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FOLLICULAR DEVELOPMENT AND ATRESIA IN THE OVARIES OF THYROXINE TREATED JUVENILE RATS

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The effect of early hyperthyroidism on folliculogenesis in the ovary of juvenile rats was investigated. Hyperthyroidism was induced by injection of 150 μg DL-thyroxine (DL-T_4) to females every other day, from the 9th to the 29th day of life. Twenty four hours after the last injection treated females and corresponding intact animals were sacrificed, and the ovaries were examined using standard light microscopy and semithin sections.

DL-T4 caused a significant decrease of pituitary and ovary mass, compared to intact animals. The population of healthy primary follicles was increased in the ovaries of treated rats. At the same time, the total number of atretic follicles, especially growing, was also significantly increased. Antral follicles without apoptotic changes were scarcely observed. Furthermore, granulosa cells displayed morphological changes characteristic for apoptosis: widened intercellular spaces, condensation of chromatin, cellular fragmentation and formation of atretic bodies.

Key words: DL-thyroxine, hyperthyroidism, ovary, juvenile rat

INTRODUCTION

It is well documented that thyroid hormones (TH) are important for the development of both the central nervous system and the autonomous system. The rat brain is thyreosensitive during the postnatal period (especially the first 14. days), which is characterized by nerve cell differentiation and glial cell multiplication. Application of T_4 accelerates nerve maturation, and reduces the number of nerve cells (Nicholson and Altman, 1972, Tsukada et al. 1977). Recent investigations confirm the presence of a T_3 binding protein in the nuclei of oligodendrocytes at the time of myelination (Besnard et al. 1994).

However, the role of TH in the regulation of the ovarian function of juvenile animals is still poorly understood. Varlamova (1988) reported a normal concentration of progesterone, decreased concentration of estrogen and cystic changes in the ovaries in hyperthyroid girls. These changes were directly proportional to the level of TH.

In vitro findings have demonstrated that TH act as biological amplifiers and together with follicle-stimulating hormone (FSH) exert a stimulatory effect on morphological and functional differentiation of granulosa cells (Maruo et al., 1987, 1992). Contrary to this, Chan and Tan (1986) reported that TH exert inhibitory effects on FSH induced aromatase activity in cultured porcine granulosa cells. The results of Fitko and Szlezyngier (1994) suggest that hyperthyroidsm decreases the LH/hCG receptor population in rat ovaries.

Our previous studies have shown that TH has a negative feedback action on receptors in the nucleus paraventricularis (PVN) and the nucleus ventromedialis (VMN) of the hypothalamus in juvenile male rats, and also delays differentiation of thyrotrophs and gonadotrophs in the pituitary (Stošić 1980, Stošić et al., 1990). Structural changes in the germ epithelium with retardation of spermatogenesis in juvenile rat testicles after DL-thyroxine treatment were also demonstrated (Stošić and Radovanović, 1993).

The present study was undertaken to investigate the effect of high doses of DL-T_4 on follicular development and atresia in the ovaries of juvenile rats.

MATERIAL AND METHODS

In our experiment juvenile female Albino Oxford (AO) rats were used. They were kept under controlled laboratory conditions and a 12h/12h light/dark cycle. The rats were fed on standard dry pellets (VZ Zemun) and tap water ad libitum. Nine day old females were treated subcutaneously every other day with DL-thyroxine (Roche), in doses of 150 μg per animal, up to the 29th postnatal day. Treated and corresponding intact animals were sacrificed 24 hours after the last injection. Isolated ovaries were weighed, fixed in Bouin's solution, and embedded in paraffin. Five μm thick serial sections were stained with haematoxylin-eosin or azan methods. Every fifth section was examined histologically and the follicles were classified and counted.

Immediately after isolation, pieces of ovary were fixed in cold 4% glutaraldehyde buffered in Miloning, postfixed in 1% buffered osmium acid and embedded in araldite or epon resin. Semithin sections, 1μm thick, were stained with methylene blue, and the cytological characteristics of oocytes and other follicular structures were examined using light microscopy.

Using morphological and cytological criteria, healthy and atretic follicles in different stages of development were classified as: primordial follicles with a single layer of flattened follicular cells surrounding the oocyte; primary follicles with a continuous layer of cuboidal cells; growing follicles, with two or more layers of follicular cells; preantral follicles, with one or more small irregular intercellular spaces, and antral, with a single cavity. The results were summarized as an average number of healthy and atretic follicles per section, and statistical significance was assessed using Student's t-test.

RESULTS

Values for pituitary and ovary mass of intact and DL-T_4 treated rats are summarized in Table 1. It is evident that DL-T4 caused a significant decrease of

both pituitary and ovary mass, compared to intact animals. However, the inhibitory effect on ovary development was more pronounced.

Table 1. Pituitary and ovary mass of intact and DL-T_4 treated rats

	Intact (n=11)	DL-T4 (n=7)
Pituitary mass (mg)	3.92 ± 0.5#	2.85 ± 0.92*
Ovary mass (mg)	36.53 ± 3.68	29. 93 ± 3.18**

n number of animals
Mean ± SE
Significantly different
* p< 0.05 ** p <0.01

Histological analysis revealed that there was a difference in the number of follicles in each particular stage of development between the ovaries of T_4-treated and intact rats (Figure 1). The population of healthy primary follicles was in-

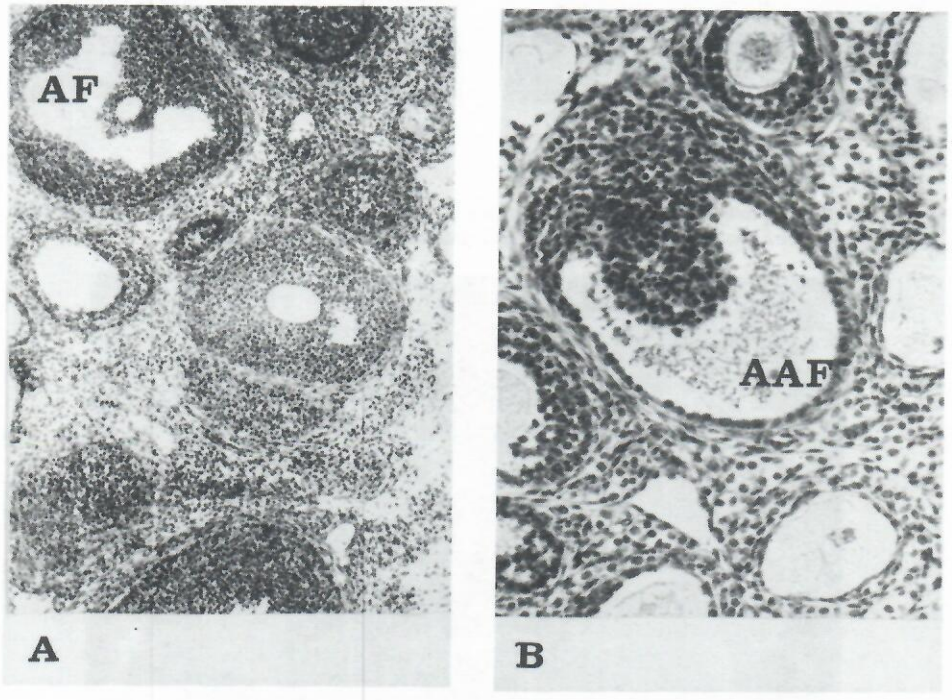

Figure 1. Follicles in various stages of folliculogenesis in the ovaries of intact (A) and treated (B) juvenile rats haematoxylin-eosin, A-x75, B-x187 / AF-antral follicle, AAF-atretic antral follicle.

creased in treated females. However, the number of atretic follicles, especially derived from growing ones (Figure 2), was also significantly increased in these

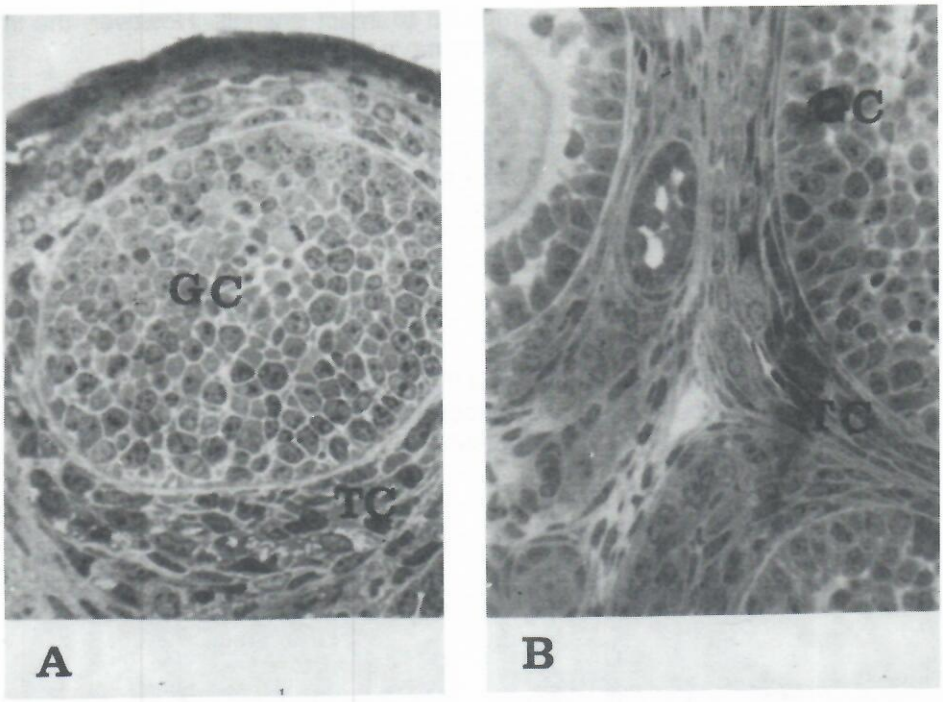

Figure 2. Growing follicle in the ovary of an intact (A) and a DL-T_4-treated (B) juvenile rat /semithin sections, methyelene blue, x1200 / GC-granulosa cells, TC-theca cells.

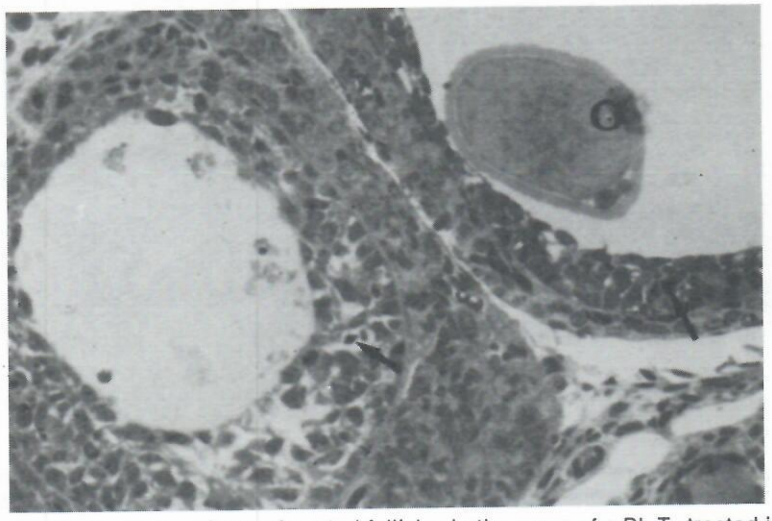

Figure 3. Apoptotic changes in atretic antral follicles in the ovary of a DL-T_4-treated juvenile rat / semithin sections, methylene blue, x1020/O-oocyte with polar bodies, arrow - apoptotic body.

animals (Figure 4). Compared to intact juvenile females, the oocytes in growing follicles of treated animals were larger, but other follicular structures frequently revealed signs of arrested development and degeneration.

Due to this, the number of healthy antral follicles was significantly decreased and follicles without atretic changes were scarcely observed (Figure 4). Furthermore, some granulosa cells were hypertrophic, while most of them displayed morphological changes characteristic for apoptosis: widened intercelullar spaces, condensation of chromatin, cellular fragmentation and formation of atretic bodies (Figures 1B and 3). Oocytes with polar bodies lay free in the antrum of follicles in late stages of atresia and lacked corona radiata (Figure 3).

The follicular theca of treated females was rich with fibroblast-like cells, containing condensed chromatin, and poorly vacuolated cytoplasm (Figure 2B).

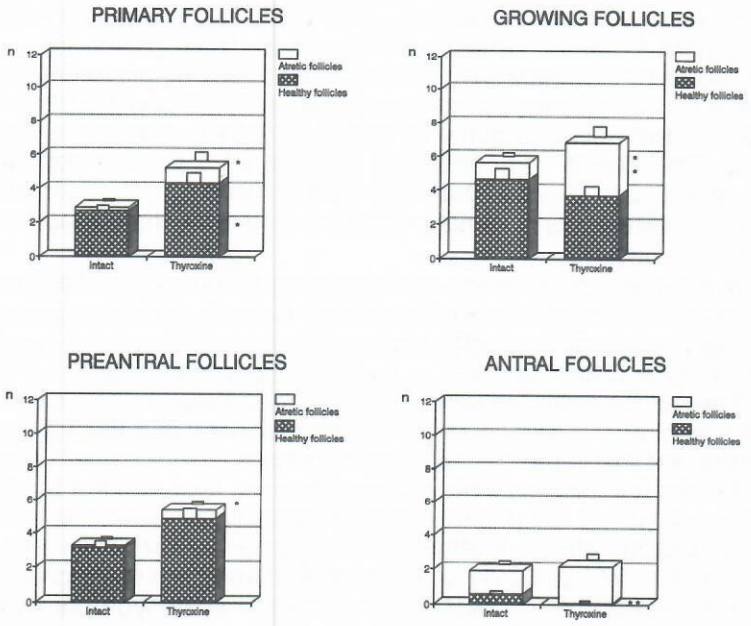

Figure 4. Number (n) of healthy and atretic follicles per section in ovaries of intact and DL-T4 treated rats (mean ± SE, * – $p < 0.05$, ** – $p < 0.01$)

DISCUSSION

The significant reduction of pituitary and ovary mass observed in DL-T_4 treated juvenile female rats in our experiment is comparable with similar results reported by other authors. Thyroxine applied as an implant or injection during

the postnatal period reduced both pituitary and serum FSH concentration (Bakke et al., 1974). As a consequence ovarial mass was reduced, puberty delayed and fertility of rats diminished. Our previous results showed that multiple doses of T_4 inhibited the differentiation of pituitary GTH, TSH and GH cells in infantile male rats also (Stošić et al., 1990). Many of these cells revealed different signs of regression.

In mature female rats T_4 treatment decreased serum FSH and increased serum LH in all phases of the estrous cycle (Žunić, 1990). Schneider et al. (1979) also reported that T_4 decreases FSH concentration in the serum of adult female rats and they suggested that this is mediated via direct suppression of FSH cell activity or via acceleration of FSH cell metabolism. Another possible explanation applicable to adult rats can be based on a suggestion given by Tonooko (1979) that neonatal hyperthyroidism induces hypofunction of the hypothalamo-pituitary axis. The first two weeks of postnatal life is the period when many morphological and biochemical TH dependant changes in the brain occur: nerve differentiation including arborization of the dendritic processes, and multiplication of glial cells (Nicholson and Altman, 1972, Tsukada et al. 1977, Besnard et al. 1994). Thus, levels of thyroid hormone receptors (TR-α1 and Tr-β1) in the brain rise to maximum values by postnatal day 10 (Strait et al., 1990). Moreover Stošić (1980) reported that DL-T_4 treatment of male rats, between the 3rd and the 18th postnatal days, reduced the nuclear volume of PVN and VMN hypothalamic cells, and inhibited the release of neurosecretory material from Gomori positive cells. Therefore, these data suggest that high levels of thyroid hormones affect the hypothalamo-pituitary axis in immature rats, and indirectly the development and function of the ovary.

There are many other reports demonstrating the possibility of a direct effect of TH on ovarian function at the cellular level. Granulosa cells of preovulatory antral follicles from nonstimulated human ovaries showed the presence of thyroid hormone receptor mRNA for both α and β receptors. Estimation of the total number of granulosa cells that were positive ranged between 25% and 50% per follicle (Wakim et al., 1994). Maruo et al. (1987) reported that thyroid hormones play an important role in the differentation of porcine granulosa cells (harvested from small sized follicles) growing *in vitro*. However, they needed to synergize with FSH to exert direct stimulatory effects on granulosa cell functions including morphological differentiation. Furthermore, TH stimulated FSH mediated LH/hCG receptor induction in these cells and increased conversion of pregnenolone to progesterone by acting at the level of 3β-hydroxysteroid dehydrogenase (Maruo et. al. 1992). They also reported that T_3 binding to granulosa cell nuclei decreased as the ovarian follicle matured. There was little difference in the affinity of T_3 receptors in granulosa cells during follicular maturation, but the number of T_3 receptors was higher in granulosa cells of small follicles compared to that in large follicles. These results obtained in *in vitro* conditions correspond to a certain degree with our results. Namely, the population of primary healthy follicles in our experiment was significantly increased in treated females which means that folliculogenesis has been activated. Since this phase is independent of FSH it is necessary to investigate whether this activation can be atributed to TH. However,

the significant increase of number of atretic follicles in different stages of maturation is probably a result of the decreased concentration of FSH reported in hyperthyroid rats (Schneider et al. 1979, Stošić et al., 1990, Žunić, 1990). Since Hsueh et al. (1994) and Tilly and Tilly (1996) reported that gonadotrophins are likely to be survival factors in preventing follicular apoptosis, the presumed decrease of FSH in our hyperthyroid juvenile females probably induces apoptotic cell death of granulosa cells at later stages of folliculogenesis.

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RAZVIĆE I ATREZIJA FOLIKULA U JAJNICIMA JUVENILNIH PACOVA TRETIRANIH TIROKSINOM

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SADRŽAJ

Ispitivan je uticaj ranog hipertireoidizma na jajnike juvenilnih pacova. Hipertieoidizam je izazvan injiciranjem DL-tiroksina ženkama pacova od 9. do 29. dana starosti u dozi 150 μg po životinji svakog drugog dana. Dvadeset četiri časa od poslednjeg injiciranja, tretirane i odgovarajuće intaktne ženke su žrtvovane, a jajnici fiksirani za svetlosnu ili elektronsku mikroskopiju i uklopljeni u parafin ili araldit. Parafinski preparati su bojeni hematoksilinom i eozinom ili azanom, a polutanki metilenskim plavim i analizirani svetlosnim mikroskopom.

DL-tiroksin je izazvao značajno smanjenje mase hipofize i jajnika u poređenju sa intaktnim životinjama. Kod tretiranih ženki uočeno je značajno povećanje broja normalnih primarnih folikula u jajniku. U isto vreme značajno je povećan i ukupan broj atretičnih folikula, a naročito folikula u rastu. Retko se uočavaju antralni folikuli bez apoptotičnih promena. Većina ćelija zone granuloze pokazuje morfološke promene karakteristične za apoptozu: kondenzovan hromatin, povećane međućelijske prostore i formiranje apoptotičnih tela.