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Nada Stošić-Bogdanović and Anita Radovanović

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THE EFFECT OF HYPOTHYROIDISM IN PREGNANT AND LACTATING DAMS ON THE TESTICLES OF JUVENILE PUPS

NADA STOŠIĆ-BOGDANOVIĆ and ANITA RADOVANOVIĆ

(Department of Histology and Embryology, Faculty of Veterinary Medicine, Bulevar JNA 18, Belgrade, Yugoslavia

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The experiments were performed with juvenile rat pups of dams in wich hypothyroidism had been induced by ingestion of propyl-thiouracil (PTU). Hypothyroidism was induced during the embryonal, fetal and postnatal period by addition of PTU to the drinking water used by the pregnant females and in the course of lactation. The germ epithelium of the testicles was analyzed by light and electron microscopy.

The results showed that prenatal and postnatal periods of development are sensitive to changes in thyroid hormone levels. The mass of the body, the testicles, and the pituitary were significantly reduced. Prolonged hypothyroidism of the dams affected maturation of the testicles of juvenile pups by postponing the development of the first generation of spermatogenesis. Examination of the germ epithelium revealed degeneratively affected spermatocytes, a decreased spermatid count and gradual regression of Sertoli cells with accumulation of a large number of electron dense lipid drops, dense and necrotic bodies and affected mytochondria in the cytoplasm. Changes in the Sertoli cells resulted in disorders of germ cell differentiation control and maturation of the testicles of the pups.

INTRODUCTION

Thyroid hormones are necessary for normal growth and development of animals. The same hormones modulate acitivity of numerous biochemical processes. However, their action and mechanism of action on the reproductive processes in males have not been elucidated. Our previous results indicated an intrinsic correlation of activities of the thyroid, pituitary-hypothalamic system and testicles of both juvenile and sexually mature rats (Stošić et al., 1969, 1973, 1977, 1980, 1981, 1990). Some referential data indicate that hypothyroidism impairs testicular function, while some deny that it has an influence on the testicles. However, insufficiency of thyroid hormones in the developing organism

severely affects its reproductive function. Delayed sexual maturation and reduced brain development have been detected in mice and rats in which hypothyroidism was induced by surgical or radiological thyroidectomy or by ingestion of propylthiouracil, thiouracil, carbimazole or methimazole (Meserve and Leathem, 1973; Meserve and Rhodes 1979; Sowers et al. 1981). Beamer (1981) states that hypothyroid male mice are sterile and their fetility can be reestablished by ingestion of food with the addition of dried powdered thyroid gland. Last year, the results reported by Francavlla et al. (1991), indicated that postnatal hypothyroidism in rats decreased serum gonadotropin levels and postponed spermatogenesis in puberty.

Contrary to previous data, hypothyroidism of sexually mature rams did not affect their spermatogenesis in spite of the fact that testosterone levels were changed as well as basal levels of luteotropic hormone (LH) in the serum (Chandrasekhar et al., 1985). Similar results were reported by Kalland et al., (1988) after treating adult rats with PTU. Exposure of adult rats to PTU failed to change testicle weight, while the seminal vesicles and prostates were somewhat lighter than in controls. The same animals had unaffected testosterone, follicle-stimulating hormone (FSH) and luteotropin (LH) levels. The findings of Chubb and Henry (1988) demonstrate that genetically induced hypothyroidism does not induce infertility.

Having in mind the conradictory reports on the effect of thyroid hormones on reproduction, we studied the effect of maternal hypothyroidism induced by exposure of the dams to propyltiouracil on days 4 and 5 of pregnancy and during lactation, on the testicles of the male pups. Possible structural and substructural changes of some types of cells in the germ epithelium and blood-testicle barrier of juvenile pups, were sought.

MATERIAL AND METHODS

Healthy adult rats of the Albert Oxford strain (AO) were used for the experiment. The animal were acclimatized to laboratory conditions. Females were separated from males. All animals were kept in the same conditions in terms of food, temperature and humidity. Vaginal smears were taken from females daily for two weeks always between 8:00 and 10:00 a.m. Mated female rats were given propylthiouracil (PTU) in the drinking water (1 mg/l) on days 4 and 5 of pregnancy and during lactation. After birth, 30 day old pups born to experimental (15) and control (10) dams were killed by cervical dislocation. The isolated testicles were measured, weighed and fixed in Bouin's solution and, after embedding in paraffin wax, 5 μm thick sections were cut and stained with Weigert hematoxylin and eosin. For the purpose of electron microscopy the sections of the testicles were fixed in 4 % glutarldexyde in Miloniq buffer and postfixed in Miloniq-buffered 1 % osmic acid and embedded in epon or araldite. Ultrathin sections were contrasted with saturated solutions of uranyl acetate and lead citrate. Sperm production per rat was determined from the number of spermatids. Body, testicle and pituitary mass, as well the number of spermatids were subjected to statistical analysis using Student's t-test.

RESULTS

Propyltiouracil ingested by the dams during pregnancy and lactation significantly reduced body mass ($p<0.001$), testicular mass ($p<0.01$) and pituitary mass ($p<0.05$).

Table 1. Body, tasticular and pituitary mass of the control pups (C), and those from dams treated with PTU (Pt)

Tissue	C	Pt
Body mass (g)	58 ± 8.838	43.1 ± 6.685 ***
Testicular mass (mg)	539.99 ± 129.61	349.81 ± 98.65 **
Pituitary mass (mg)	3.089 ± 0.61	2.27 ± 1.017 *

Values represent mean ± SD
*** -significantly different ($p<0.001$)
** -significantly different ($p<0.01$)
* -significantly different ($p<0.05$)

Components of the blood-testicle barrier in 30 day old pups from PTU treated dams were represented by complex structures surrounding the seminiferous tubules and Sertoli cells. The barrier was composed of cellular and intercellular structures containing homogenous electron-light zones in which collagen fibers were clearly visible. In the cellular part elongated myoid cells with a small number of micropinocytotic vesicles participating in transport were seen. The substructure of the myoid cells, collagen fibers and basal membrane

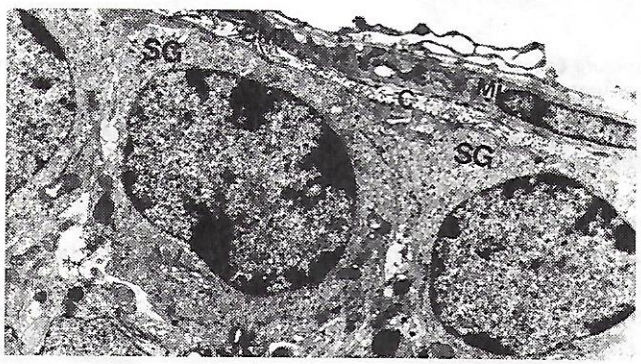

Figure 1. Unaffected substructure of myoid cells, collagen fibers of the acellular layer and basal membrane as a part of the blood-tasticle barrier of the testicler of a pup born to a PTU treated dams. 4 % glutaraldehyde, uranyl acetate and lead citrate. x 14000.
Mi – myoid cells; C – collagen fibers; BM – basal membrane, SG – spermatogonia.

was not changed (Figure 1). In the germ epithelium, Sertoli cells, as a part of the blood-testicle barrier were in gradual regression. Nucleoprotein granules were distributed along the nuclear membrane. Numerous dense drops of lipids of different sizes were present in the cytoplasm. Many of dense and necrotic bodies, short and single edematous mitochondria with vacuolized matrix were common features of the cytoplasm (Figure 2).

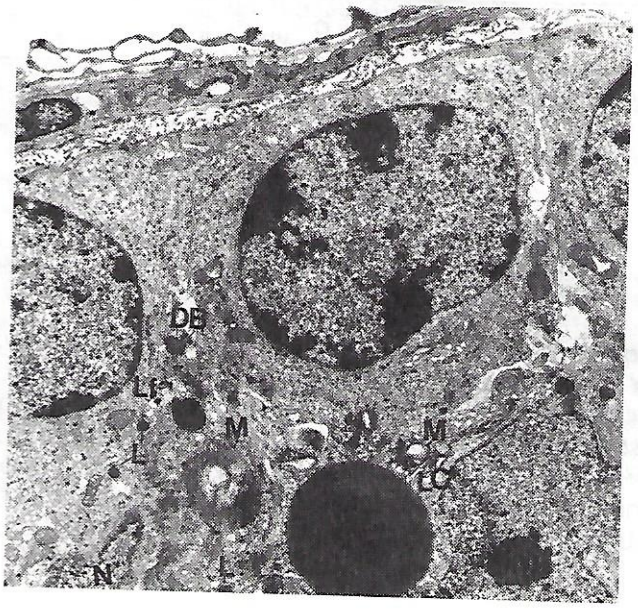

Figure 2. Substructure of testicular Sertoli cells of a pup from a PTU treated dams. 4 % glutaraldehyde, uranyl acetate and lead citrate. x 16000.
N – nucleus in regression; DB – numerous dense bodies; M – mitochondira with vacuolized matrix and disintegrated cristae; LC – dense lipid drops; L – lysosomes; Lf – fipofuscin granules.

In the germ epithelium of control pups spermatogonia, spermatocytes and spermatids were grouped into well defined cell associations distributed in definitive cycle of the germ epithelium. Spermatocytes in all phases of meiotic division were seen. More than 50 % of the seminiferous tubules were filled with spermatids (Figure 3A).

In the testicles of pups born to PTU-treated dams the adluminal compartment of the germ epithelium showed disorganization of the normal cell associations and a small number of cells had matured beyond the spermatocyte stage. In the germ epithelium preleptotene and zygotene spermatocytes, as well as

occasional pachytene spermatocytes, were seen (Figure 3B). The percentage of seminiferous tubules containing the most differentiated from of germ epsithelium - the spermatid, was decreased by 57 % (p 0.01) as compared to the controls (Figure 4).

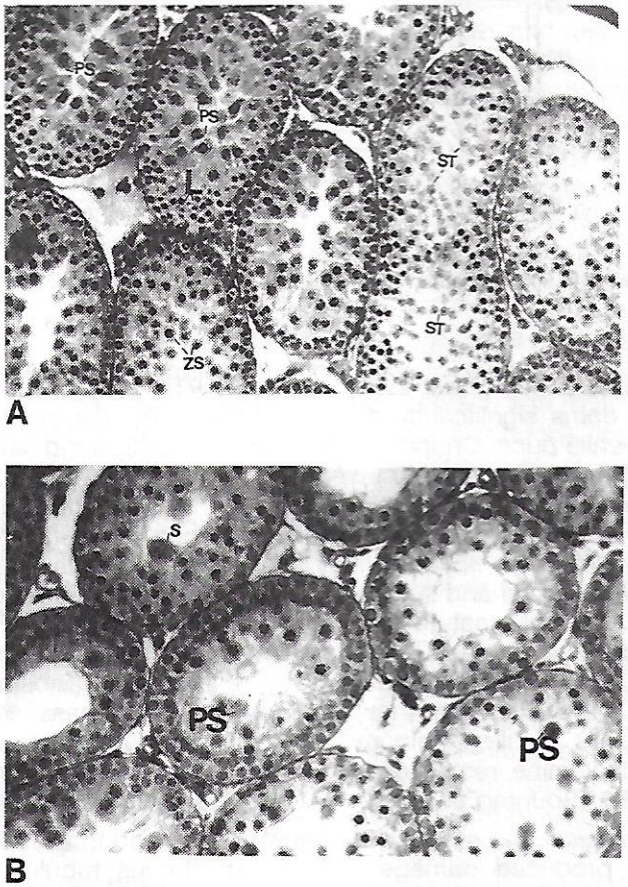

Figure 3. A: Germ epithelium of the testicles of a control pup. Bouin. Hematoxylin-eosin. x 460 x 550.
L – leptotene spermatocytes; ZS – zygotene spermatocytes; PS – pachytene spermatocytes; ST – spermatids.

B: regressively altered pachytene spermatocytes (PS) and unchanged (S) germ epithelium in the testicles of a pup from a PTU treated dam.

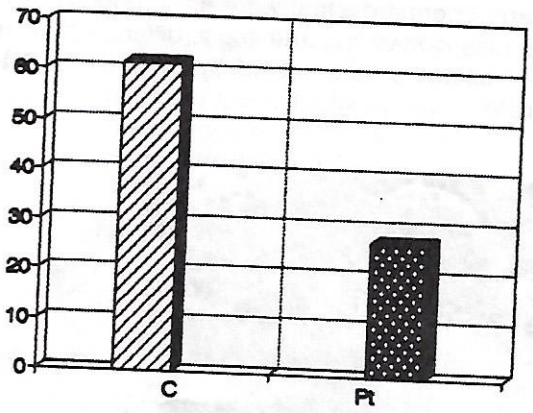

Figure 4. Spermatid number of pups from PTU -treated dams (Pt) compared to controls (C).

DISCUSSION

Our results showed that incorporation of PTU into the diet of pregnant and lactating dams significantly depressed the body, testicular and pituitary mass in all juvenile pups. Chandala and Thapliya (1972) suggested that growth hormone (GH) and prolactin (PRL) were directly or indirectly involved in growth and development of the body and the stated organs. Patricia et al. (1982) proposed that inhibited growth of 25 day old pups resulted rather from PTU presence than from hypothyroidism of the pregnant and lactating dams. According to our structural and substructural analysis of the pituitary of thyroidectomized male, sexually mature rats, differentiation of GH and PRL cells had been inhibited. Thyroxine also had a marked stimulatory effect on the differentiation of GH and PRL cells in the pituitary of intact rats (Stosic et al., 1981). Leung et al., (1981) believe that the reduced body mass of thyroxinated hypothyroid birds resulted from reduced GH levels, which agrees with our results. Catecholamine restriction is primarily responsible for the decreased secretion of GH (Sountag et al., 1980, 1982; Morimota et al., 1988).

Our cytological and substructural analysis showed that PTU consumption by the dams produced damage to the seminiferous tubules. In the germ epithelium derangement of the cells, degeneration of spermatocytes, gradual regression of Sertoli cells and decreased spermatid counts were recorded.

Our previous studies had revealed that exogenous thyroxine (T4) and surgical thyroidectomy of sexually mature rats and juvenile pups inhibited or stimulated the activity of neurosecretory cells of hypothalamic nuclei, thyreotropic (TSH), gonadtropic (GTH), GH and PRL pituitary cells with corresponding changes in the germ epithelium (Stošić et al., 1969, 1973, 1980, 1981, 1990).

Hypothyroidism and hyperthyroidism of sexually mature merino rams did not affect their spermatogenesis and daily *production* of sperm. However, the

mobility of ejaculated spermatozoa and serum testosterone levels were changed. Basal LH levels were lower and TSH concentrations also differed from those of controls (Chandrasekhar et al., 1985). The results of studies conducted by Kalland et al., (1988) showed no difference between the testicular mass of adult rats before and after ingestion of PTU. Nevertheless, weights of seminal vesicles and the prostate were significantly reduced. In the same rats TSH was elevated, while T4 was lowered and LH, FSH and testosterone were unchanged. Contrary to the previous findings, in the earlier stages of development, hypothyroidism of male rats reduced the biological activity of FSH, probably due to a direct effect of thyroidal hormones on FSH production (Ruiz et al., 1989). According to their own results, Francavilla et al., (1991) suggested that postnatal hypothyroidism of rats reduces serum gonadotropin levels and postpones spermatogenesis in puberty. Numerous studies have indicated a correlation between testicular changes and changes of pituitary hormones or corresponding receptors for these hormones in the testicles. Thus, fetal gonads like the testicles, have receptors for pituitary GTH and PRL. The beta TSH subunit (ßTSH) reversibly binds, with relatively high affinity, to receptors in the rat testicles. It has been proposed that the same receptors are used for ßTSH, hCG (choriogonadotropin) and LH. or that they interact. The ßTSH subunit, like hCG, stimulates cAMP production in Leydig cells (testosterone production) and Sertoli cells (including androgen-binding protein) (Amir et. al., 1977). Propylthiouracil-induced hypothyroidism significantly decreased PRL binding to the testicles (50 %). The modulation of PRL receptors by hypothyroidism suggests that thyroid hormones play a role in the regulation of PRL receptors (Kharroubi et al., 1984). Luteotropin is synergic with testosterone in stimulation of the seminal vesicles and the prostate.

It has already been established that thyroid hormones are important for development and maturation of the central nervous system. The rat brain is thyreosensitive from 8 to 14 days of postnatal life. This period is characterized by differentiation and multiplication of glial cells prepared for myelinization(Tsukada et al., 1977). Thyroxine stimulates the development of glial cells and the production of myelin in the brain neuron culture (Amur et al., 1984; Kawasa et al., 1988).

The results of our previous studies, as well as the results of the aforementioned authors suggest that prenetal and postnatal disbalance of thyroid hormones affects the development of neuroendocrinal mechanisms. thyroid hormone disbalance affects the development and maturation of the hypothalamic mechanism involved in the regulation of biosynthesis and release of GH, PRL and the glycoprotein hormones. The alteration of TSH, GH, PRL and GTH cells are accompanied by cellular alteration in the germ epithelium. The delay in the first generation spermatogenesis in the testicles in rat pups delivered from hypothyroid dams results from an alteration in the Sertoli cells and degeneration of spermatocytes, thus preventing maturation of the germ cells.

REFERENCES

1. *Amir, S. M., Sulivan, C. R., Sidney, H. J.* 1977. Binding of bovine thyrotropin to receptors in rat testis and its interaction with gonadotropins. *Reprint by the Endocrine Society, 103,* 101.
2. *Amur, S. G., Shanker, G., Pilringer, R. A.* 1984. Regulation oof myelin basic protein (arginine) methyltransferase by thyroid hormone in myelinogenic cultures of cells dissociated from mouse brain. *J. Neurochem. 43,* 494-498.
3. *Beamer, W. G. and Cresswell, L. A.* 1982. Defective thyroid ontogenesis in fetal hypothyroid mice. *Anat. Rec., 202,* 387-393.
4. *Chandrasekhar, Y., Holland, M. K., D'Occhio, M. J., Setchell, B. P.* 1985. Spermatogenesis, seminal characteristics and reproductive hormone levels in mature rams with induced hypothyroidism and hyperthyroidism. *J. Endocrinol., 105,* 39-46.
5. *Chandola, A. and Thaphiyal, J. P.* 1972. Effect of growth hormone and prolactin on the body weight and thyroid activity of Spotted Munia. *Annales d'Endocrinol., 33,* 583-591.
6. *Chubb, C., Nolan, C.* 1985. Animal models of male infertility mice bearing single-gene mutations that induce infertility. *Endocrinol., 117,* 338-346.
7. *Chubb, C. and Henry, L.* 1988. The fertility of hypothyroid male mice. *J. Reprod. Fert., 83,* 819-823.
8. *Francavilla, S., Cordeshic, G., Properzi, G., Di Cicco, L., Jannini, E. A., Palmero, S., Fugassa, E., Lorar, B., D'Armiento, M.* 1991. Effect of thyroid hormone on the pre- and postnatal development of the rat testis. *J. Endocrinol., 129,* 35-42.
9. *Kalland, G. A., Vera, A., Peterson, M., Swerdloff, R. S.* 1978. Reproductive hormonal axis of the male rat in exprerimental hypothyroidism. *Endocrinol., 102,* 476-484.
10. *Kawada, J., Mino,, H., Nishida, M., Yoshimura, T.* 1988. An appropriate model for congenital hypothyroidism in the rat induced by neonatal treatment with propylthiouracil and surgical thyroidectomy: studies on learning ability and biochemical parameters. *Neuroendocrinol. 47,* 424-430.
11. *Kharroubi, A. T., Shaunwhite, W. R.,* 1984. Hormonal regulation of prolactin receptors in male rat target tissues. The effect of hypothyroidism and adrenelectomy. *Endocrinol. 115,* 1283-1288.
12. *Leung, F. C., Taylor, I. E. and Van Inderstine, A.* 1985. Effects of dietary thyroid hormones in normal and hypothyroid chickens. General. and Compar. *Endocrinol., 59,* 91-99.
13. *Meserve, L. A. and Leathem, J. M.* 1973. Hypothyroidism and the maturation of the hypothalamo-typophyseal-adrenal axis Develop. *Psyhobiol., 6,* 123-129.
14. *Meserve L. A. and Rhodes, A. B.* 19790. Influence of pre- and postnatal thiouracil administration on pituitary growth hormone content in mice. *Ohio. J. Sci., 76,* 267-271.
15. *Morimoto, N., Kawakami, F., Makino, Ch., Chilara, K., Hasegawa, M., Ibota, Y.* 1988. Age-related changes in growth hormone releasing factor and somatostatin in the rat hypothalamus. *Neuroendocrinol. 47,* 459-464.
16. *Patrica, A., Froelich, L. and Meserve, A.* 1982. Altered growth patterns and depressed pituitary growth hormone content in young rats; effects of pre-and postnatal thiouracil administration. *Growth, 46,* 296-305.
17. *Ruiz, M., Diego, A. M., Reyes, A., Alonso, A., Morell, M.*1989. Influence of thyroidectomy on serum and pituitary FSH in control and orchiedectomized rats. *Res. Exp. Med. (Berl.). 189,* 85-90.
18. *Sowers, J. R., Resch, G., Walker, S., Sollars,E.* 1981. Developmental patterns of the hypothalamic-pituitary axis in the euthyroid and hypothyroid male rat. *Neuroendocrinol. 30,* 257-261.
19. *Sountag, E. E., Steger, R. W., Formann, L. J., Meites, J.* 1980. Decreased pulsatile release of growth hromone in old rats. *Endocrinol., 107,* 1875-1879.
20. *Stošić, N., Pantić, V., Pavlović-Hournac, M. Radovanović, D.* 1969. Systeme hypothalamo-hypophysaire des rats thyroidectomises porteurs de greffes intraoculaires de thyroide. *Z. Zellforsch. 102,* 554-569.
21. *Stošić, N., Pantić, V.* 1973. The hypothalamo-pituitary system of intact and thyroidectomised rats treated with thyroxine. Iugoslav. Physiol. *Pharmacol. Acta. 9,* 335-348.
22. *Stošić, N.* 1980. The effect of thyroxine on some hypothalamic nuclei, pituitary cells producing glycoprotein hormones and spermatogenesis of immature rats. Iugoslav. Physiol. *Pharmacol. Acta. 16,* 209-220.

23. *Stošić, N., Kilibarda, M., Lovren, M.* 1981. The pituitary cells of throidectomized and thyroxine treated intact and thyroidectomized mature rats. *Iugoslav. Physiol. Pharmacol. Acta. 17,* 31-39.
24. *Stošić-Bogdanović, N., Gledić, D., Radovanović, A.* 1990. The pituitary cells of infantile rats after thyroxine treatment. *Acta Veterin. 40,* 173-182.
25. *Tsukada, Y., Nomura, M., Nugai, K., Kohasaka, S., Kawahata, H., Ito, M., Matsutani, T.* 1977. Neurochemical correlates of learning ability. In: *Behavioral neuroshemistry. Delgado, J. N. R., De Feudis, F. V. (eds), 63-84 (Spectrum New York, 1977).*

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NADA STOŠIĆ-BOGDANOVIĆ i ANITA RADOVANOVIĆ

SADRŽAJ

Eksperimenti su izvođeni na juvenilnim mladuncima poreklom od majki kod kojih je indukovan hipotireodizam unošenjem Propiltiuracila (PTU). Hipotireodizam je izazvan tokom embrionalnog, fetalnog i postnatalnog perioda dodavanjem PTU u pijaćoj vodi gravidnim majkama i u toku laktacije. Germinativni epitel semenika je analiziran primenom svetlosnog i elektronskog mikroskopa.

Rezultati ukazuju da je prenatalni postnatalni period razvoj organizma osetljiv na promenu količine hormona štitaste žlezde. Značajno je smanjena masa tela, semenika i hipofize. Dugi hipotireoidizam majki deluje na sazrevanje semenika juvenilnih mladunčadi odlaganjem razvoja prve generacije procesa spermatogeneze. U germinativnom epitelu su degenerativno promenjeni spermatociti, značajno smanjenje broja spermatida ($p<0.01$) i postupna regresija Sertolijevih ćelija. U citoplazmi Sertolijevih ćelija su brojna gusta i nekrotična tela, akumulacija lipidnih kapi i izmenjenih mitohondrija.

Posledica promena u Sertolijevim ćelijama je poremećaj kontrole diferenciranja germinativnih ćelija i sazrevanja semenika mladunčadi.