are absent and most convoluted tubules do not contain any mature spermatozoa in their lumen. This is a manifestation of spermatogenesis atrophy and a morphological equivalent of azoospermia. These changes are illustrated on Fig. 1.

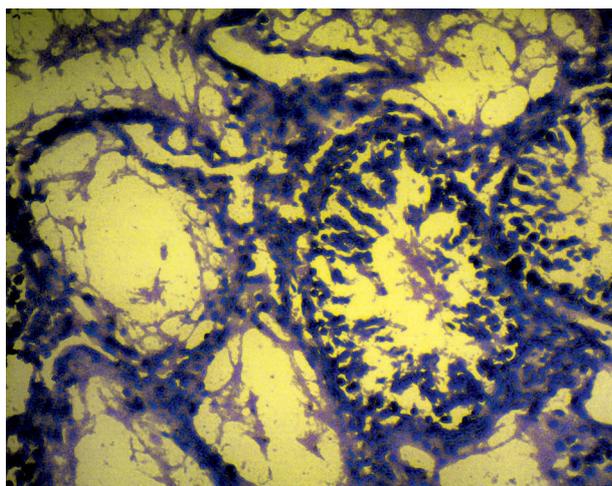


Fig. 1. Pathomorphological changes in the testis of hypothyroid rats consisting in a reduced number of dividing spermatogonia and mature spermatozoa, a reduced thickness of seminiferous epithelium layers and a considerable interstitial edema. Hematoxilin and eosin staining (x 400)

Table 3 demonstrates the amount of the spermatogenic cells. Their total number decreases in hyperthyroidism and hypothyroidism as well - by 5,7 times in the testes of hypothyroid and by two times in the testes of hyperthyroid rats when compared to that of the control rats. The atrophic changes are more outlined in hypothyroidism. The signs of vascular insufficiency such as plethora of vessels, considerable interstitial edema and enlarged intertubular spaces are detected in male rats of all the experimental groups. There are features of hypertension such as isolated sclerosis of arteriolar walls in hypothyroid male rats or generalized one in hyperthyroid male rats only.

The morphometric study of the testes reveals the parenchymal-mesenchymal index. It amounts to  $8,48\pm0,02$  in control rats, to  $0,92\pm0,0006$  - in hypothyroid rats ( $p<0,001$ ) and to  $3,55\pm0,008$  - in hyperthyroid rats ( $p<0,001$ ). In hypothyroidism, this index is by 9 times lower than that in control animals. It can be concluded that the content of connective tissue in the testicles of hypothyroid rats is significantly (by 9 times) higher than of the control animals. Stroma content in the testes of hyperthyroid rats is by two times higher than that of the control group.

In both hyperthyroid and hypothyroid rats, there is a great number of spermatogenic epithelium, spermatozoa in the seminiferous tubules as well as a focal accumulation of spermatozoa in the lumen of the tubules. These patterns are typical of normal and unchanged testes. Another normal finding is that Leydig cells are characterized by irregular groups with perivascular location.

Microscopically, uterus of rodents from the control group presents with a dense connective tissue-like stroma with endometrial glands. In this stroma, there are lymphocytes and granulocytes. Their concentration is maximal at the stromal-ep-

Table 3. Number of the spermatogenic cells in male rats

Spermatogenic cells	control rats	hypothyroid rats	hyperthyroid rats
spermatids	880,83±80,06	166,98±7,56*	655,6±63,5*
spermatozoa	1169,6±83,39	188,5±6,2*	292,72±23,56*
total	2050,43±163,45	355,48±13,76	948,32±87,06

Data are presented as mean±SEM; \* $p < 0,001$  versus control rats

ithelial border. There are numerous spiral vessels with thin walls. The surface of endometrial epithelium contains almost always numerous rows (in one observation, however, it deals with a single row only). There are many light secretory cells, too.

The histological study of the uterus demonstrates the signs of acute vascular insufficiency. They consist in uneven venous plethora, moderate interstitial edema, venous stasis and sludge in hyperthyroid female rats. In such animals, the signs of inflammation consisting in moderate focal interstitial infiltration of the mucosa by lymphocytes, histiocytes, diffuse eosinophilic infiltration of mucosal and submucosal layers, moderate edema of submucosa, diffuse infiltration by fibroblasts of any layers are more common. There are signs of hypertension such as irregular spasm of arterioles, formation of endothelium of fence type, goffering of elastic membrane, thickening of the walls of arteries and arterioles due to hyperplasia of muscle cells, thin perivascular collagen fibers in both experimental groups being, however, more outlined in hyperthyroidism. The features of old hemorrhages represent a deposition of brownish pigment in the mucous layer and of granules in the macrophages in hypothyroid female rats only. The fibrotic changes in both experimental groups are the following: presence of thin collagen fibres in the submucous and subserous layers and in the mesenterial interstitium; a band-like deposition of collagen fibres under the mesothelium; a moderate sclerosis and deposition of collagen fibres in the mucous layer, and a focal deposition of collagen fibres in the interstitium. Penetration of collagen fibres between smooth muscle cells and muscle bundles as well is an evidence of fibrosis distribution. The fibrotic changes are of equal frequency in both hyperthyroidism and hypothyroidism. Maturation of granulation tissue with delicate net of collagen fibres in the submucosal layer results from necrosis and focal replacement by con-

nective tissue. These alterations are demonstrated in Fig. 2.

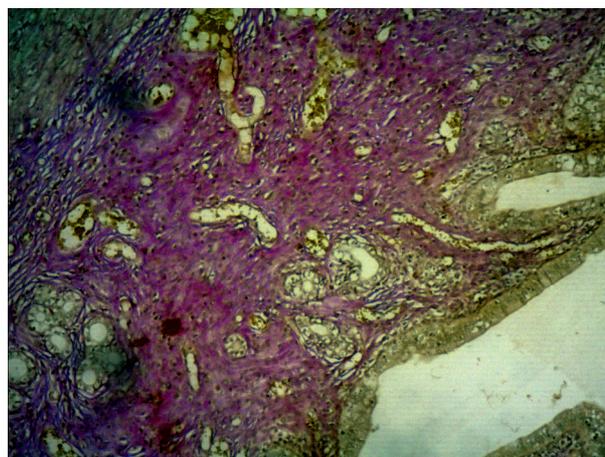


Fig. 2. Pathomorphological changes in the uterus of hyperthyroid rats consisting in a mild interstitial edema. Van Gieson staining identifies a deposition of collagen and elastic fibres around the blood vessels and focally in the interstitium (x 200)

The content of connective tissue in the uterus is examined by morphometry. There is a significant difference between stroma content in hyperthyroid and hypothyroid female rats when compared with the control rats. The parenchymal-mesenchymal index of endometrium is the following:  $1,48 \pm 0,001$  in the control group,  $2,18 \pm 0,03$  - in hypothyroidism and  $4,43 \pm 0,001$  - in hyperthyroidism. It is by three times higher in hyperthyroid female rats ( $p < 0,001$ ) and by 1,5 times higher - in hypothyroid ones ( $p < 0,001$ ) than in the control animals.

## DISCUSSION

In hypothyroidism, a reduced TH concentration occurs in females and males being more clearly manifested in males. There is no evidence of any gender-specific differences of TH level in hypothyroidism (12). In hyperthyroidism, an increased TH

concentration is observed in any rodents being more significant in males. There is no data about gender differences of TH level changes in hyperthyroidism (12). Thus, some changes of TH concentration occur in experimental hyperthyroidism and hypothyroidism. There are gender differences of TH changes under the conditions of impaired thyroid status. These results clearly show that the experimental models of primary hyperthyroidism and hypothyroidism are effective.

In hypothyroidism, the decreased TH level is accompanied by an increased concentration of sex hormones in female rats. These results coincide with literature data that in mature female rats, hypothyroidism inhibits follicular development and estradiol secretion, whereas progesterone and prolactin plasma concentrations increase in hypothyroidism (4,5,10). According to some investigators (2), the concentrations of estradiol and progesterone are reduced in hypothyroid female rats. We could suppose that such a variety of the influence of the hypothyroid status on the level of sex hormones and related reproductive properties could be due to different experimental models and duration of experiments.

Testosterone concentration in hyperthyroid male rats decreases by 2,6 times when compared with that in control rats. Hypothyroidism in males is associated with decreased (5,7,11), increased and normal (7) free testosterone level.

It is known that TH stimulates synthesis of a specific glycoprotein, the sex hormone binding globulin (SHBG), in the liver. It could bind testosterone and dehydrotestosterone (DHT) with a high affinity and estradiol with a lower affinity. Hypothyroidism is characterized by decreased blood SHBG level that leads to elevation of the biologically active testosterone, which in turn inhibits ovulation. The low systemic level of TH inactivation of estrogens changes the formation of less active forms that elevate the estrogen levels and violate an adequate feedback mechanism in gonadotropins such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) secretion regulation. Thus, lack of TH leads to changes in the synthesis, transport, metabolism and peripheral effects of sex hormones.

In our experiment, a significant gender-specific difference in TH level in hyperthyroidism was

established. T3 level was by 1,4 times higher and T4 was by 1,8 times higher in male than in female rats. There are various opinions about the influence of hyperthyroidism on the hormonal activity of gonads (3,4,6). Increased blood estradiol concentrations during all the phases of the estrous cycle caused by elevated SHBG level (2) or unbound estrogen concentration (10).

There is on-going discussion about the possible association between the elevated plasma estrogen and increased SHBG or free estrogen level. The metabolic rate of estradiol decreases in hyperthyroidism mostly due to increased estradiol binding to SHBG (6). There are changes in the level of circulating androgens in hyperthyroid females consisting in significantly increased secretion rates of testosterone and androstenedione, too. The conversion frequency of androstenedione to estrone and of testosterone to estradiol increases in hyperthyroidism, too (6). Estradiol concentration was not changed in 70% of the cases with hyperthyroidism and was elevated in 30% of the cases (10).

We establish a significant reduction of testosterone level in both experimental groups. Hyperthyroidism is associated with increased total testosterone level (11) and normal concentration of free or biologically active testosterone (6). These changes are due to increased SHBG level that is associated with hyperthyroidism. The elevated SHBG level leads to free testosterone reduction that results in an increase of serum LH (negative feedback), testosterone and estradiol by peripheral conversion.

Testosterone and estradiol synthesis reduces in hyperthyroidism while androstenedione and estrone one remains within normal limits. Conversion of testosterone into androstenedione and of testosterone into DHT decreases while that of androstenedione into testosterone and of androstenedione into DHT increases (6). These changes can be explained by the increased serum SHBG level that binds testosterone and DHT rather than androstenedione. Synthesis rates of testosterone and estradiol are within normal limits while those of androstenedione and estrone raise in hyperthyroidism (6,11).

In healthy males, testosterone, DHT and estradiol are circulating in plasma and partially binding to SHBG, which is synthesized in the liver. The se-

rum SHBG level directly depends on blood TH level. Sex hormones that are bound with SHBG are inactive. The sex hormones which are unbound or bound with albumin remain biologically active. Testosterone concentration in the testis is maintained at a high level in contrast with its serum one by androgen-binding protein secreted by Sertoli cells under the influence of FSH. The high intratesticular testosterone concentration may be necessary for the normal functioning of Sertoli cells and thus play a role in spermatogenesis.

Therefore, in experimental hyperthyroidism and hypothyroidism, there is an increased and decreased TH concentration, respectively. Testosterone concentration reduces in hyperthyroidism and does not change in hypothyroidism in males. Hyperthyroidism results in increased estradiol and progesterone levels in females. In experimental hypothyroidism, there are increased sex hormone levels of females and unchanged testosterone levels in males. There is a gender difference in TH change in hyperthyroidism, i.e., elevation of T3 and T4 is more outlined in males than in females. Thus, in females with hypothyroidism, the concentration of TH decreases and that of sex hormones increases while in experimental hyperthyroidism, the levels of TH and sex hormones increase.

The effects of hyperthyroidism on the male reproductive system are manifested by the signs of hypertension in the testis. Hypothyroidism results in initial signs of spermatogenic epithelium atrophy, vascular insufficiency, and hypertension. One can conclude that hypothyroidism exerts a crucial influence on the pathomorphological alterations in rat testes.

The systemic hyperthyroidism effects on the uterus present with the signs of acute vascular insufficiency, inflammation, hypertension and fibrosis. Hypothyroidism results in hemorrhage, hypertension and fibrosis in the uterus. One can conclude that there is a significant effect of TH and, especially of systemic hyperthyroidism, on the uterine tissue.

## CONCLUSION

The experimental hypothyroidism causes a decreased concentration of TH and an increased one of sex hormones in females, while the experimental hy-

perthyroidism elevates the levels of these hormones. Hypothyroidism presents with a decreased TH level and unchanged level of testosterone while hyperthyroidism does with an increased TH and a decreased testosterone concentration. The gender difference in the change of TH in hyperthyroidism consists in the higher elevation of T3 and of T4 in male than in female rats.

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