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Comparative study of the effects of pre and post natal administration of a thyroid drug on testicular activity in adult rat

Z. Hamouli-Said, F. Tahari, F. Hamoudi, F. Hadj-Bekkouche

L.B.P.O/Section Endocrinology, Faculty of Biological Sciences, BP 32, U.S.T.H.B., 16123 Bab-Ezzouar, Algiers, Algeria

Abstract: Thyroid hormone is known to play a critical role in growth and development of rat testes with a specific effect on the differentiation of Sertoli cells leading to a normal evolution of germ cells. In the present study, we aimed to compare the effect of induced hypothyroidism during fetal and post-natal life on the structure and function of the testis in adult. Pregnant or lactating mothers were treated with 6-propyl-2-thiouracil (PTU) during 21 days and weight gain of pups was steady until adult age. Plasma hormonal levels were determined by RIA and morphology of testis was studied on sections stained with Masson's trichrome. Pre and early post natal hypothyroidism resulted in an impairment of body development and a diminution of thyroid hormone levels of treated rats. No significant effect on testicular development has been observed when hypothyroidism is induced in fetal life while it was associated with reduction in testis weight, diameter of seminiferous tubules and hormonal levels and delay in maturation of germ cells, when induced during early post natal life.

Key words: Hypothyroidism - Thyroid hormones - Growth - Testis - Steroidogenesis

Introduction

The thyroid gland develops from the floor of the primitive pharynx and begins to synthesize and secretes T4 by 17 days of gestation [27,28]. The most numerous cell population in the gland is the thyroid follicular cells which are responsible of secretion of ioded tyrosine derived hormones which exert important effects on development, growth, and metabolism [29,41]. Some of their most prominent effects occur during foetal development and early childhood while several organs undergo significant morphological and physiological changes. First, it has been thought that the male gonad was unresponsive to the thyroid hormones [2] so the delayed gonadal maturation observed in hypothyroid experimental animals and man has been attributed to a reduction of gonadotrophins secretion [38,40]. Since then in several studies, a hypothyroid state was induced by thyroidectomy [1,4,9,10] or by administration of anti-thyroid drugs [1,11-15,17-19,22,24,30-31,33-34,38-39] and the effects on testicular structure or/and function have been evaluated. Likewise, in most of these studies the hypothyroidism was induced after birth and little has been known about effects of pre-natal transient hypothyroidism on the testis development [17]. In this study, we aimed to compare morphological variations and hormonal status in adult rats after transient hypothyroidism induced by PTU during gestation and lactation periods.

Correspondence: Z. Hamouli-Said, L.B.P.O/Section Endocrinology, Faculty of Biological Sciences, BP 32, U.S.T.H.B., 16123 Bab-Ezzouar, Algiers, Algeria;
e-mail: hamouli_z@hotmail.com

Materials and methods

Animals and induction of hypothyroidism. Wistar female rats were made pregnant and kept in a animal room with a controlled temperature (20-25°C) and under natural photoperiod with pelleted commercial chow *ad libitum*. Experiments were conducted according to recommendations edicted in the Guide to the Care and Use of Experimental Animals. Prenatal treatment was started 1 day *post coitum* and stopped at birth, by giving pregnant rats a drinking water containing 0.025% (w:v) 6-propyl-2-thiouracil (PTU; Sigma Chemical Co., St. Louis, MO) and 0.075% saccharine-aspartam (Cologan, Lidl UK GmBH) to mask the bitter taste of PTU (PTU), control pregnant rats received water laced with 0.075% saccharine-aspartam (C). After birth, to render newborns hypothyroid, lactating rats were provided with drinking water containing 0.025% PTU and 0.075% sweetener (PTU). Control mothers received sweetened water (C). Treatment has been maintained to day 21. Treated and control litters were followed up until adulthood and decapitated

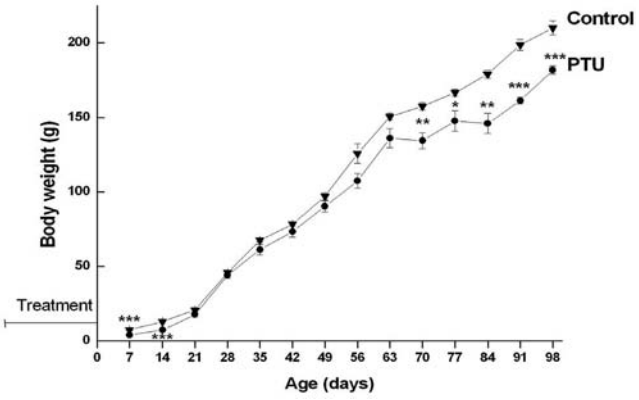

Fig. 1. Body weight in control and prenatal PTU treated rats. Bars represent mean ± SEM;treated vs. control:*p<0.05, **p<0.01, ***p<0.001; n=4.

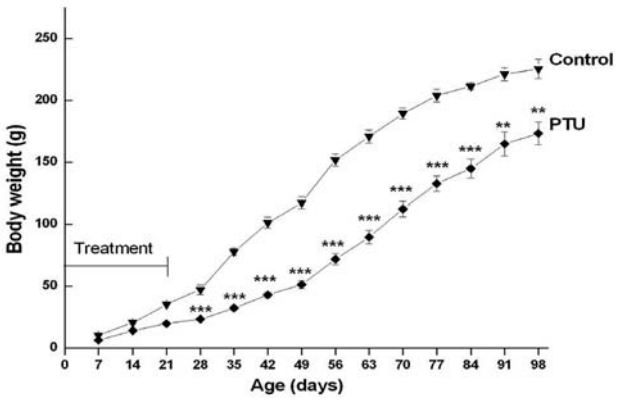

Fig. 2. Body weight in control and postnatal PTU treated rats. Bars represent mean ± SEM;treated vs. control:**p<0.01, ***p<0.001; n=14.

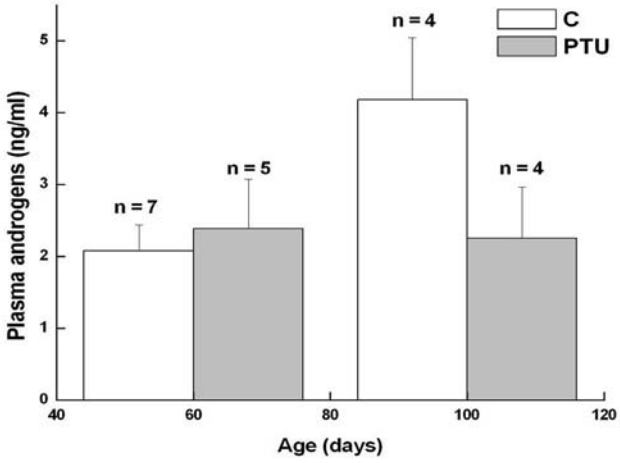

Fig. 3. Plasma androgens levels in control and prenatal PTU treated rats. Bars represent mean ± SEM;treated vs. control: p>0.05.

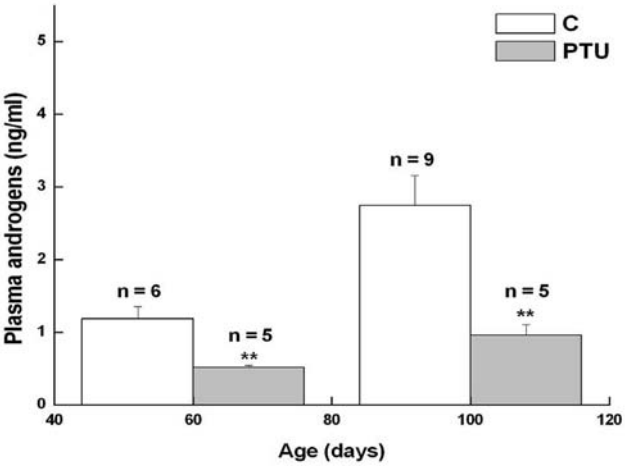

Fig. 4. Plasma androgens levels in control and postnatal PTU treated rats. Bars represent mean ± SEM;treated vs. control: **p<0.01.

60 and 100 after birth. Blood was collected on 2% EDTA, plasma were separated by centrifugation and stored at -20°C for hormones measurements.

Hormone measurements. Plasma levels of total androgens were evaluated by Radio Immunoassay method after Cyclohexane/Ethylacetate (V/V) extraction, all samples were assayed in duplicate in a single RIA run using [1,2,6,7] ^{3}H testosterone (Amersham, GB) and an anti-testosterone rabbit serum antibody produced in laboratory. Plasma free triiodothyronine (FT_3) and free thyroxin (FT_4) were measured using a single antibody direct radioimmunoassay kits (Immunotech, France, FT_3: ref. IM1579; FT_4: ref. IM1363).

Histology. Testes of PTU treated rats and controls were fixed in an aqueous fixative, embedded in paraffin and 5 m sections were stained with Masson's trichrome dyes solutions and studied in a Zeiss photomicroscope equipped with ocular grid.

Statistical analysis. Body and testis weights and hormone measurements are presented as mean ± SEM. Two-way analyses of variance were used for statistical evaluation of data using Student's t test. The level of significance was taken as $p < 0.05$.

Results

Body weight (BW)

Body weight of prenatal PTU treated animals was significantly lower the two first weeks after birth (respectively 3.99 ± 0.36 g vs. 7.5 ± 0.46 g and 7.5 ± 0.29 g vs. 13.07 ± 0.43 g; $p< 0.001$). It evolved similarly until 60 dpp and became significantly lower over the rest of experiment (Fig. 1). Body weight of postnatal PTU treated rats evolved parallel to control's one until adult age but remained significantly lower from the fourth week onwards. The mean weights of 9 and 14-week-old rats being 89.68 + 5.43 g vs. 170.89 ± 5.22 g ($p< 0.001$) and 173.26 ± 9.27 g vs. 225.44 ± 7.76 g ($p< 0.01$; Fig. 2).

Testis weight

Testis weight in prenatal PTU treated rats was weakly decreased, respectively 6% and 4%, at 60 and 100 days

Table 1. Testis weight and diameter of seminiferous tubules in control and PTU treated rats. Treated vs. control rats:*p<0.05.

Ages	Testis weight (g)		Diameter of seminiferous tubules (□m)	
	C	PTU	C	PTU
Prenatal treatment				
60 dpp	1.242 ± 0.017 (n = 4)	1.170 ± 0.018* (n = 4)	242.01 ± 0.46	256.41 ± 1.61
100 dpp	1.295 ± 0.024 (n = 8)	1.242 ± 0.013 (n = 8)	234.50 ± 8.12	238.32 ± 6.46
Postnatal treatment				
60 dpp	1.189 ± 0.031 (n = 10)	0.460 ± 0.049* (n = 18)	256.62 ± 0.49	168.23 ± 2.95*
100 dpp	1.577 ± 0.023 (n = 14)	1.665 ± 0.034* (n = 14)	241.35 ± 12.43	251.00 ± 8.54

Table 2. Plasma FT4 and FT3 in control and PTU treated rats. Treated vs. control rats: *p<0.05, **p<0.01, ***p<0.001.

Ages	FT_4 (pM)		FT_3 (pM)	
	C	PTU	C	PTU
Prenatal treatment				
60 dpp	24.33 ± 2.26 (n = 4)	23.80 ± 1.94 (n = 4)	2.35 ± 0.10 (n = 4)	1.69 ± 0.16* (n = 4)
100 dpp	20.00 ± 3.14 (n = 4)	16.88 ± 3.63 (n = 4)	4.62 ± 0.28 (n = 4)	4.74 ± 1.46 (n = 4)
Postnatal treatment				
60 dpp	21.52 ± 1.89 (n = 7)	12.30 ± 1.15*** (n = 9)	5.66 ± 0,67 (n = 7)	2.93 ± 0,31** (n = 9)
100 dpp	19.24 ± 1.41 (n = 11)	18.51 ± 1.07 (n = 12)	5.84 ± 0.74 (n = 11)	4.49 ± 0.23 (n = 12)

of age ($p < 0.05$ and $p > 0.05$). An important decrease (61%) was noted in postnatal PTU treated rats when compared with control rats at 60 days of age ($p < 0.05$). At 100 days of age, testis weight in this PTU treated group was 6% greater (1.665 ± 0.034 g vs. 1.577 ± 0.023 g; $p < 0.05$) despite the 23% decrease in body weight (Table 1).

Hormonal levels

Mean plasma FT_4 of prenatal treated rats was respectively 23.80 ± 1.94 pM and 16.88 ± 3.63 pM respectively at 60 and 100 days of age. No significant difference was observed when means were compared to those of controls. In postnatal treated rats, FT_4 plasma level was significantly decreased 60 days after birth when compared to control rats (12.30 ± 1.15 pM vs. 21.52 ± 1.89 pM; $p < 0.001$; Table 2).

Prenatal and postnatal PTU treated rats together showed reduced FT3 60 days after birth (1.69 ± 0.16 pM vs. 2.35 ± 0.10 pM; $p < 0.05$ and 2.93 ± 0.31 pM vs. 5.66 ± 0.67 pM; $p < 0.01$) and no significant variations 100 days after birth (Table 2).

Plasma androgens concentrations in prenatal PTU treated group were not different compared with control group (Fig. 3). These concentrations were significantly lowers when treatment was provided after birth: 0.52 ± 0.03 ng/ml vs. 1.19 ± 0.17 ng/ml in 60-day-old rats and 0.96 ± 0.15 vs. 2.74 ± 0.41 ng/ml in 100-day-old ones ($p < 0.01$; Fig. 4).

Testicular morphology

No significant differences were obtained in tubule diameter and morphology of testis in prenatal PTU treated rats when compared with their respective controls contrary to postnatal PTU treated 60-day-old rats in which mean diameter tubule was significantly decreased and spermatogenesis delayed. In fact, while tubules in control rats contained spermatozoa, PTU treated rats showed tubules with few elongated spermatids (Table 1, plates 1 and 2).

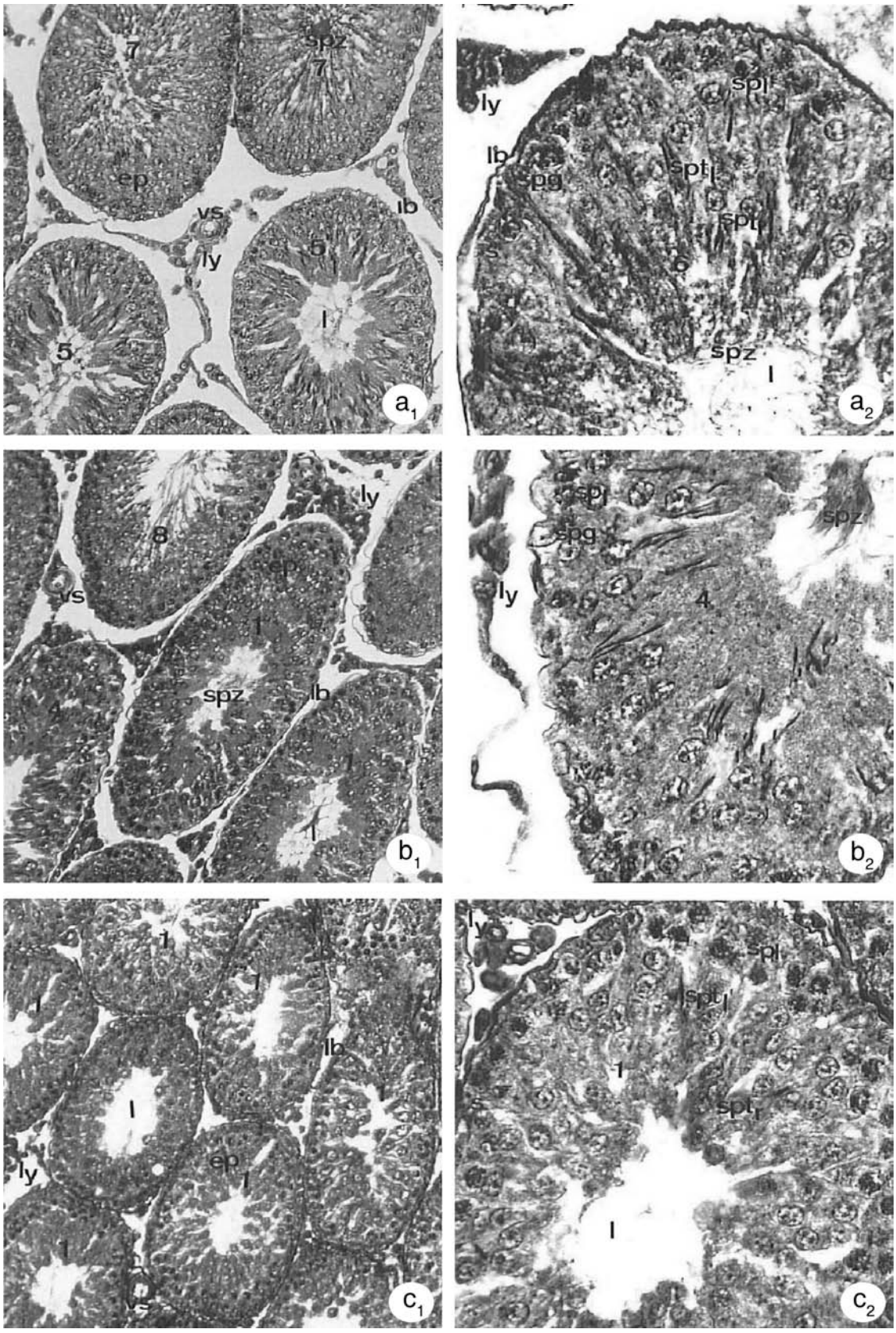

Fig. 5. Testis sections in control and PTU 60-day-old-rats. a_1-a_2: Control, b_1-b_2: PTU prenatal treated rats, c_1-c_2: PTU postnatal treated rats. ep:seminiferous epithelium, L: lumen, lb:basal lame, Ly: Leydig cell, Sn:Sertoli cell nucleus, SpI: primary spermatocyte, Spg: spermatogonia, Sptl: elongate spermatide, Sptr:round spermatid, Spz: spermatozoa, Vs: blood vessel, 1-4-5-8-7:spermatogenesis stages. a_1-b_1-c_1: Gx200, a_2-b_2-c_2: Gx630.

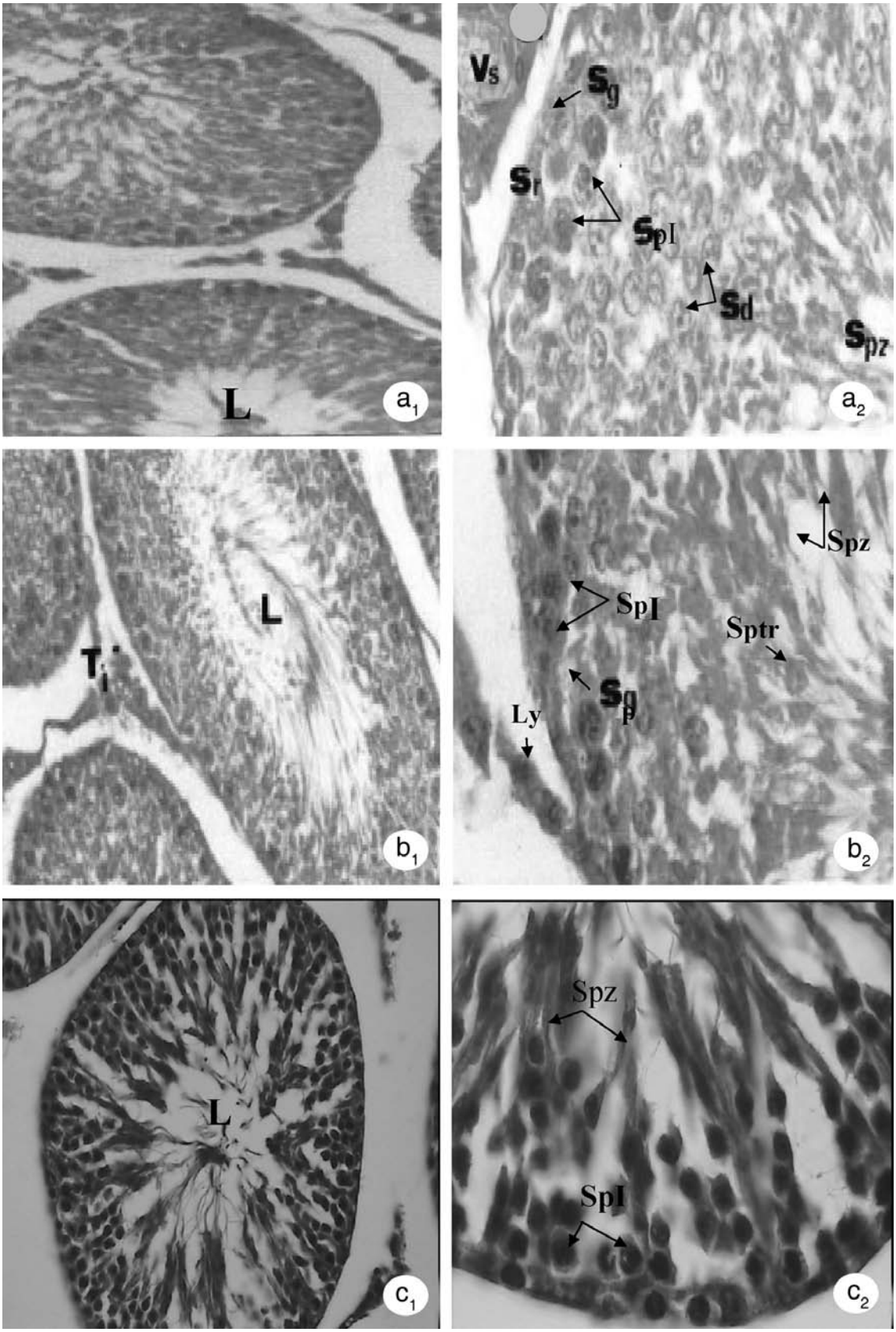

Fig. 2. Testis sections in control and PTU 100-day-old-rats. a_1-av: Control, b_1-b_2: PTU prenatal treated rats, c_1-c_2: PTU postnatal treated rats. L: lumen, Ly:Leydig cell, Sd: Spermatid, SpI: primary spermatocyte, Spg: spermatogonia, Sr: Sertoli cell, Sptr: round spermatid, Spz: spermatozoa, Ti: interstitial tissue, Vs: blood vessel. a_1-b_1-c_1: Gx240, a_2-b_2-c_2: Gx480.

Discussion

The PTU treatment induced a decrease in thyroid hormones levels by inhibiting oxidation of iodide to oxidized iodine and its binding to tyrosine moieties in thyroglobulin: a central step in thyroid hormone synthesis catalysed by the thyroperoxydase and decreasing D1 deiodinase's activity and peripheral transformation of T4 [35]. This drug is known to be able to cross the placenta [25] and to be present in milk of lactating mothers [3,16] inducing an hypothyroidy in fetus and newborns. Our treated animals were hypothyroids like it was demonstrated by decreased FT4 and/or FT3 plasma levels at 60 dpp and like it is reported [17, 34]. The results of this study demonstrate a significant weight loss at birth and adulthood in prenatal treated rats and over all the experiment for postnatal treated ones. No significant difference was observed in the body weight of fetuses taken from 0.025% MMI treated mothers by Francavilla et al [17] while similar variations have been observed after postnatal thyroidectomy [1,4], 0.1% [1, 30], 0.05% [17] or 0.025% MMI treatment [31] and 0.1% PTU administration [12-14,19]. This effect on body growing was foreseen since numerous data demonstrate that thyroid hormone is strongly involved in the regulation of body growth during fetal and postnatal periods through their complex metabolic effects, stimulation of growth factors production and secretion and action of GH and IGF-1 [7,37].

Adult rats made hypothyroid during fetal period did not show any morphological differences when compared with control rats while postnatal transient hypothyroidism resulted in delayed maturation of testis. These results complete those of Francavilla for fetuses [17] and agree with those obtained during prepubertal and pubertal periods [1,17,22,30-31,33-34,39] and adulthood [12-14,18,19] after postnatal induced hypothyroidism.

Early induced hypothyroidism slows the transition from mitogenic to differentiated state of Sertoli cells since it is associated with a marked delay in Sertoli cell development [15] resulting in a dramatic impairment of protein metabolism and level of specific biochemical markers [30] and consequently a significant decrease in the number of germ cells per tubule during pubertal period [17,34] supported by the presence of active forms of thyroid hormone receptors α in testes during postnatal period [6,8,20,21]. The reduced androgens levels in postnatal PTU treated rats, in agreement with results obtained in thyroidectomized rats [1,4] and animals made hypothyroid during different periods [23,24,38] can be explained by lower serum levels of LH [17,38], reduced number of LH receptors per Leydig cell [18,38] and a delay in the onset of mesenchymal precursor cells differentiation into Leydig progenitor cells and newly formed adult Leydig cells [26,36]. The enhanced gonadal function in adulthood after transient hypothyroidism seems to be dependent on activity of enhanced Sertoli cells number in spite of the androgens essential role for the maintenance of spermatogenesis [5, 32]. In conclusion, this study demonstrates that neonatal hypothyroidism results in delayed maturation of the testis and reduced plasma androgens levels while prenatal thyroid hormone deficiency remains without effect on testis development.

References

[1] Ando S, Panno ML, Beraldi E, Tarantino G, Salerno M, Palmero S, Prati M, Fugassa E. Influence of hypothyroidism on in vitro testicular steroidogenesis in adult rats. *Exp Clin Endocrinol.* 1990;96:149-156.

[2] Barker SB, Klitgard HM. Metabolism of tissu excised from thyroxine injected rats. *Am J Physiol*. 1952;170:81-86.

[3] Ben Hamida F, Soussia L, Guermazi F, Rebai T, Zeghal N. Effets de deux antithyroïdiens de synthèse (propylthiouracile et perchlorate) sur la fonction thyroïdienne de la souris en période d'allaitement. *Ann Endocrinol.* 2001;62:446-453.

[4] Biswas NM, Ghosh PK, Biswas R, Ghosh D. Effect of thyroidectomy and thyroxine and α_{2u}-globulin replacement therapy on testicular steroidogenic and gametogenic activities in rats. *J Endocrinol.* 1994;140:343-347.

[5] Bremner WJ, Millar MR, Sharpe RM, Saunders PT. Immunohistochemical localisation of androgen receptors in the rat testis: evidence for stage-dependent expression and regulation by androgens. *Endocrinology.* 1994;135:227-1234.

[6] Buzzard JJ, Morrison JR, O'Bryan MK, Song Q, Wreford NG. Developmental expression of thyroid hormone receptors in the rat testis. *Biol Reprod.* 2000;62:664-669.

[7] Cabello G, Wrutniak C. Thyroid hormone and growth: relationships with growth hormone effects and regulation. *Reprod Nutr Develop.* 1989;29:387-402.

[8] Canale D, Agostini M, Giorgilli G, Caglieresi C, Scartabelli G, Nardini V, Jannini EA, Martino E, Pinchera A, Macchia, E. Thyroid hormone receptors in neonatal, prepubertal and adult rat testis. *J Androl.* 2001;22:284-288.

[9] Chowdhury AR, Arora U. Role of thyroid in testicular development of immature rat. Arch Androl. 1984;12:49-51.

[10] Chowdhury AR, Gautam AK, Chatterjee BB. Thyroid-testis interrelationship during the development and sexual maturity of rat. *Arch Androl.* 1984;13:233-239.

[11] Cooke PS, Hess RA, Porcelli J, Meisami E. Increased sperm production in adult rats after transient neonatal hypothyroidism. *Endocrinology*. 1991;129:244-248.

[12] Cooke PS, Kirby JD, Porcelli J. Increased testis growth and sperm production in adult rats following transient neonatal goitrogen treatment:optimisation of the propylthiouracile dose and effect of methimazole. *J Reprod Fertil,* 1993;97: 493-499.

[13] Cooke PS, Meisami E. Early hypothyroidism in rat causes increased adult testis and reproductive organ size but does change testosterone levels. *Endocrinology.* 1991;129:237-243.

[14] Cooke PS, Porcelli J, Hess RA. Induction of increased testis growth and sperm production in adult rats by neonatal administration of goitrogen propylthiouracile (PTU):The critical period. *Biol Reprod*. 1992;46:146-154.

[15] Cooke PS, Zhao YD, Bunnick D. Triiodothyronine inhibits proliferation and stimulates differenciation of cultured neonatal Sertoli cells:possible mechanism of increased adult testis weight and sperm production induced by neonatal goitrogen treatment. *Biol Reprod*. 1994;51:1000-1005.

[16] Cooper DS. Substances that affect thyroid function or thyroid hormone metabolism. In: The thyroid gland. Greer MA [Eds]. Raven Press Ltd, New york, 1990;323-344.
[17] Francavilla S, Cordeshi G, Properzi G, Di Cicco L, Jannini EA, Palmero S, Fugassa E, Loras B, D'armiento M. Effect of thyroid hormone on the pre and postnatal development of rat testis. *J Endocrinol*. 1991;129:35-42.
[18] Hardy MP, Kirby JD, Hess RA, Cooke PS. Leydig cells increase their numbers but decline in steroidogenic function in the adult rat after neonatal hypothyroidism. *Endocrinology*. 1993;132:2417-2420.
[19] Hess RA, Cooke Ps, Bunick D, Kirby JD. Adult testicular enlargement induced by neonatal hypothyroidism is accompanied by increased Sertoli and germ cell numbers. *Endocrinology*. 1993;132:2607-2613.
[20] Jannini EA, Olivieri M, Francavilla S, Gulino A, Ziparo E, D'Armiento M. Ontogenesis of the nuclear 3, 5, 3' triiodothyronine receptor in the rat testis. *Endocrinology*. 1990;126:2521-2526.
[21] Jannini EA, Ulisse S, D'Armiento M. Thyroid hormone and male gonadal function. *Endocr Rev.* 1995;16:443-457.
[22] Loveland K, Schlatt S, Sasaki T, Chu ML, Timpl R, Dziadek M. Developmental changes in the basement membrane of the normal and hypothyroid postnatal rat testis:Segmental localisation of fibulin-2 and fibronectin. *Biol Reprod.* 1998;58: 1123-1130
[23] Maran RR, Ravichandran K, Arunakaran J, Aruldhas MM. Impact of neonatal hypothyroidism on Leydig cell number, plasma, and testicular interstitial fluid sex steroids concentration. *Endocr Res.* 2001;27:119-141.
[24] Maran RR, Sivakumar R, Arunakaran J, Ravisankar B, Ravichandran K, Sidharthan V, Jeyaraj DA, Aruldhas MM. Duration-dependant effect of transient neonatal hypothyroidism on Sertoli and germ cell number, and plasma and testicular interstitial fluid androgen binding protein concentration. *Endocr Res.* 1999;25:323-340.
[25] Marchant B, Brownlie BEW, McKay Hart D, Horton PW, Alexander WD. The placental transfer of propylthiouracil, methimazole and carbimazole. *J Clin Endocrinol Metab.* 1977;45:1187-93.
[26] Mendis-Handagama SMLC, Ariyaratne HBS. Differentiation of the adult Leydig cell population in the postnatal testis. *Biol Reprod*. 2001;65:660-671.
[27] Morreale de Escobar G, Pastor R, Obregon MJ, Escobar del Rey F. Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues, before and after onset of fetal thyroid function. *Endocrinology*. 1985;117:1890-1900.
[28] Obregon MJ, Mallol J, Pastor R, Morreale de Escobar G, Escobar del Rey F. L-thyroxine and 3, 5, 3'-triiodo-L-thyronine in rat embryos before onset of fetal thyroid function. *Endocrinology*. 1984;114:305-307.
[29] Oppenheimer J, Schwartz H, Mariash C. Advances in our understanding of thyroid hormone action at the cellular level. *Endocr Rev*. 1987;8:288-308.
[30] Palmero S, De Marchis M, Gallo G, Fugassa E. Thyroid hormone affects the development of Sertoli cell function in the rat. *J Endocrinol*. 1989;123:105-111.
[31] Panno ML, Bereldi E, Pezzi V, Salerno M, Delula G, Lanzino M, Leper M, Sis D, Prati M, Palmero S, Bolla F, Fugassa E, Ando S. Influence of thyroid hormone on androgen metabolism in prepubertal rat Sertoli cell. *J Endocrinol.* 1994;140: 349-355
[32] Sharpe RM, Maddocks S, Millar M, Kerr JB, Saunders PT, Mckinnell C. Testosterone and spermatogenesis. Identification of stage-specific, androgen-regulated proteins secreted by adult rat seminiferous tubules. *J Androl*. 1992;13:172-184.
[33] Simorangkir DR, De Kretser DM, Wreford NG. Increased numbers of Sertoli and germ cells in adult rat testes induced by synergic action of transient neonatal hypothyroidism and neonatal hemicastration. *J Reprod Fertil.* 1997;104:207-213.
[34] Simorangkir DR, Wreford NG, De Kretser DM. Impaired germ cell development in the testes of immature rats with neonatal hypothyroidism. *J Androl.* 1997;18:186-193.
[35] Taurog A. Hormone synthesis:thyroid iodine metabolism. In:the thyroid. Ingbar SH, Baverman LE [Eds], Lippincott, Philadelphia. 1986;53-97.
[36] Teerds KJ, DeRooj DG, DeJong FH, Van Haaster LH. Development of adult-type Leydig cell population in the rat is affected by neonatal thyroid hormone levels. *Biol Reprod.* 1998;59:344-350
[37] Tsai JS, Samuels HH. Thyroid hormone action:stimulation of growth hormone and inhibition of prolactin secretion in cultured GH1 cells. *Biochem Biophys Res Commun*. 1974;59: 420-428
[38] Valle LB, Oliveira-Filho RM, Romaldini JH, L'Ara PF. Pituitary testicular axis abnormalities in immature male hypothyroid rats. *J Steroid Biochem.* 1985;23:253-257.
[39] Van Haaster LH, Dejong FH, Docter R, Derooij DG. The effect of hypothyroidism on Sertoli cell proliferation and differenciation and hormone levels during testicular development in rat. *Endocrinology.* 1992;131:1574-1576.
[40] Wong CC, Dohler KD, Muhlen AVZ. Effects of tri-iodothyronine, thyroxine and iso-propyl-di-iodothyronine on thyroid stimulating hormone in serum and pituitary gland and on pituitary concentrations of prolactine, growth hormone, luteinizing hormone and follicle-stimulating hormone in hypothyroid rats. *J Endocrinol.* 1980;87:255-264.
[41] Yen PM. Physiological and molecular basis of thyroid hormone action. *Physiol Rev.* 2001;81:1097-1142.