THE JOURNAL OF EXPERIMENTAL ZOOLOGY 273:160–169 (1995)

Effect of Hypothyroidism Induced by Propylthiouracil and Thiourea on Male and Female Reproductive Systems of Neonatal Mice

W.Y. CHAN AND T.B. NG
Departments of Anatomy (W.Y.C.) and Biochemistry (T.B.N.), Faculty of Medicine, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong

ABSTRACT The effect of hypothyroidism induced by 6-propyl-2-thiouracil (PTU) or thiourea (TU) on the development of the reproductive system in male and female neonatal ICR mice was investigated. PTU or TU was injected subcutaneously into experimental animals from postnatal day 1 (PD1) onward. The histological changes of the reproductive organs, formation of ovarian follicles, and spermatogenesis were examined on PD 14, 21, and 28, and the fertility of the hypothyroid mice in adulthood was followed. It was found that PTU or TU treatment did not produce an effect on the histology of the neonatal uterus and oviduct. In contrast, the drugs induced a decrease in the number of primordial follicles, multilaminar follicles, and Graafian follicles in the ovary. The number of follicles with degenerated follicular cells was increased. In the testis both PTU and TU treatments brought about a decrease in the number of seminiferous tubules with developing spermatids although the mean diameter of seminiferous tubules and the histology of the testis, epididymis, seminal vesicle, and coagulating gland was unaffected. The mating between hypothyroid females and euthyroid males and that between hypothyroid males and euthyroid females were normal with regard to the pregnancy rate, litter size, and sex ratio of offspring. The somatic growth of the resulting offspring was normal. It is concluded that the retarding effect on ovarian and testicular development in mice during neonatal period was not serious enough to adversely affect reproduction in the hypothyroid animals.

Hypothyroidism is the most common disorder in thyroid function. It has already been well documented that the effects of hypothyroidism are widespread. The skin and appendages, cardiovascular system, digestive tract, bones and joints, muscles, nervous system, adrenal, blood, and carbohydrate, lipid, and protein metabolism are all affected (De Visscher and Inglebleek, '80).

The consequences of hypothyroidism on the male reproductive system have recently been studied by Francavilla et al. ('91) and Senthikumaran et al. ('91). Francavilla et al. ('91) noted that treatment of pregnant rats with methimazole did not influence fetal testicular development. However, hypothyroidism induced in neonatal rats by methimazole produced a delay in testicular maturation which consisted of reductions in seminiferous tubule diameter and germ cell number, increased degeneration, and arrested germ cell maturation. Increased degeneration of spermatogonia and primary spermatocytes occurred, and immature spermatids were present in the epididymis. Sertoli cells without cytoplasmic lipid droplets and seminiferous tubules lacking a lumen appeared. Neonatal hypothyroidism induced by 6-propyl-2-thiouracil retards the morphological differentiation of Sertoli cells and prolongs the proliferation of these cells (van Hasster et al., '92). The normal increases in γ-glutamyl transpeptidase activity, androgen-binding protein, and lactate production in Sertoli cells were inhibited. The finding that the nuclear triiodothyronine receptor in rat Sertoli cells was expressed maximally during late fetal and early postnatal life and progressively diminished infers a direct effect of thyroid hormones on early postnatal life (Jannini et al., '90). Rats rendered hypothyroid during the early postnatal period exhibited lower serum concentrations of gonadotropins during puberty than control rats, and the serum gonadotropin levels could be normalized by replacement therapy with triiodothyronine, suggesting that thyroid

Received December 20, 1994; revision accepted April 28, 1995.
Address reprint requests to Dr. W.Y. Chan, Department of Anatomy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

hormones play a role in the early postnatal period to bring about the onset of puberty by stimulating gonadotropin secretion (Francavilla et al., '91). The ability of testicular membranes to bind luteinizing hormone or human chorionic gonadotropin was reduced in hypothyroid rats (Minetti et al., '86). The activities of seminal vesicular glycosidase including β-D-glucosidase, β-D-galactosidase, *N*-acetyl-β-D-glucosaminidase, and *N*-acetyl-β-D galactosaminidase were suppressed in rat after thyroidectomy at 30 days post-partum. Simultaneously there were reductions in the weights of seminal vesicles, epididymis, prostate, coagulating gland, and plasma testosterone levels (Senthikumaran et al., '91). On the other hand, Cooke and his co-investigators (Cooke and Meisami, '91; Cooke et al., '91) reported that transient neonatal hypothyroidism in rats increased testicular size and sperm production in adults. Chandrasekhar et al. ('85) observed that hypothyroidism did not affect testicular development in prepubertal lambs.

Hypothyroidism has also been associated with a wide range of reproductive abnormalities in female including menstrual disorders, amenorrhea, infertility, and frequent abortions (Longcope, '91). The existence of a specific nuclear triiodothyronine receptor in immature porcine granulosa cells has been demonstrated (Wakim et al., '87). In the rat, hypothyroidism induced polycystic ovaries (Gold et al., '65; Scommegna et al., '65). Hypothyroidism also diminished the stimulatory effect of estrogen on cell division in the luminal epithelium, stroma, and myometrium of the uterus (Kirkland et al., '81). In hypothyroid humans delay in the onset of puberty or menarche (Styne, '91) and anovulation (Yen, '86) may occur.

The intent of the present study was to examine the effects of hypothyroidism induced by the antithyroid drugs 6-propyl-2-thiouracil (PTU) or thiourea (TU) on the development of the reproductive system in neonatal ICR mice of both sexes, in view of the inconsistency in the reported effects of hypothyroidism on testicular development and the fewer reports dealing with hypothyroidism in the female. It was found that postnatal hypothyroidism affected the development of ovarian follicles in females and retarded the spermatogenesis in males. However, the effects on ovarian and testicular development in mice during the neonatal period were not serious enough to adversely affect reproduction in adulthood.

MATERIALS AND METHODS

Histology

Newborn ICR mice were divided into groups, and each group consisted of ten to 15 mice. One group received daily subcutaneous injections of 6-propyl-2-thiouracil (PTU, Sigma) at 50 μg/g body wt per day. Another group received two subcutaneous injections of thiourea (TU, Sigma) per week at 200 μg/g body wt per injection. A vehicle-injected group served as the control. The injections commenced on postnatal day 1 (PD1) and continued until the animal was sacrificed. From each group some of the animals were sacrificed on PD14, PD21, and PD28. The male reproductive organs including testes, epididymis, and seminal vesicles and female reproductive organs including ovaries, oviducts, and uteri were dissected out and fixed directly either in Bouin's fixative overnight for histological examinations or in 2.5% glutaraldehyde in cacodylate buffer for 2 hr for morphometric measurements. Blood samples were collected for determination of the level of triiodothyronine (T_3). Hypothyroidism was established in the drug-treated animals by a statistically significantly lower plasma T_3 level which was only about 50–60% of the control values. A previous study using a similar protocol for induction of hypothyroidism in mice also demonstrated obvious distention of colloid-filled follicles in the thyroid after treatment with antithyroid drugs (Ng et al., '92).

Follicular development

For assessing follicular development, the ovaries were sectioned at 5 μm thick and stained with hematoxylin and eosin. The stages of follicular development and the incidence of each type of follicles were scored. For each ovary, at least five sections were selected, and the total numbers of primordial, multilaminar, Graafian, and preovulatory follicles [classification according to Pedersen and Peters ('68)] were counted. Special attention was taken in looking for any abnormalities in follicular development.

Spermatogenesis

The diameters of the seminiferous tubules were determined by planimetric computation of camera lucida drawings of sections on a digitizer (Grafbar GP-7, Science Accessories Corporation, Gainesville, FL). The shape of the tubules varied from round to elliptical depending on the plane of section. Since the true diameter of the tubule is always the short

axis of a random section through it, the shortest diameter of the tubule section was measured. Using a light microscope (Nikon Labophot) equipped with a 10× eyepiece and 20× (for measuring the diameter of tubules on PD14) or 40× (for measuring the diameter of tubules on PD21 and PD28) objective, at least 50 tubules randomly selected from each of the sections were measured for their diameters, and at least five sections from each testis were taken for measurement. The mean was then calculated. Measurement of more tubules or more sections had no effect on the mean.

For computing the percentage of Sertoli cells, germ cells, and seminiferous tubules containing elongated spermatids, testes were post-fixed in 1% osmium tetroxide and embedded in Spurr plastic. Sections of 1 μm were stained with toluidine blue. The number of cells was estimated from the number of nuclear profiles. No attempt was made to specify germ cells at various developmental stages. The total number of cells, the number of Sertoli cells, and the number of germ cells within seminiferous tubules were obtained from 20 cross sections of seminiferous tubules. For counting the total number of tubules and the number of the tubules containing elongated spermatids at steps 8–16 (Russell et al., '90), five sections were randomly selected from each of the testes, and five areas each in the upper right, lower right, middle, upper left, and lower left regions of each section were chosen at a magnification of 200× (for samples on PD14) or 100× (for samples on PD21 and PD28).

Fertility

To determine whether the hypothyroid mice were fertile and able to produce normal offspring, mice at 8–9 weeks which had been treated with either PTU or TU were housed individually with normal adult mice of the opposite sex (proven breeders) for a maximum period of 4 days. The males and females were separated once vaginal plugs were found. The resultant pregnancies were noted. When the mice gave birth, the number of pups was recorded, and the body weights and tail lengths were measured weekly for 5 consecutive weeks.

RESULTS

Female reproductive system

The uteri of the control mice underwent histological changes during the postnatal period. Two weeks after birth (PD14), the uterine epithelium consisted of a layer of columnar epithelium cells with glandular downgrowths into the stroma in certain areas. The stroma and the inner circular and outer longitudinal muscular layers were not well differentiated. By 3 weeks (PD21), uterine epithelium became increasingly more columnar. The stroma had increased in thickness, and more glandular tissue was found underneath the epithelium in the stroma. The circular and longitudinal muscles became more differentiated. By 4 weeks (PD28), the uteri had become more mature, with a very thick columnar epithelium, thick stromal tissue, and an abundance of glandular tissue. The circular and longitudinal muscles were well differentiated. The treatment with propylthiouracil (PTU) or thiourea (TU), however, did not alter the histology of the mouse on PD14, PD21, or PD28. The uteri of the treated mice were similar to those of control mice with respect to their morphological appearance, thickness of individual layers, and the rate of maturation.

The oviductal epithelium of the control mice changed from cuboidal to low columnar during the postnatal period from PD14 to PD28. The epithelium exhibited numerous mucosal folds and contained ciliated and non-ciliated cells. The oviducts of the PTU- and TU-treated mice were histologically similar to those of control mice when examined on PD14, PD21, and PD28. The epithelial structure, muscocal folds, and the diameter of the oviducts of the treated mice all appeared normal.

Histologically, the ovaries of the control mice and the mice treated with PTU or TU were similar (Fig. 1), but differences were found in the numbers of the ovarian follicles at different maturation stages between the control and treated mice (Table 1; Fig. 1). When examined on PD14, both the ovaries of control and experimental animals contained numerous primordial and multilaminar follicles. Some Graafian follicles were present (Fig. 1; Table 1). There was a reduction in the number of Graafian follicles in the PTU- and TU-treated group and multilaminar follicles in the PTU-treated group (Table 1). On PD21, the ovaries of both control and experimental animals contained primordial, multilaminar, Graafian, and preovulatory follicles (Fig. 1). The numbers of multilaminar follicles and Graafian follicles were again fewer in both PTU- and TU-treated groups (Table 1). The number of primordial follicles was diminished in the TU-treated animals. By PD28, the ovaries of control animals contained more Graafian follicles and there was a reduction in the number of primary follicles compared with the ovaries of younger control animals (Table 1; Fig.

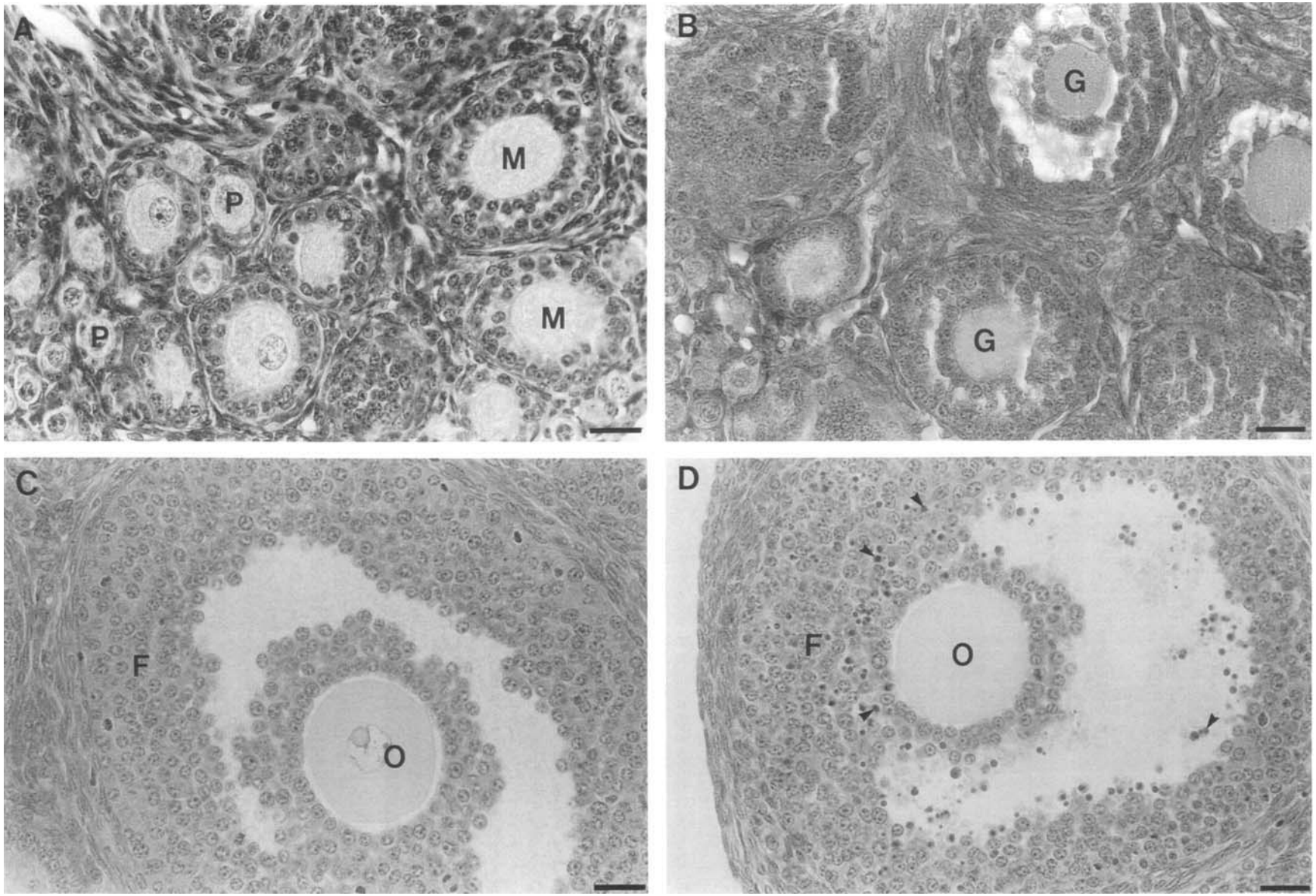

Fig. 1. Cross sections of **(A)** a control ovary at PD14, **(B)** a control ovary at PD21, **(C)** a control ovary at PD28 and **(D)** an ovary at PD28 which has been treated with TU since birth. At PD14 (A), the ovary consists of mainly primordial follicles (P) and multilaminar primary follicles (M) although Graafian follicles are occasionally seen (not shown). At PD21 (B), more multilaminar primary follicles and Graafian follicles (G) are found. The overall histological structures of the control and treated ovaries (treated either with PTU or TU) at PD14 and PD21 are similar. At PD28 (C), the Graafian follicles enlarge and a layer of follicular cells (F) surrounds the developing oocytes (O) in the control ovary, while in the ovary treated with TU (**D**), dead cells (arrowheads) are frequently found among follicular cells. H and E staining. Bar = 25 μm.

TABLE 1. Developmental stages of ovarian follicles at different postnatal periods

Postnal period	Treatment group[1]	Number of ovaries examined	Number of ovarian follicles at stages per section (means ± SD)[2]				Number of follicles with degenerated follicular cells/section (mean ± SD)
			A	B	C	D	
PD 14	Control	10	45.6 ± 4.5	6.0 ± 0.5	1.2 ± 0.3	0	0
	PTU	8	59.0 ± 3.0	2.2 ± 0.7*	0.2 ± 0.1*	0	0
	TU	6	51.8 ± 5.3	4.3 ± 0.4	0.6 ± 0.1*	0	0
PD 21	Control	8	23.0 ± 1.0	3.6 ± 0.3	2.2 ± 0.2	1.3 ± 0.1	0
	PTU	6	28.2 ± 2.0	1.8 ± 0.1*	0.4 ± 0.1*	1.8 ± 0.1	2.6 ± 0.4*
	TU	7	17.2 ± 0.9*	2.4 ± 0.4*	0.2 ± 0.1*	1.0 ± 0.1	1.6 ± 0.1
PD28	Control	8	15.0 ± 1.4	3.6 ± 0.4	2.4 ± 0.2	2.2 ± 0.3	0
	PTU	7	6.5 ± 0.5*	2.0 ± 0.1*	1.0 ± 0.1*	1.8 ± 0.1	2.1 ± 0.1*
	TU	7	8.5 ± 0.5*	3.8 ± 0.2	2.0 ± 0.1	2.3 ± 0.1	2.0 ± 0.2*

[1]PTU, neonatal mice injected with 6-propyl-2-thiouracil; TU, neonatal mice injected with thiourea.
[2]Stages of developing follicles (Pedersen and Peters, '68): A = primordial or growing primary follicles; B = multilaminar primary follicles; C = Graafian or antral follicles; D = pre-ovulatory follicles.
*$P < .05$, significantly different from control values by Student's t-test or χ^2-square test.

1). There was a reduction in the number of primordial follicles in the TU-treated group (Table 1). The decrease in the numbers of primordial, multilaminar, and Graafian follicles was evident in the PTU-treated animals. In the ovaries of PTU-treated and TU-treated mice examined 4 weeks after birth (PD28), dead follicular cells were found surrounding the oocytes although the oocytes themselves appeared to be normal (Fig. 1). There was an increased incidence of Graafian follicles with degenerated follicular cells, which was not obvious in the case of control ovaries (Table 1).

Male reproductive system

The testes of the control mice on PD14 contained many seminiferous tubules where Sertoli cells, spermatogonia, and spermatocytes were easily identified (Fig. 2). In the interstitial supporting tissues between the seminiferous tubules, Leydig cells were found singly or in clusters and were in close contact with blood vessels. Both PTU and TU showed little if any effect on the histology of the testes on PD14. Neither the PTU nor TU treatment affected the diameter of the seminiferous tubules or the percentage of Sertoli and germ cells within the tubules (Table 2). On PD21, the diameter of the seminiferous tubules in control testes was slightly increased, and a higher percentage of germ cells was found in the tubules (Table 2). The overall histological appearance of the testes was similar to that found on PD14 except that more interstitial tissues were found between the tubules. When the mice were treated with PTU or TU, the testes did not show any significant changes. The diameter of the tubules, the percentage of Sertoli cells and germ cells, and the histology of the seminiferous tubules and interstitial tissues were comparable to those in the control (Fig. 2; Table 2). By PD28, spermatids and spermatozoa began to appear in some of the seminiferous tubules of the control testes (Fig. 2). The diameter of the tubules was increased, the percentage of the germ cells increased, and the interstitial tissues became highly vascularized. Under the influence of PTU or TU, there was a reduction in the percentage of seminiferous tubules containing elongated spermatids at steps 8–16 (Fig. 2; Table 2), suggesting that the spermatogenesis was retarded. The diameter of seminiferous tubules and the percentage of germ cells in the tubules were, however, close to control values (Table 2).

The epididymis of the control mice on PD14 consisted of a low columnar epithelium with microvilli on its luminal surface. The epithelium was lined externally by layers of muscular tissues. The epithelium gradually changed to a tall columnar on PD21 and PD28. The secretory epithelium of the seminal vesicle of the control mice was cuboidal on PD14 and changed to tall columnar with basally located nuclei on PD21 and PD28. The coagulating gland of the control mice showed a columnar epithelium on PD14, and luminal secretion was found on PD21 and PD28 when the epithelium changed to a tall columnar appearance. Neither PTU nor TU affected the histology of the epididymis, seminal vesicles, or coagulating gland on PD14, PD21, or PD28.

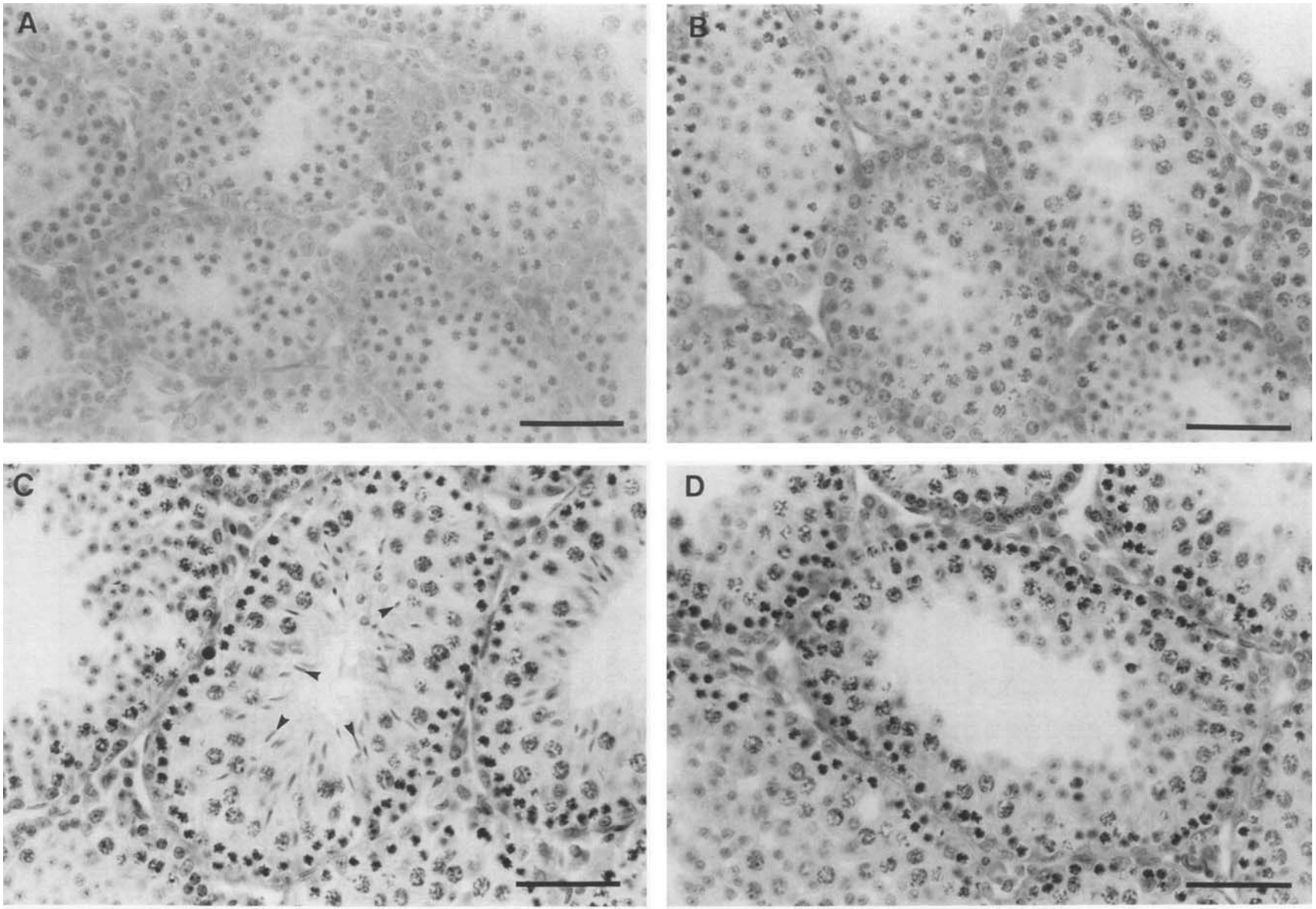

Fig. 2. Cross sections of **(A)** a control testis at PD14, **(B)** a control testis at PD21, **(C)** a control testis at PD28, and **(D)** a testis at PD28 which has been treated with TU since birth. At PD14 (A) and PD21 (B), seminiferous tubules in either the control and treated testes (treated either with PTU or TU) contain mainly spermatogonia, spermatocytes, and Sertoli cells although developing round spermatids are occasionally seen in some seminiferous tubules. At PD28, round and elongated spermids (arrowheads) are observed in seminiferous tubules of the control testis (C). In the testis of a treated animal (D), a decrease in the number of seminiferous tubules with elongated spermatids is observed, and some of the tubules show a retardation in spermatogenesis in which developing germ cells were arrested at the stage of secondary spermatocytes or round spermatids. H and E staining. Bar = 50 μm.

TABLE 2. Effect of drug-induced hypothyroidism on spermatogenesis

Postnatal period	Treatment group[1]	Number of testes examined	Percentage of Seroti cells*	Percentage of germ cells*	Percentage of seminiferous tubules containing elongated spermatids[2]	Average diameter of seminiferous tubules (μm)**
PD 14	Control	10	44.5 ± 6.1	55.4 ± 5.2	0	88.4 ± 9.8
	PTU	10	41.7 ± 4.5	58.1 ± 4.8	0	83.4 ± 9.8
	TU	10	42.1 ± 4.3	57.9 ± 6.2	0	87.6 ± 9.7
PD21	Control	12	21.6 ± 2.9	78.3 ± 3.9	0.9 ± 0.1	108.2 ± 15.7
	PTU	10	20.0 ± 3.2	79.9 ± 8.6	0.8 ± 0.0	109.6 ± 11.8
	TU	10	19.1 ± 2.8	80.8 ± 5.4	0.3 ± 0.1	106.7 ± 14.5
PD 28	Control	12	11.5 ± 2.1	88.1 ± 9.0	48.1 ± 1.5	127.6 ± 10.6
	PTU	12	12.1 ± 2.4	88.0 ± 4.2	14.1 ± 2.2***	120.1 ± 10.8
	TU	12	10.1 ± 1.3	90.3 ± 4.9	17.8 ± 1.5***	121.0 ± 11.1

[1]PTU, neonatal mice injected with 6-propyl-2-thiouracil; TU, neonatal mice injected with thiourea.
*Values are expressed in mean ± SD. Twenty seminiferous tubule cross sections from each testis were evaluated, and statistical anlayses using one-way ANOVA reveal no significant difference ($P > .1$) among values in the control and treatment groups.
[2]Values are expressed in mean ± SD.
**At least 50 tubules were randomly chosen from each section for the measurement of the diameter, and at least five sections from each testis were taken for the measurement. Values are expressed in mean ± SD obtained from five animals in each group. No significant difference ($P > .1$) was found between the experiment group and the control group at each time point.
***$P < .5$, significantly different from the control value at the same postnatal period, Peritz's F test (Harper, '84).

Fertility

The results of mating between hypothyroid males and normal females and that between hypothyroid females and normal males did not differ much from mating between normal males and normal females with respect to the percentage of successful mating, percentage of pregnancy and litter size (Table 3), and the sex ratio of the resulting offspring (Table 4).

Somatic growth of offspring of hypothyroid mice

Somatic growth of the offspring of hypothyroid mice, as reflected by body weight (Fig. 3) and tail length (Fig. 4), was similar to that of the progeny of euthyroid mice.

DISCUSSION

A previous study showed that 6-propyl-2-thiouracil or thiourea treatment effectively induced neonatal hypothyroidism in the mouse (Ng et al., '92). The thyroid of the treated mice became hypertrophied and contained thyroid follicles distended with colloid. The plasma T_3 level of the treated neontal mice was significantly lower than that of the control value. Results of the present study disclosed that hypothyroidism induced by propylthiouracil (PTU) and thiourea (TU) impaired the development of ovarian follicles in females and spermatogenesis in males. However, the impaired development in ovary and testis was not serious enough to adversely affect reproduction after puberty. Both hypothyroid males and females were

*TABLE 3. Reproductive performance of drug-induced hypothyroid animals**

Mating	Treatment group[1]	Percent mated (n)[2]	Percent pregnant (n)[2]	Litter size (mean ± SD)[2]
Hypothyroid female normal male	Control	86 (14)	92 (12)	13.7 ± 1.6 (11)
	PTU	75 (8)	83 (6)	12.0 ± 1.1 (5)
	TU	73 (11)	100 (8)	12.1 ± 1.1 (8)
Hypothyroid male normal female	Control	75 (8)	83 (6)	12.0 ± 1.2 (5)
	PTU	70 (10)	85 (7)	12.2 ± 1.1 (6)
	TU	79 (14)	73 (11)	12.5 ± 1.1 (8)

[1]PTU, neonatal mice injected with 6-propyl-2-thiouracil; TU, neonatal mice injected with thiourea.
[2]Nos. in parentheses represent sample no.
*Statistical analyses using Student's t-test or χ^2-test revealed no significant difference ($P > .1$) between the control and experimental values.

*TABLE 4. Sex ratios of offspring of drug-induced hypothyroid animals at PD28**

Treatment groups[1]	Hypothyroid female × normal male		Hypothyroid male × normal female	
	Number of litters analysed	Female/male ratio (mean ± SD)	Number of litters analysed	Female/male ratio (mean ± SD)
Control	11	0.98 ± 0.16	5	1.00 ± 0.18
PTU	5	1.01 ± 0.22	6	1.01 ± 0.18
TU	8	0.98 ± 0.23	8	0.96 ± 0.13

*No statistically significant differences ($P > .1$) were revealed by χ^2-test between control and experimental values.
[1]Mothers had been injected with either 6-propyl-2-thiouracil (PTU) or thiourea (TU) during their neonatal development.

fertile and when mated with euthyroid partners they produced litters of offsprings with normal somatic growth. In the male, adverse effects of hypothyroidism on the testis have been reported in the case of mice (Hamolsky, '75) and man (Barnes et al., '73; Gilberg and Walfish, '80), but there were no effects in rams (Chandrasekhar et al., '85). In the rat, the influences of hypothyroidism on the testis and serum luteinizing hormone and testosterone levels reported by different groups were different, ranging from stimulation (Cooke and Meisami, '91; Cooke et al., '91), to no alterations (Vilchez-Martinez, '73; Weiss and Burns, '88), and degenerative changes (Baksi, '73; Bruni et al., '75; Amin and El-Sheikh, '77; Chowdury and Arora, '84; Chowdury et al., '84), depending on whether immature (Chowdury and Arora, '84; Chowdury et al., '84) or mature (Vilchez-Martinez, '73; Weiss and Burns, '88) animals were used and whether hypothyroidism was induced permanently by thyroidectomy (Chowdury and Arora, '84; Chowdury et al., '84) or transiently by treatment with a reversible goitrogen followed by return to euthyroidism (Cooke and Meisami, '91; Cooke et al., '91, '93; Joyce et al., '93; Hess et al., '93; Hardy et al., '93; Kirby et al., '92). In the female, fetal hypothyroidism resulted in small ovaries in rats (Leathem, '59) but not in sheep (Hopkins and Thorburn, '72). Thyroidectomy of prepubertal rats resulted in delayed sexual maturation and vaginal opening and underdeveloped uterus and vagina (Leathem, '72). Hypothyroidism in the adult rat and hamster was associated with abnormal estrous cycles and interfered with gestation (Ortega et al., '90; Vriend et al., '87). In hypothyroid sheep, the uterus showed endometrial hyperplasia, and hypothyroid rats exhibited a decrease in the uterine response to estrogen (Kirkland et al., '81). When taken together the results of the present and previous investigations suggest that the effects of hypothyroidism on gonadal development may vary with the animal species, the age of the animal, the method used to induce

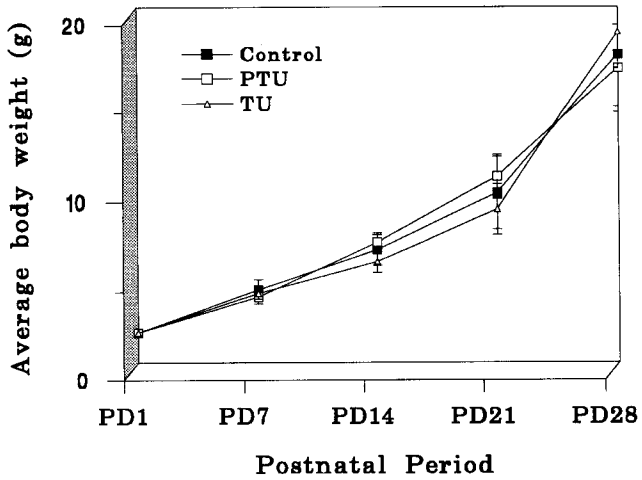

Fig. 3. The average body weight of the offsprings born to the parents one of which was treated with either PTU or TU. Each group consisted of five to seven litters. Statistical analyses using one-way ANOVA revealed no significant difference ($P > .1$) among the values in the control and experimental groups.

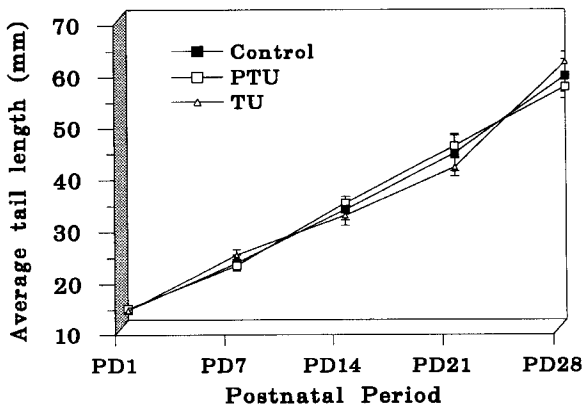

Fig. 4 The average tail length of the offsprings born to the parents one of which was treated with either PTU or TU. Each group consisted of five to seven litters. Statistical analyses using one-way ANOVA revealed no significant difference ($P > .1$) among values in the control and experimental groups.

hypothyroidism, and the duration of hypothyroidism. It appears that the developing gonad in the neonatal animal is susceptible to a deficiency of circulating thyroid hormones, but the deleterious effect of hypothyroidism is not sufficiently strong to seriously inhibit the reproductive function of the gonads.

The specific role of the thyroid hormones in the growth of the reproductive tract and the mechanism through which the impairing effect of hypothyroidism on ovarian and testicular development is mediated remained unclear. It has already been shown that the reproductive effects induced by the antithyroid drug PTU were due to hypothyroidism and not to the drug used to induce hypothyroidism (Cooke and Meisami, '91). Control of growth and development of the reproductive system was thought to be primarily regulated by sex hormones, growth factors (e.g., prolactin, growth hormone) and the secretion of gonadotropic hormones (e.g., follicle stimulating hormone, luteinizing hormone) from the hypothalamus–pituitary neuroendocrine axis (Kosco et al., '87; Heidel and Treinen, '89). Hence the effect of the hypothyroidism on the ovary and testis may be indirect, acting through the hypothalamus–pituitary axis. However, this does not exclude the possibility of an additional direct effect of the thyroid hormones on the reproductive tract. It was demonstrated in male rats that high levels of T_3 receptors which were present in immature Sertoli cells (Palmero et al., '88; Jannini et al., '90) declined during development until they disappeared in the adult. The thyroid hormone T_3 also directly affected production of insulin-like growth factor I by the Sertoli cells, which has both mitogenic and differentiative effects on various cell types (Palmero et al., '90), and perturbed the development of biochemical functions of immature Sertoli cells cultured in vitro (Fugassa et al., '87; Palmero et al., '89). In the female, T_3 receptors were also found in the porcine granulosa cells of immature ovarian follicles (Wakim et al., '87). Thyroid hormones also enhanced the actions of follicle stimulating hormone in the functional differentiation of the porcine granulosa cells in vitro (Maruo et al., '87). These findings suggested that thyroid hormones may have a direct effect on the development of the reproductive system.

In conclusion, neonatal hypothyroidism induced either by PTU or TU during the period from birth to postnatal day 28 impaired the normal development of the follicles in the ovary and spermatogenesis in the testis although obvious histological changes were not observed in other structures of the reproductive systems. The impaired prepubertal development in the ovary and testis, however, did not result in abnormalities in reproduction of the hypothyroid mice. The offsprings produced by the mice were also found to be normal with regard to the litter size, body weight, and tail length. The investigation of the mechanism of action of hypothyroidism on the reproductive system is underway using different types of tissue culture techniques.

ACKNOWLEDGMENTS

The expert secretarial assistance of Mrs. Doris Ling is gratefully acknowledged.

LITERATURE CITED

Amin, S.O., and A.S. El-Sheikh (1977) Pituitary-testicular function in hypothyroid male rats. Acta Anat. (Basel), *98*:121–129.

Baksi, S.N. (1973) Effect of propylthiouracil-induced hypothyroidism on serum levels of luteinizing hormone and follicle stimulating hormone in the rat. J. Endocrinol., *59*:655–656.

Barnes, M.D., A.B. Hayles, and R.J. Ryan (1973) Sexual maturation in juvenile hypothyroidism. Mayo Clin. Proc., *48*:849–853.

Bruni, J.F., S. Marshall, J.A. Dibbett, and J. Meites (1975) Effect of hyper- and hypothyroidism on serum LH and FSH levels in intact and gonadectromized male and female rats. Endocrinology, *97*:558–563.

Chandrasekhar, Y., M.J. D'Occhio, M.K. Holland, and B.P. Setchell (1985) Activity of the hypothalamo-pituitary axis and testicular development in prepubertal ram lambs with induced hypothyroidism or hyperthyroidism. Endocrinology, *117*:1645–1651.

Chowdury, A.R., and U. Arora (1984) Role of thyroid in testicular development of immature rat. Arch. Androl., *12*: 49–51.

Chowdury, A.R., A.K. Gautam, and B.B. Chatterjee (1984) Thyroid-testis interrelationship during the development and sexual maturity of the rat. Arch. Androl., *13*:233–239.

Cooke, P.S., and E. Meisami (1991) Early hypothyroidism in rats caused increased adult testis and reproductive organ size but does not change testosterone levels. Endocrinology, *129*:237–243.

Cooke, P.S., R.A. Hess, J. Porcello, and E. Meisami (1991) Increased sperm production in adult rats after transient neonatal hypothyroidism. Endocrinology, *129*:244–248.

Cooke, P.S., J.D. Kirby, and J. Porcelli (1993) Increased testis growth and sperm production in adult rats following transient neonatal goitrogen treatment: optimization of the propylthiouracil dose and effects of methimazole. J. Reprod. Fertil., *97*:493–499.

De Visscher, M., and Y. Inglebleek (1980) Hypothyroidism. In: The Thyroid Gland. M. De Visscher, ed. Raven Press, New York, pp. 377–412.

Francavilla, S., G. Cordeschi, G. Properzi, L. DiCicco, E.A. Jannini, S. Palmero, E. Fugassa, B. Loras, and M. D'Armiento (1991) Effect of thyroid hormone on the pre-

and postnatal development of the rat testis. J. Endocrinol., *129:*35–42.

Fugassa, E., S. Palmero, and G. Gallo (1987) Triiodothyronine decreases the production of androgen-binding protein by rat Sertoli cells. Biochem. Biophys. Res. Commun., *143:*241–247.

Gilberg, K., and P.G. Walfish (1980) Thyroid function and male reproduction. In: Diagnosis in Andrology. J. Bain and E.S.E. Hafez, eds. Martinus Nijhoff, The Hague, p. 56–68.

Gold, J.J., E. Castillo, S. Borushek, and A. Scommegna (1965) Factors in polycystic ovary development. I. Gonadotropins and altered thyroid function in the rat. Fertil. Steril., *16:*560–570.

Hamolsky, M.W. (1975) Thyroid factor. In: Progress in Infertility. S.J. Behrman and P.W. Kitsner, eds. Little Brown & Co., Boston, pp. 25–31.

Hardy, M.P., J.D. Kirby, R.A. Hess, and P.S. Cooke (1993) Leydig cells increase their numbers but decline in steroidogenic function in the adult rat after neonatal hypothyroidism. Endocrinology, *132:*2417–2420.

Harper, J.F. (1984) Peritz' F test: basic program of a robust multiple comparison test for statistical analysis of all differences among group means. Comput. Biol. Med., *14:*437–441.

Heidel, J.J., and K.A. Treinen (1989) Physiology of the male reproductive system: endocrine, paracrine and autocrine regulation. Toxicol. Pathol., *17:*411–445.

Hess, R.A., P.S. Cooke, D. Bunick, and J.D. Kirby (1993) Adult testicular enlargement induced by neonatal hypothyroidism is accompanied by increased Sertoli and germ cell numbers. Endocrinology, *132:*2607–2613.

Hopkins, P.S., and G.D. Thorburn (1972) The effects of foetal thyroidectomy on the development of the ovine foetus. J. Endocrinol., *54:*55–67.

Jannini, E.A., M. Olivier, S. Francavilla, A. Gulino, E. Ziparo, and M. D'Armiento (1990) Ontogenesis of the nuclear 3,5,3′-triiodothyronine receptor in the rat testis. Endocrinology, *126:*2521–2526.

Joyce, K.L., J. Porcelli, and P.S. Cooke (1993) Neonatal goitrogen treatment increases adult testis size and sperm production in the mouse. J. Androl., *14:*448–455.

Kirby, J.D., A.E. Jetton, P.S. Cooke, R.A. Hess, D. Bunick, J.F. Ackland, F.W. Turek, and N.B. Schwartz (1992) Developmental hormonal profiles accompanying the neonatal hypothyroidism-induced increase in adult testicular size and sperm production in the rat. Endocrinology, *131:*559–565.

Kirkland, J.L., R.M. Gardner, U.R. Mukku, M. Akhtar, and G.M. Stancel (1981) Hormonal control of uterine growth: the effect of hypothyroidism on estrogen-stimulated cell division. Endocrinology, *108:*2346–2351.

Kosco, M.S., D.J. Bolt, J.E. Wheaton, K.J. Loseth, and B.G. Crabo (1987) Endocrine response in relation to compensatory testicular growth after neonatal hemicastration in boars. Biol. Reprod., *36:*1177–1185.

Leathem, J.H. (1959) Extragonadal factors in reproduction. In: Recent Progress in the Endocrinology of Reproduction. C.W. Lloyd, ed. Academic Press: New York, pp. 170–221.

Leathem, J.H. (1972) Role of the thyroid. In: Reproductive Biology. H. Balin and S. Glasser, eds. Excerpta Medica, Amsterdam, pp. 23–33.

Longcope, C. (1991) The male and female productive systems in hypothyroidism. In: The Thyroid: A Fundamental and Clinical Text. L.E. Braverman, R.D. Utiger, S.C. Werner, and S.H. Ingbar, eds. Lippincott, Philadelphia, pp. 1052–1055.

Maruo, T., M. Hayashi, H. Matsuo, T. Yamamoto, H. Okada, and M. Mochizuki (1987) The role of thyroid hormone as a biological amplifier of the actions of follicle-stimulating hormone in the functional differentiation of cultured porcine granulosa cells. Endocrinology, *121:*1233–1241.

Minetti, C.A.S.A., P.Y. Su, L.B.S. Valle, and R.M. Oliveira-Filho (1986) Testicular function in prolactin-treated hypothyroid rats. In: Frontiers in Thyroidology. G. Medeiros-Neto and E. Gaitan, eds. Plenum Publishing Corporation, vol. 2, pp. 1231–1234.

Ng, T.B., W.Y. Chan, and P.P.L. Tam (1992) Correlation of acid phosphatase but not alkaline phosphatase activity with myelination in the developing mouse brain. Int. J. Biochem. *25:*247–251.

Ortega, E., E. Rodriquez, and E. Ruiz (1990) Activity of the hypothalamo-pituitary ovarian axis in hypothyroid rats with or without triodothyronine replacement. Life Sci. 46:391–405.

Palmero, S., S. Maggiani, and E. Fugassa (1988) Nuclear triiodothyronine receptors in rat Sertoli cells. Mol. Cell. Endocrinol., *58:*253–256.

Palmero, S., M. De Marchis, G. Gallo, and E. Fugassa (1989) Thyroid hormone affects the development of Sertoli cell function in the rat. J. Endocrinol., *123:*105–111.

Palmero, S., M. Prati, A. Barreca, F. Minuto, G. Giordano, and E. Fugassa (1990) Thyroid hormone stimulates the production of insulin-like growth factor I (IGF-I) by immature rat Sertoli cells. Mol. Cell. Endocrinol., *68:*61–65.

Pedersen, T., and H. Peters (1968) Proposal for a classification of oocytes and follicles in the mouse ovary. J. Reprod. Fertil., *17:*555–557.

Russell, L.D., R.A. Ettlin, A.P. SinhaHikim, and E.D. Clegg (1990) Histological and Histopathological Evaluation of the Testis. Cache River Press, Clearwater, FL.

Scommegna, A., E. Castillo, S. Borushek, and J.J. Gold (1965) Factors in polycystic ovary development: II. Role of the pituitary in the rat. Fertil. Steril., *16:*571–578.

Senthikumaran, B., M.M. Araldhas, R.E.R. Udhayakumar, and P. Govindarajula (1991) Influence of thyroidectomy on seminal vesicular glycosidases of young albino rats. Ind. J. Exp. Biol., *29:*205–207.

Styne, D.M. (1991) Puberty. In: Basic and Clinical Endocrinology. F.S. Greenspan, ed. Appleton and Lange, Norwalk, CT, Chapter 19, pp. 519–542.

van Haaster, L.A., F.H. de Jong, R. Docter, and D.G. de Rooij (1992) The effect of hypothyroidism on Sertoli cell proliferation and differentiation and hormone levels during testicular development in the rat. Endocrinology, *131:*1574–1576.

Vilchez-Martinez, J.A. (1973) Study of the pituitary-testis axis in hypothyroid adult male rats. J. Reprod. Fertil., *35:*123–126.

Vriend, J., F.D. Bertalanffy, and T.A. Ralcewicz (1987) The effects of melatonin and hypothyroidism on estradiol and gonadotropin levels in female Syrian hamsters. Biol. Reprod., *36:*719–724.

Wakim, N.G., N. Ramani, and C.V. Rao (1987) Triiodothyronine receptors in porcine granulosa cells. Am. J. Obstet. Gynecol., *156:*237–240.

Weiss, S.R., and J.M. Burns (1988) The effect of acute treatment with two goitrogens on plasma thyroid hormones, testosterone and testicular morphology in adult male rats. Comp. Biochem. Physiol. A, *90:*449–452.

Yen, S.S.C. (1986) Chronic anovulation caused by peripheral endocrine disorders. In: Reproductive Endocrinology, Physiology, Pathophysiology and Clinical Management. S.S.C. Yen and R.B. Jaffe eds. W.B. Saunders Co., Philadelphia, pp. 441–499.