Histol Histopathol (2004) 19: 985-997

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Effects of thyroid hormones on Leydig cells in the postnatal testis

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Summary. Thyroid hormones (TH) stimulate oxidative metabolism in many tissues in the body, but testis is not one of them. Therefore, in this sense, testis is not considered as a target organ for TH. However, recent findings clearly show that TH have significant functions on the testis in general, and Leydig cells in particular; this begins from the onset of their differentiation through aging. Some of these functions include triggering the Leydig stem cells to differentiate, producing increased numbers of Leydig cells during differentiation by causing proliferation of Leydig stem cells and progenitors, stimulation of the Leydig cell steroidogenic function and cellular maintenance. The mechanism of action of TH on Leydig cell differentiation is still not clear and needs to be determined in future studies. However, some information on the mechanisms of TH action on Leydig cell steroidogenesis is available. TH acutely stimulate testosterone production by the Leydig cells *in vitro* via stimulating the production of steroidogenic acute regulatory protein (StAR) and StAR mRNA in Leydig cells; StAR is associated with intracellular trafficking of cholesterol into the mitochondria during steroid hormone synthesis. However, the presence and/or the types of TH receptors in Leydig cells and other cell types of the Leydig cell lineage is still to be resolved. Additionally, it has been shown that thyrotropin-releasing hormone (TRH), TRH receptor and TRH mRNA in the testis in many mammalian species are seen exclusively in Leydig cells. Although the significance of the latter observations are yet to be determined, these findings prompt whether hypothalamo-pituitary-thyroid axis and hypothalamo-pituitary-testis axis are short-looped through Leydig cells.

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Key words: Thyroid hormone, Leydig cells, Stem cell differentiation, Leydig progenitor cells, Steroidogenesis, Aging

Introduction

Leydig cells are the main source of androgens in the mammalian male. In this paper, efforts have been made to review the available literature on TH on Leydig cells in the postnatal testis.

Leydig Cells

Franz Leydig, a scientist from Germany, first described the Leydig cells in the testis interstitium in 1850 (Leydig, 1850), in a comparative study of various mammalian species (primates, carnivores, rodents, etc.). Pol Bouin (1870-1962) and Paul Ancel (1873-1961) were the first to strongly emphasize a possible endocrine role for Leydig cells, i.e. that internal secretion of Leydig cells controls male secondary characteristics (Christensen, 1996). It is established now that the Leydig cells are the main source of androgens in the mammalian male and that the population of Leydig cells in the adult testis, identified as the adult Leydig cells, are differentiated postnatally during the neonatal pre-pubertal period (Roosen-Runge and Anderson, 1959; Mancini et al., 1963; Niemi and Kormano, 1964; Baillie, 1964; Lording and de Kretser, 1972). The adult Leydig cells, hereafter referred to as Leydig cells in many places in the text, reside in the testis interstitium (Fig. 1) as large polyhedral cells. Among species, variations are seen in Leydig cell number, size, morphological characteristics and their relationship to blood vessels and other surrounding structures; these are unique to each species (Fawcett et al., 1973) and will not be discussed in this review. Luteinizing hormone (LH) produced by the gonadotrophs of the anterior pituitary gland, is

considered as the primary regulator of Leydig cell structure and function in the postnatal testis.

Thyroid Hormones (TH)

J.F. Gudernatsch (1912) provided the first evidence for TH and their role in cellular differentiation. It is now established that thyroxine (T_4) and triiodothyronine (T3) are produced by the thyroid gland and T_3 is at least five times more potent than T_4. The most characteristic effect of TH is their ability to stimulate oxidative metabolism in many tissues in the body, however, in this sense, testis is not considered as a target organ for these hormones. TH secretion is regulated by thyroid hormone releasing hormone (TRH) and thyroid stimulating hormone (TSH) from the hypothalamus and the anterior pituitary, respectively.

The rest of the text of this paper is divided into six subheadings, namely, Leydig cell lineage, TH on postnatal Leydig cell differentiation and steroidogenic function, mechanism of action of TH on Leydig cell steroidogenesis, TH receptors in Leydig cells and the presence of TRH in Leydig cells; possible significance of the latter is also discussed.

Leydig cell lineage

Figure 2 shows a schematic representation of the Leydig cell lineage. Mesenchymal cells of the testis interstitium are the precursors/stem cells to Leydig cells (Mancini et al., 1963; Baillie, 1964; Niemi and Kormano, 1964; Lording and de Kretser, 1972; Fawcett et al., 1973) and they embryologically originate either from the mesonephric tubules or loose connective tissue of the developing gonad derived from the embryonic mesoderm (Byscove, 1986). In the postnatal testis they are either found at the peritubular region or central interstitium (randomly scattered); those in the peritubular region have been identified as the precursor cell type for Leydig cells (Lording and de Kretser, 1972; Haider et al., 1995; Russell et al., 1995; Chemes, 1996) and several recent studies have confirmed this observation in the pre-pubertal rat (Ariyaratne et al., 2000a,c,d) and adult rat testes following ethane-

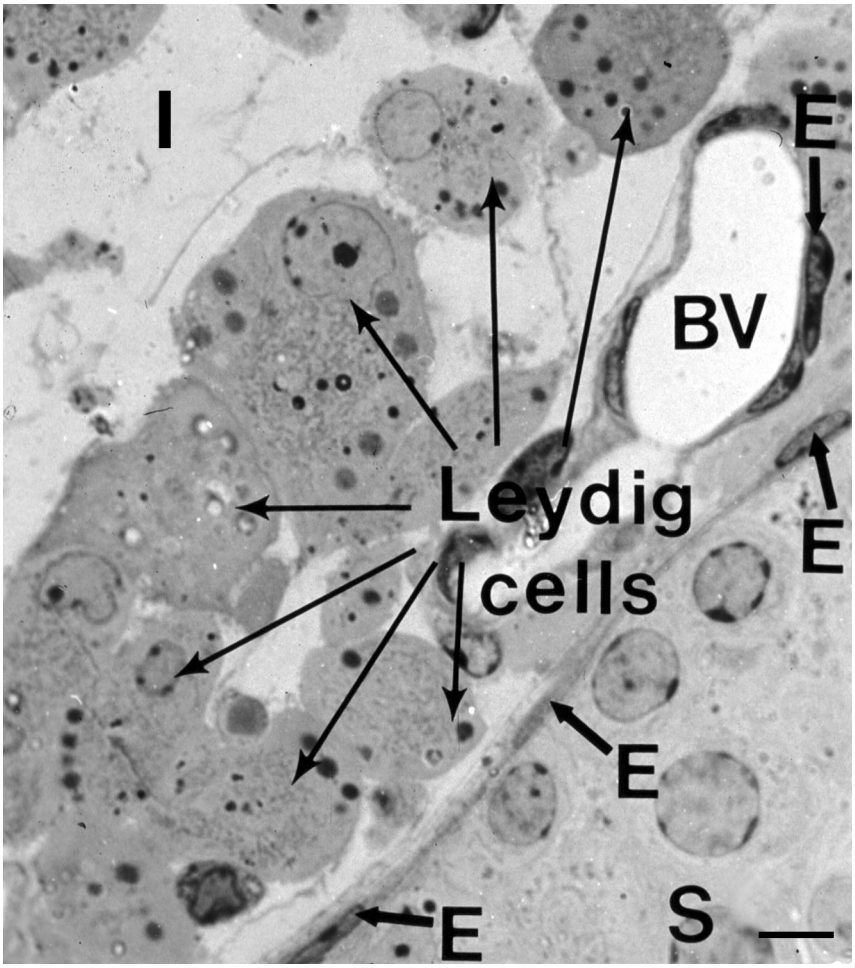

Fig. 1. A light micrograph of a guinea pig testis interstitium (I) to show Leydig cells, which appear as large polygonal-shaped structures. BV: blood vessels; E: elongated spindle-shaped cells in the testis interstitium; those present at the periphery of the seminiferous tubules (S) are commonly known as peritubular mesenchymal cells and are the stem/precursor cells to Leydig cells. Bar: 20 μm

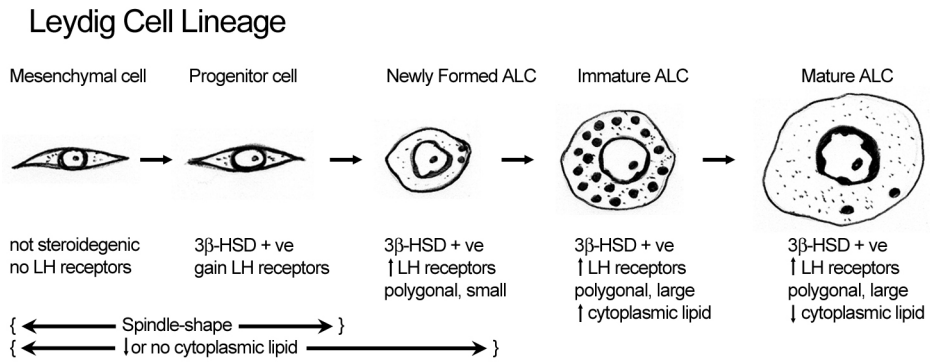

Fig. 2. Schematic diagram of Leydig cell lineage.The stem cells for Leydig cells are the mesenchymal cells in the testis intersitium, which are spindle-shaped and non-steroidogenic. They first differentiate into progenitor cells, which are also spindle-shaped, but possess few steroidogenic enzymes (e.g. 3ß-HSD) and LH receptors. Thyroid hormone is critical to stimulate the mesenchymal cells to differentiate into the progenitor cells (the first step in Leydig cell differentiation) to begin the process of Leydig cell differentiation. Progenitor cells differentiate into mature adult Leydig cells through stages of newly formed adult Leydig cells and immature adult Leydig cells, respectively. (used with permission from the publisher, Mendis-Handagama and Ariyaratne, 2001, Biol.Reprod. 65, 660-671).

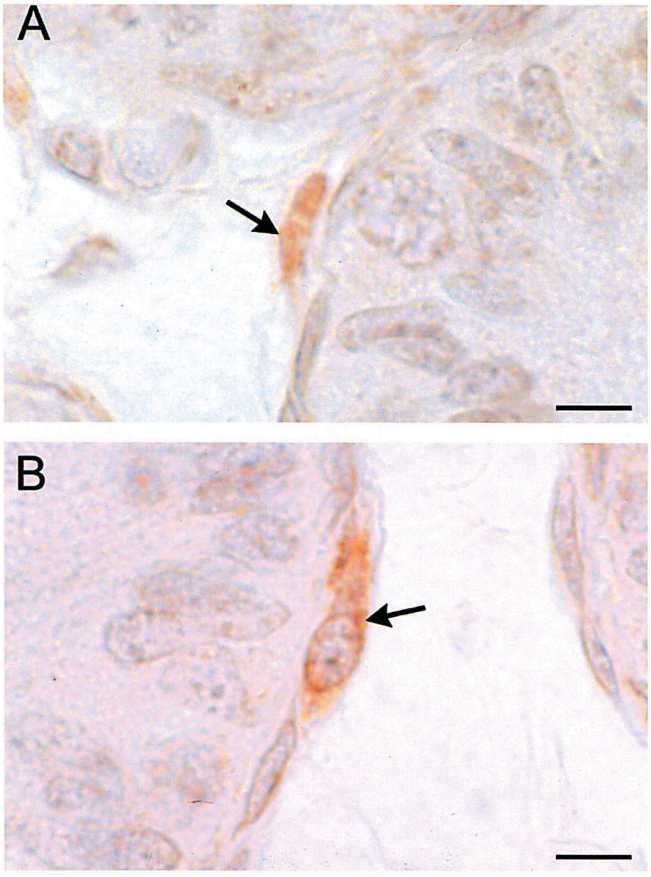

Fig. 3. Representative light micrographs from a 10 day old rat testis immunolabeled for 3ß-HSD (shown in brown color) and demonstrate early steps in Leydig cell differentiation. With thyroid hormone stimulation, mesenchymal cells in the periphery of the seminiferous tubules (S) differentiate into progenitor cells (arrows in Figures A and B), which are still spindle-shaped; with the progression of their differentiation towards the newly formed adult Leydig cells, they become rounder in shape (compare cells depicted by arrows arrow in Figure B, with A) and move gradually away from the peritubular region towards the central part of the testis interstitium. (used with permission from the publisher, Ariyaratne et al., 2000, Biol. Reprod. 65, 660-671). Bar: 20 μm.

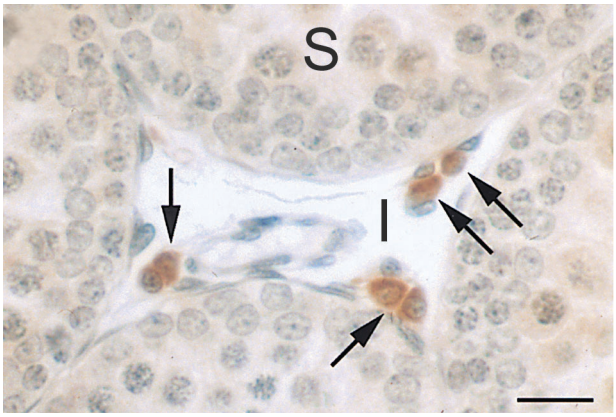

Fig. 4. A representative light micrograph immunolabeled for 3ß-HSD to demonstrate newly formed adult Leydig cells (arrows) at postnatal day 21 in a rat testis. Note that the newly formed adult Leydig cells appear as rounder profiles and are found near the peritubular region as well as away from the tubules (used with permission from the publisher, Ariyaratne et al., 2000, Biol. Reprod. 63, 493-502). Bar: 22μm

dimethane sulfonate (EDS)-treatment (Ariyaratne et al., 2000b; EDS kills Leydig cells within 48hrs and therefore, EDS-treated adult rat testis is a good model to study Leydig cell differentiation) using the universally accepted marker, 3ß-hydroxy steroid dehydrogenase (3ß-HSD) for all steroid secreting cells. At the onset of the process of Leydig cell differentiation, a mesenchymal cell which is a non-steroidogenic cell is differentiated into the second cell type in the lineage, the progenitor cell, which is steroidogenic (Mendis-Handagama and Ariyaratne, 2001; see Figure 3). Following differentiation, these progenitor cells proliferate and also differentiate into the next cell stage, the newly formed adult Leydig cells; progression of these events accompany the movement of these cells away from the peritubular location towards the central interstitium (Mendis-Handagama and Ariyaratne, 2001). With advancement of age and the progression of the Leydig

cell differentiation process, the newly formed adult Leydig cells (Figs. 4, 5) transform into immature Leydig cells and finally attain the status of mature adult Leydig cells found in the sexually mature testis (Fig. 1).

Thyroid hormones in postnatal Leydig cell differentiation

Until recent years, little was known about the effects of TH on the differentiation of Leydig cells in the postnatal testis. The observation that the adult rats subjected to transient neonatal hypothyroidism contain twice the number of Leydig cells per testis compared to the age-matched untreated controls (Mendis-Handagama and Sharma, 1994) raised the question of the mechanism of this Leydig cell hyperplasia following transient neonatal hypothyroidism. It was shown later that Leydig cell differentiation in the neonatal-prepubertal testis is arrested with hypothyroidism (Mendis-Handgama et al., 1998; Teerds et al., 1998) and increased numbers of precursor cells/mesenchymal cells are generated during this period (Mendis-Handgama et al., 1998). Although it is reported elsewhere (Hardy et al., 1996) that increased proliferation of postnatally differentiated Leydig cells from day 8 through 50 postpartum is the principal mechanism responsible for this Leydig cell hyperplasia in adult rats subjected to transient neonatal hypothyroidism, evidence is available to discard this conclusion. First, the postnatally differentiated Leydig cells are not present at day eight in the prepubertal rat testis (Mendis-Handagama et al., 1987; Mendis-Handagama and Ariyaratne, 2001) and the only Leydig cell type present at this time (i.e. at postnatal day 8) is the fetal Leydig cell (16, 19-21). Second, it is a proven fact that Leydig cell differentiation in the prepubertal testis (Mendis-Handagama et al., 1998; Ariyaratne et al., 2000a) and in the adult testes following EDS treatment (Ariyaratne et al., 2000b) is arrested with hypothyroidism. As revealed by immunocytochemistry for 3ß-HSD, the universal marker for all steroidogenic cells, not only Leydig cells, but all other cell types in the Leydig cell lineage (i.e. progenitors, newly formed Leydig cells and immature Leydig cells) were absent in testes of these hypothyroid rats. These observations clearly showed that postnatal Leydig cell differentiation does not take place under hypothyroid conditions, i.e. TH is critical for the onset of mesenchymal cell differentiation into Leydig progenitors to begin the process.

Continuous exposure of lactating mothers to polychlorinated biphenyls (PCB) causes significant effects on Leydig cell structure and function; hypotrophy and reduced capacity to produce testosterone *in vitro* in response to LH stimulation (Kim et al., 2000). Additionally, it is reported that PCBs disrupts the thyroid gland function in humans (Langer et al., 1998; Cheek et al., 1999; Nagayama et al., 1998) as well as in many other mammalian species such as the rat (Collins, 1980;

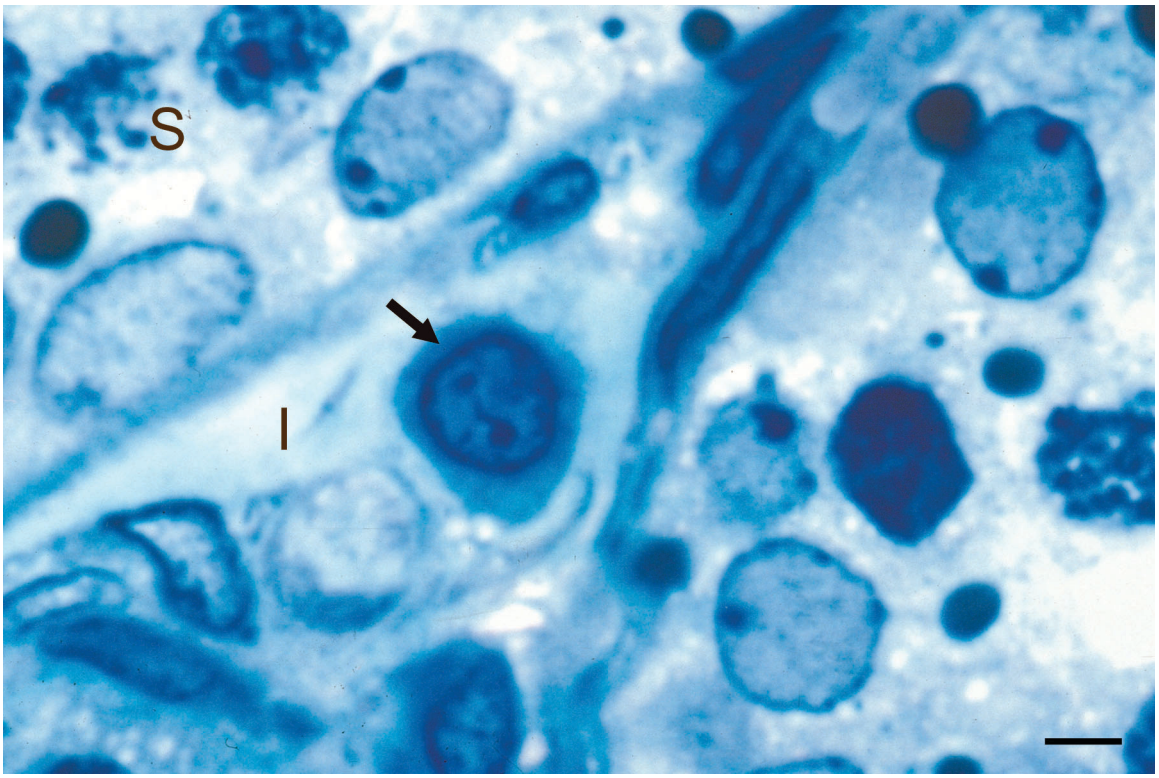

Fig. 5. A representative light micrograph of a newly formed adult Leydig cell (arrow) at postnatal day 21 in a rat testis. The newly formed adult Leydig cells are much smaller than the mature adult Leydig cell, and have relatively little cytoplasm in contrast to the adult Leydig cells in a sexually mature testis. S: seminiferous tubules; I: testis interstitium. Bar: 8 μm.

Saeed and Hansen 1985; Ness et al., 1993; Cooke et al., 1996; Kato et al., 1998, 1999; Desaulniers et al., 1999) and the grey seal (Wolstad and Jensen, 1999). Based on the observations of Cooke et. al (1996) and Kim et al. (2000) it appears that PCB exposure during the neonatal period has subjected these rats to undergo a transient hypothyroid status, which has caused an interference in the normal process of Leydig cell differentiation during prepuberty and produce a defect in the steroidogenic function of the Leydig cells in the adult testis.

It is also being demonstrated that hyperthyroidism causes accelerated Leydig cell differentiation (Teerds et al., 1998; Ariyaratne et al., 2000a,c). Additionally, it is reported that greater numbers of mesenchymal cells are recruited into Leydig cells with TH treatment in prepubertal (Ariyaratne et al., 2000c) and EDS treated (Ariyaratne et al., 2000b) adult rats. These findings indicate that thyroid hormone causes proliferation of mesenchymal precursor cells and acceleration of their differentiation into Leydig progenitors; this is in addition to its effects of enhanced proliferation of progenitors and newly formed Leydig cells in the prepubertal testis (Ariyaratne et al., 2000a,c). A yet unresolved question on the effect of TH on the initiation of mesenchymal precursor cell differentiation to begin the process of Leydig cell differentiation is whether this effect is direct or indirect. Based on the demonstration of the presence of thyroid receptor mRNA in mesenchymal precursor cells (Hardy et al., 1996), it is possible to suggest that TH act directly on the mesenchymal precursor to trigger this process. However, it still needs to be demonstrated that this mRNA expression is followed by protein synthesis to confirm this fact. Irrespective of this possible direct action of thyroid hormone on mesenchymal cells, it is also possible to predict that thyroid hormones may have an indirect effect on mesenchymal cell differentiation into progenitors in the postnatal testis. A logical hypothesis could be built on the known facts on TH action of Sertoli cell maturation and anti-Mullerian hormone (AMH) production by the Sertoli cells in the neonatal-prepubertal testis. This is because AMH is suggested as a negative regulator of Leydig cell differentiation (Racine et al., 1998).

Anti-Mullerian hormone and thyroid hormone on Leydig precursor cell differentiation

Anti-Mullerian hormone which is also called the Mullerian-inhibiting substance (MIS) or Mullerian inhibiting factor (MIF) is a member of the transforming growth factor (TGF) ß family of cytokines that includes TGF ß, activins, inhibins and the bone morphogenetic proteins. Anti-Mullerian hormone produced by the Sertoli cells in the developing male fetal testis causes regression of the Mullerian ducts (Josso, 1986; Lee and Donohoe, 1993). AMH production by the rat Sertoli cells decreases gradually and dramatically after the 3rd and 5th postnatal days, respectively and present at a very low level on the 20th postnatal day (Zhou et al., 1993). Although it is been reported that the measurement of AMH mRNA is not an accurate index of AMH production (Jannini et al., 1995) the fact that a dose-dependent decrease occur in AMH mRNA production by the immature Sertoli cells with triiodothyronine (T3) is an interesting observation with respect to the hypothesis on the indirect role of TH on Leydig cell differentiation. This is because AMH has been suggested as a negative regulator for postnatal differentiation of Leydig cells (Racine et al., 1998). A schematic diagram of the hypothesis on the mechanism of action of TH in precursor cell differentiation into the Leydig cell progenitors to begin the process of postnatal differentiation of Leydig cells is shown in Figure 6.

Thyroid hormones on testicular steroidogenesis

Thyroid abnormalities has long been known to cause reproductive disturbances in the male as well as in the female (Jannini et al., 1995). Studies on the male have largely been focused on changes in Sertoli cells because

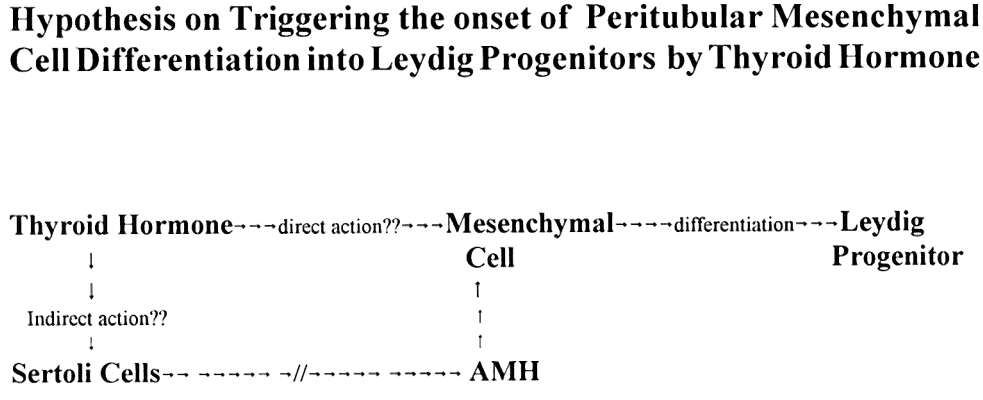

Fig. 6. Hypothesis on thyroid hormone action on mesenchymal cell differentiation into Leydig progenitor cell to begin the process of Leydig cell differentiation. Thyroid hormone could act directly on the mesenchymal cells to trigger the onset (i.e. +ve regulation). Anti-Mullerian hormone (AMH) produced by the immature Sertoli cells inhibits (-ve regulation) this differentiation. As thyroid hormones act on immature Sertoli cells to cause cell maturation, it is a possibility that this action inhibits the production of AMH, and thereby inhibits the inhibitory action of AMH on mesenchymal cell differentiation,

it was generally believed that this cell is primarily affected by TH in the postnatal testis (Jannini et al., 1995). Nevertheless, few available data indicate strongly that TH are closely associated with the function of Leydig cells in the adult testis by influencing the hypothalamo-pituitary testicular axis. It is reported that thyroid dysfunction often results in abnormalities of gonadotropin release, sex steroid metabolism and testicular function. The presence of nuclear T3 receptors in gonadotrophs of the rat pituitary gland is supportive of the effect of TH on gonadotropin release (Sasaki et al., 1991).

In intact mature animals, who were hypothyroid due to diseases, thyroidectomy, or administration of chemicals such as propylthiouracil (PTU), a marked decrease in body, testis and accessory sex organ weights (Maran et al., 2000a; Cristovo et al., 2002) occur. Additionally, serum concentrations of testosterone (Chandrasekhar et al., 1986; Hoffman et al., 1991; Antony et al., 1995; Maran et al., 2001; Cristovo et al.,

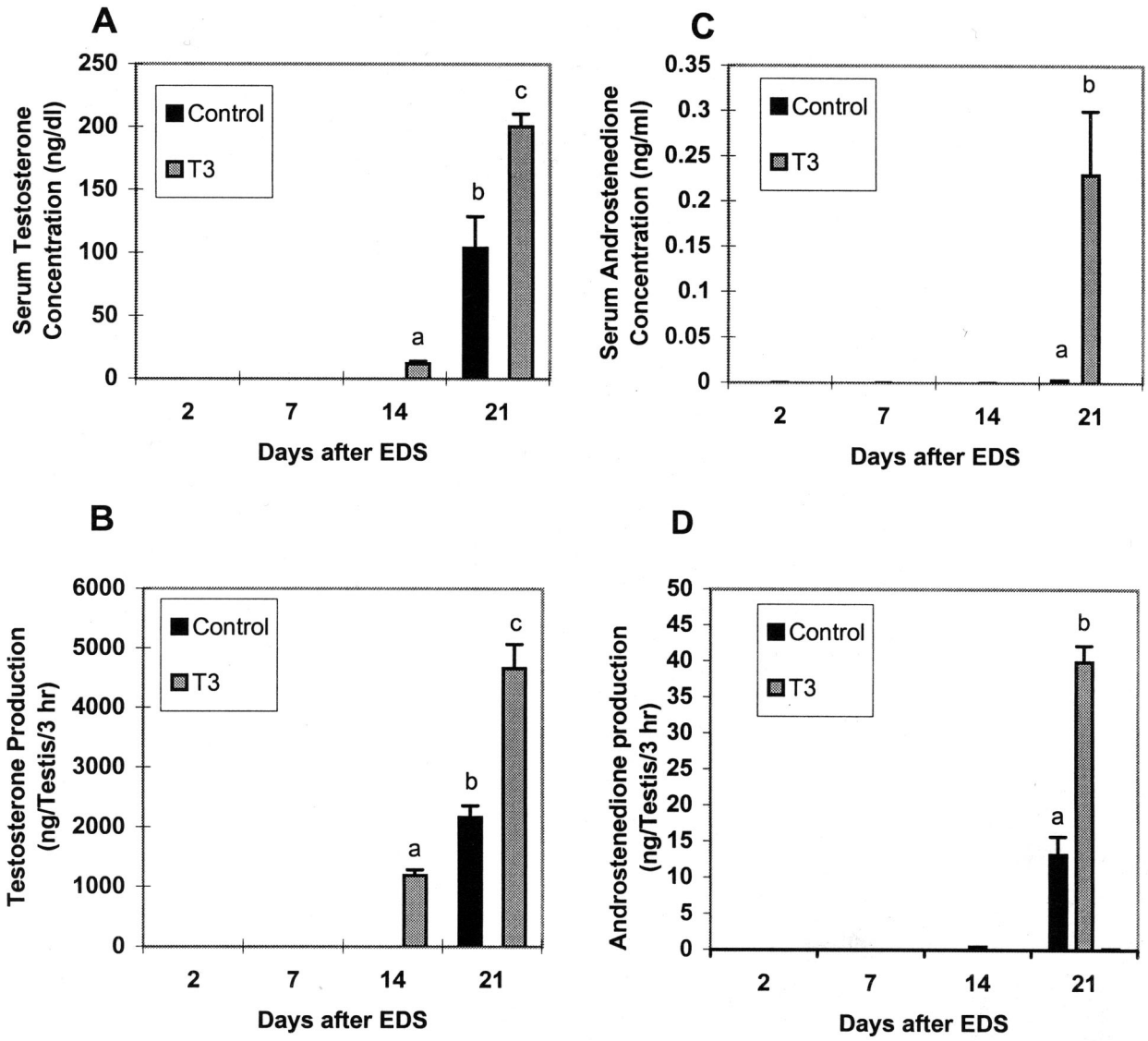

Fig 7. Androgens (testosterone and androstenedione) in serum and testicular incubates of control EDS rats and T3-treated EDS rats. Testosterone in serum **(A)** and testicular incubates **(B)** were first detected at 14 days in T3-treated EDS rats but not in control EDS rats. This finding agreed with the observation that new Leydig cell differentiation has taken place in the T3-treated EDS rats at day 14 and not in the control EDS rats. At day 21, testosterone levels in serum and testicular incubates were two-fold greater in T3-treated EDS rats compared to controls; T3-treated EDS rats had greater number of Leydig cells compared to control EDS rats. Androstenedione levels in serum **(C)** and testicular incubates **(D)** were greater in T3-treated EDS rats compared to control EDS rats and explained by the greater numbers of newly formed adult Leydig cells in the T3-treated EDS rats (used with permission from the publisher, Ariyaratne et al., 2000, Biol. Reprod. 63, 1115-1123).

2002) and Leydig cell responses to exogenous gonadotropins (Chandrasekhar et al., 1986; Wortsman et al., 1987; Hoffman et al., 1991; Antony et al., 1995) are observed to be reduced. Furthermore, morphological changes such as reduced numbers of Sertoli and Leydig cells, a reduced tubular diameter, interstitial edema and thickening of basal membrane of seminiferous tubules have been reported in the adult hypothyroid males (Wortsman et al., 1987; Hoffman et al., 1991; Tahmaz et al., 2000). By contrast, patients with Grave's disease (chronic hyperthyroidism) show high basal levels of serum testosterone, estradiol and gonadotropic hormones (Foldes et al., 1982; Kumar et al., 1996). In addition, an exaggerated pituitary and testicular response to exogenous gonadotropin releasing hormone (GnRH) is seen in hyperthyroid patients (Rojdmark et al., 1988), which demonstrates an altered hypothalamo-pituitary-testicular axis under hyperthyroid conditions.

Studies in EDS-treated adult rats have shown that daily T3 treatment following the EDS injection causes detectable serum testosterone levels on day 14 post EDS in contrast to control EDS rats, where the serum testosterone levels were still undetectable (Fig. 7A, Ariyaratne et al., 2000c). These results have been further confirmed by the observations on LH-stimulated testicular testosterone secretory capacity *in vitro* of these rats, where detectable amounts of testosterone was seen in the testicular incubates of T3-treated EDS rats in contrast to control EDS rats (Fig. 7B). At 21 days post EDS, testosterone levels in serum and testicular incubates of the T3-treated EDS rats in that study showed significantly greater amounts compared to control EDS rats (Fig. 7A,B). A somewhat similar pattern is seen for androstenedione in serum and testicular incubates in these control EDS and T3-treated EDS rats (Fig. 7C,D).

Although direct effects of TH on Leydig cell steroidogenesis *in vivo* or *in vitro* have not been studied extensively, available reports clearly show that TH have a significant role in this process. Valenty et al. (1997) observed that isolated Leydig cells from hypothyroid adult rats secreted less testosterone, both under basal conditions as well as in the presence of cAMP and non-cAMP mediated stimulatory substances. They (Valenty et al., 1997) further noticed that this reduction of testosterone production was due to decreased synthesis of cAMP and reduced activity of the enzymes in the androgen biosynthetic pathway but not due to changes in LH receptor content. Moreover, it has been demonstrated that culture of isolated Leydig cells from sexually mature rats with TH resulted in stimulated secretion of testosterone and estrogen under basal conditions as well as in response to LH stimulation (Maran et al., 2000b). Additionally, treatment of mouse Leydig cells with T3, coordinately augments the levels of steroidogenic acute regulatory (StAR) protein and StAR mRNA and steroid production (Manna et al., 1999, 2001a,b). StAR protein is involved in intracellular cholesterol transport mechanism during LH-stimulated steroidogenesis in Leydig cells (Stocco, 1996). Because the effects of T4 *in vivo* (T4 is restricted to the vascular pool) are mediated via T3 (T4 is converted to T3 in target tissues; T3 is 3- to 5-times more active than T4; Norman and Litwick, 1997), it is possible to suggest that the stimulatory effects of T3 *in vitro* on Leydig cell steroidogenesis (Manna et al., 1999, 2000a,b; Maran et al., 2000b) reflect the acute effects of TH on Leydig cells *in vivo*.

With aging, a progressive decline in circulating testosterone levels is seen in humans (Hollander and Hollander, 1958; Kirschner et al., 1968; Vermulen, 1976) and other species including the rat (Bethea and Walker, 1979; Mendis-Handagama and Gelber, 1995; Kim et al., 2002). Many studies on the effects of aging on Leydig cell structure and function have revealed that Leydig cells undergo atrophic changes in size (Mendis-Handagama and Gelber, 1995; Kim et al., 2002) and organelle content (Mori et al., 1982; Ichihara et al., 1993). It is being shown that serum TH levels are reduced with aging (Cizza et al., 1992, 1995) and exogenous supplementation of TH alone to aged Brown Norway rats (18 months of age) for 28 days could reverse the LH-stimulated testosterone secretory capacity per testis and per Leydig cell *in vitro* by 71%, Leydig cell size by 82% and serum testosterone levels by 33% compared to three month old control rats (Kim et al., 2002). 100% reversibility in LH-stimulated testosterone secretory capacity per testis and per Leydig cell *in vitro* and Leydig cell size is achieved by the combined treatment of T4 and LH (Kim et al., 2002). Representative Leydig cells of control rats of 3, 6, 12 and 19 month of age and T4-, LH- and T4+LH- treated Brown Norway rats are shown in Figure 8.

Studies in neonatal rats have shown that daily subcutaneous injections of T3 to rat pups from birth to 21 days significantly reduce the LH-stimulated testicular testosterone capacity *in vitro* up to day 21, in contrast to the control rats which show no change in the testosterone secretory capacity up to day 12; however, this capacity declines thereafter (Ariyaratne et al., 2000c; Fig. 9). These observations are correlated with the capacity of the fetal Leydig cells in the neonatal testis to secrete testosterone during this time of life as they are the primary source of androgens in the neonatal testis. As TH cause early atrophic changes in the fetal Leydig cells (Ariyaratne et al., 2000c) the testicular capacity to secrete testosterone in response to LH stimulation *in vitro* is reduced in the neonatal rat pups under hyperthyroid conditions. By contrast, testicular androstenedione secretory capacity per testis in response to LH stimulation *in vitro* in these neonatal rats under a hyperthyroid status is significantly increased and is explained by the increased numbers of newly formed adult Leydig cells in the testes of hyperthyroid rat pups (Ariyaratne et al., 2000c). Additionally, increased amounts of cAMP (Valenty et al., 1997) and StAR protein (Manna et al., 2001a,b) generated in Leydig cells of hyperthyroid rats may have been complementary to this effect.

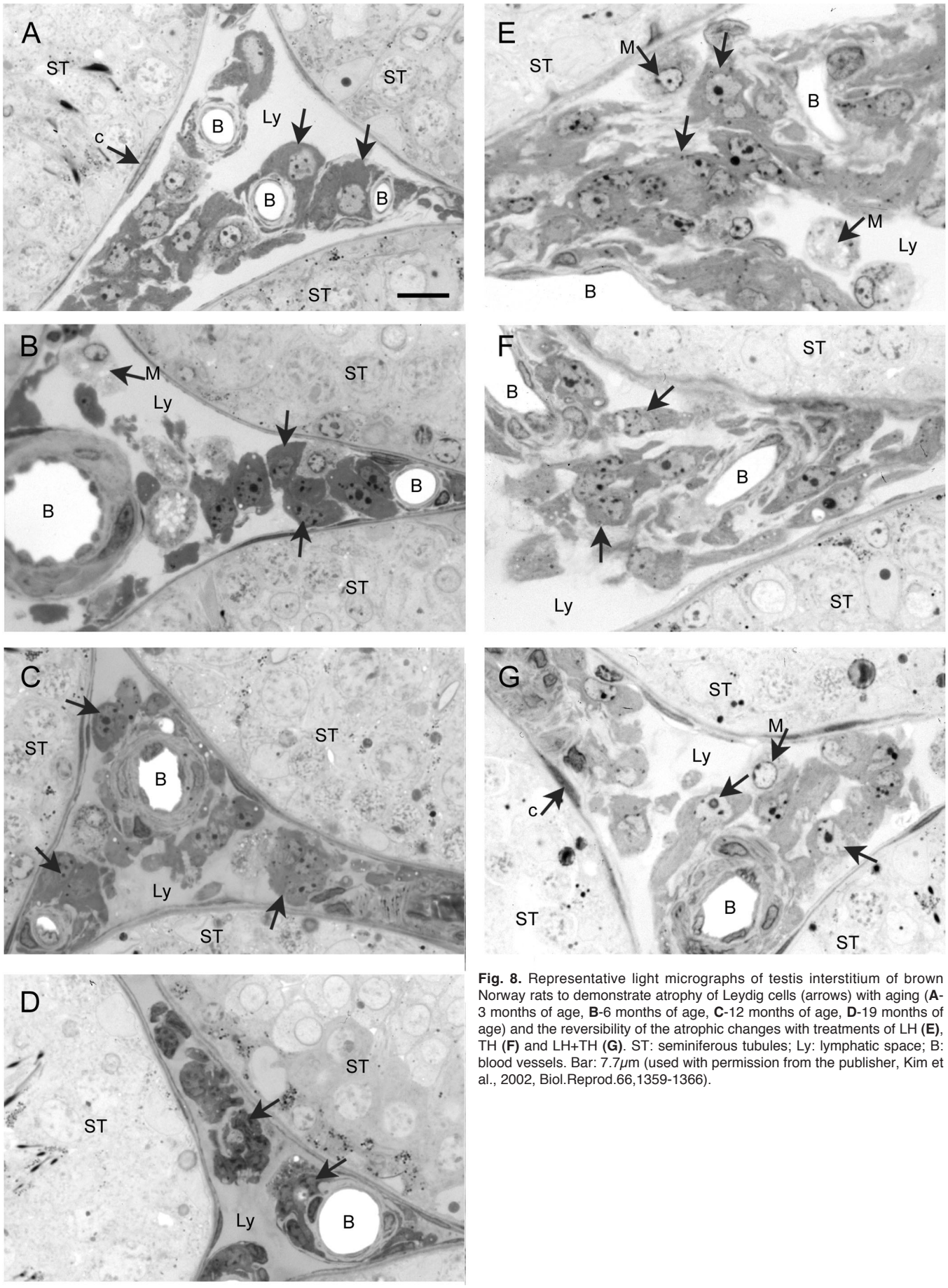

Fig. 8. Representative light micrographs of testis interstitium of brown Norway rats to demonstrate atrophy of Leydig cells (arrows) with aging (**A**-3 months of age, **B**-6 months of age, **C**-12 months of age, **D**-19 months of age) and the reversibility of the atrophic changes with treatments of LH **(E)**, TH **(F)** and LH+TH **(G)**. ST: seminiferous tubules; Ly: lymphatic space; B: blood vessels. Bar: 7.7μm (used with permission from the publisher, Kim et al., 2002, Biol.Reprod.66,1359-1366).

Mechanism of action of thyroid hormones on Leydig cell steroidogenesis

Based on the reviewed information, it is clear that TH have significant regulatory role on Leydig cell steroidogenic function. However, the precise mechanism of action of these hormones on this cell is not clearly apparent. It is possible to hypothesize that the *in vivo* effects of TH are at least in part mediated via the Sertoli cells. Nevertheless, direct actions of TH on Leydig cells comes from *in vitro* studies. Study of Jana et al. (1996) is the first to report on production of a 52 kDa soluble protein by goat Leydig cells *in vitro* when exposed to TH. This protein was capable of stimulating testosterone secretion by the Leydig cells when added to their incubation medium. More recent studies using molecular biological techniques to analyze the effects of TH on Leydig cells has shown that stimulatory effect of this hormone on steroidogenesis is brought about by increased synthesis of StAR protein mediated through steroidogenic factor-1 (SF-1; Manna et al., 1999, 2001a,b). Moreover, these investigators were able to demonstrate an acute stimulatory but a chronic inhibitory effect of thyroid hormone on steroidogenic enzymes and LH receptor content in mouse tumor Leydig cells (Manna et al., 2001b). It is clear that further studies are required to establish the precise mechanism of action of TH on Leydig cell steroidogenesis.

Thyroid hormone receptors in leydig cells

Biological effects of TH on the target cells are brought about by binding the hormones to their specific receptors which are localized to nuclear as well as cytoplasmic compartments of the cell (D'Armiento and Jannini, 1992). These receptors basically act as nuclear transcription factors and when bound with the hormone, the hormone-receptor complex stimulates the metabolism, growth and differentiation of the organism by binding to regulatory regions of the responsive genes (Openheimer, 1991). Thyroid hormone receptors are encoded by two different genes α and ß. By alternative splicing of the each of these transcripts, hormone binding α_1, $ß_1$ and $ß_2$ and non-hormone binding α_2 and α_3 isoforms of the receptor are produced (Evans, 1988; Chin, 1991; Lazar, 1993). The distribution of different isoforms of the TH receptors in an animal, appears to be dependant on developmental stage and the tissue (Jannini et al., 1995). Presence (or absence) of TH receptors in the testis has been investigated by using the techniques of evaluating the specific nuclear binding of the hormone, expression of receptor mRNA or localization of receptor protein with the help of specific antibodies. In early studies using isolated nuclei from adult testis no nuclear binding of hormone was observed (Openheimer et al., 1974) leading to the conclusion that testis is an un-responsive organ as far as TH is concerned. Further more, these early studies were also unable to detect mRNAs of α_1, $ß_1$ or $ß_2$ in the adult testis (Murray et al., 1988; Santos et al., 1988; Barsano et al., 1990; Strait et al., 1990). However, a renewed interest on this subject was generated when Palmero and co-workers (Palmero et al., 1988) demonstrated a specific binding of TH to nuclei of Sertoli cells isolated from immature rats. Since then, number of studies have shown an age dependent TH receptor mRNA in the testis

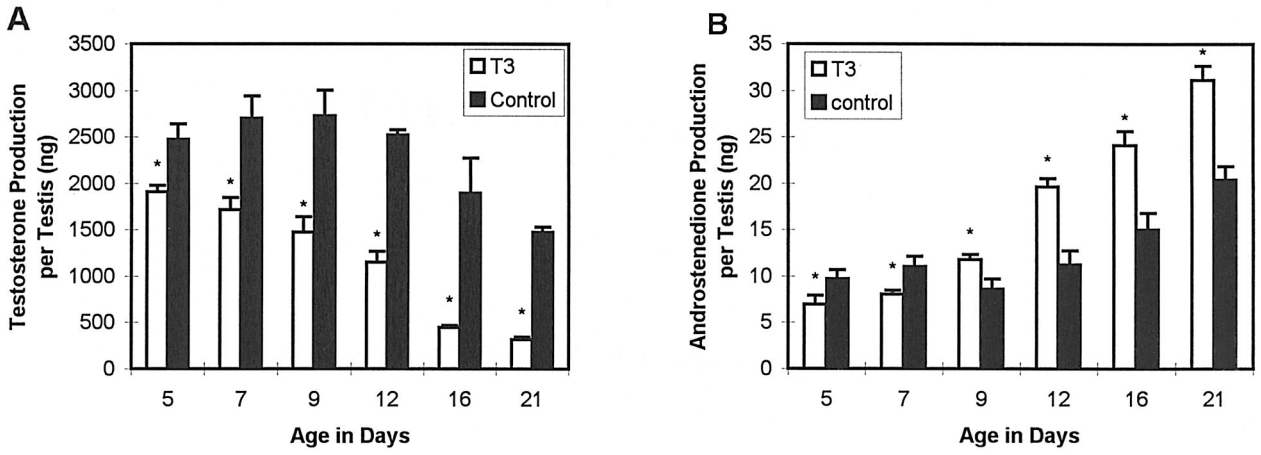

Fig. 9. LH-stimulated testicular testosterone **(A)** and androstenedione **(B)** production *in vitro* by the control and T3-treated rats during the neonatal-prepubertal period. Testicular testosterone production (main source during this period is fetal Leydig cells) is maintained from birth to 16 days in control rats, which shows a decline at day 21. In contrast, in T3-treated rats a concomitant decline in testicular testosterone production *in vitro* is observed with the advancement of age, due to the atrophy of fetal Leydig cells in response to T3-treatment. By contrast, testicular androstenedione production *in vitro* shows a concomitant increase with age in both control and T3-treated rats due to the differentiation of adult type Leydig cells; T3-treated rats had significantly greater values than the control group from day 9 to 21 due to increased numbers of newly differentiated adult type Leydig cells. (used with permission from the publisher, Ariyaratne et al., 2000, Biol. Reprod. 63, 493-502).

(Jannini et al., 1990, 1994; Bunick et al., 1994); binding of the hormone and expression of the mRNA was highest in fetal testis, gradually declined during prepubertal age and totally absent in adult testis. No expression of ß receptor mRNA was detected in any of these studies. In contrast, recent studies were able to detect considerable amounts of receptor α1 mRNA even in adult testis (Buzzard et al., 2000, Jannini et al., 2000; Canale et al., 2001).

The question of the presence or absence of TH receptors in Leydig cells and/or their precursor cell types remains unsolved as the available reports have discrepancies. Until recent, Leydig cell was not even considered as a target cell for TH because many studies reported that specific binding of the hormone or expression of receptor mRNA is not seen in this cell type (Santos et al., 1988; Jannini et al., 1990, 1994, 1995; Palmero et al., 1992). However, TH receptor protein has been localized to testicular interstitium in several previous studies (Lue et al., 1988; Macchia et al., 1990; Tagami et al., 1990). Recent studies using purified Leydig cells and their precursors from rats at different age groups, the presence of TRH α1 mRNA (but not the protein) has been shown in Leydig cells and their precursors, even in the adult animals (Hardy et al., 1996). However, it is not yet clear whether the T3 receptor proteins are expressed in Leydig cells. According to Palmero et al (1992), T3 receptors are absent in nuclei of immamture pig Leydig cells. By contrast, Tagami et al. (1990) demonstrated the presence of nuclear T3 receptors in rat Leydig cells using immunocytochemistry. Presence of TH receptor protein in a subset of interstitial cells in rats has also been reported by Buzzard et al. (2000) using immunolabeling as well. However, the latter study was focused on T3 receptors in seminiferous tubules and therefore, no detailed information is available in this study on the testicular interstitial cell types that are positive for T3 receptors. Hence, the spatial and temporal expression of TH receptors in the adult Leydig cell and the cells types of its lineage remains to be established in future investigations.

Thyrotropin releasing hormone in Leydig cells

Thyrotropin-releasing hormone (TRH) is a hypothalamic tripeptide-releasing factor that stimulates thyrotropin (TSH) synthesis and secretion by the thyrotrophs of the anterior pituitary gland (Schally et al., 1969; Burgus et al., 1970); TSH stimulates the thyroid gland to synthesize and secrete T3 and T4. TRH plays the central regulatory role in the hypothalamic-pituitary-thyroid axis. Therefore, it is interesting to note that TRH, TRH mRNA and TRH receptor (TRH-R) gene expression has been observed in Leydig cells in many species including human (Wilber and Xu, 1998), rat (Fang et al., 1993; Satoh et al., 1994; Liu, et al., 2001; Li et al., 2002), mouse (Zhang et al., 1995), bull (Zraly et al., 1997) and Siberian hamster (Rao et al., 1997). In humans (Wilber and Xu, 1998), and rats (Liu et al., 2001). TRH and TRH-R expression in the testis is exclusively seen in Leydig cells. Suppression of circulating TRH to non-detectable levels in bulls by oral administration of nitrates cause suppression of Leydig cell steroidogenic function in bulls (Zraly et al., 1997). Moreover, developmental studies in rats have shown that TRH mRNA expression in the rat testis is development dependent; the earliest detection was at postnatal day 15, and the signal was increased progressively on days 20, 35, 60 and 90 (Liu et al., 2001). Although the precise role of TRH in Leydig cells is not clear at present, some investigators suggest that TRH may function as paracrine (Fang et al., 1993; Wilber and Xu, 1998;) or autocrine (Fang et al., 1993; Satoh et al., 1994; Liu et al., 2001) regulator of testicular function. One such paracrine role of TRH is thought to be to serve as an inhibitory modulator of gonadotropin-stimulated testosterone secretion (Wilber and Xu, 1998). All of the above findings suggest that the presence of TRH in Leydig cells have a significant role in the testis. Whether hypothalamo-pituitary-thyroid axis and hypothalamo-pituitary-testis axis are short-looped through Leydig cells is a novel concept that needs to be tested in future investigations.

References

Antony F.F., Aruldhas, M.M., Udhayakumar R.C., Maran R.R. and Govindaraju P. (1995). Inhibition of Leydig cell activity in-vivo and in-vitro in hypothyroid rats. J. Endocrinol. 144, 293-300.

Ariyaratne H.B.S., Mason J.I. and Mendis-Handagama S.L.M.C. (2000a). Effects of thyroid and luteinizing hormone on the onset of precursor cell differentiation into Leydig progenitor cells in the prepubertal rat testis. Biol. Reprod. 63, 898-904.

Ariyaratne H.B.S., Mason J.I. and Mendis-Handagama S.L.M.C. (2000b). Effects of thyroid hormone on Leydig cell regeneration in the adult rat following ethane dimethane sulphonate treatment. Biol. Reprod. 63,1115-1123.

Ariyaratne H.B.S., Mason J.I. and Mendis-Handagama S.L.M.C. (2000c). Effects of triiodothyronine on testicular interstitial cells and androgen secretory capacity of the prepubertal rat. Biol. Reprod. 63,493-502.

Ariyaratne H.B.S., Mendis-Handagama S.M.L.C, Hales D.B. and Mason J.I. (2000d).Studies on the onset of Leydig precursor cell differentiation in the prepubertal rat testis. Biol. Reprod. 63,165-171.

Baillie A.H. (1964). Further observations on the growth and histochemistry of Leydig tissue in the postnatal prepubertal mouse testis. J. Anat. 98, 403-429.

Barsano C.P., Iqbal Z., Pullen G.L. Munoz E. and Singh S.P. (1990). Tissue-specific differences in the compartmentalization of rat nuclear triiodothyronine receptors. Acta Endocrinol. (Copenh) 122,181-190.

Bethea C.L. and Walker R.F. (1979). Age-related changes in reproductive hormones and in Leydig cell responsivity in the male Fisher 344 rat. I. Gerontology 34, 21-27.

Bunick D., Kirby J., Hess R.A. and Cooke P.S. (1994). Developmental expression of testis messenger ribonucleic acids in the rat following propylthiouracil-induced neonatal hypothyroidism. Biol. Reprod. 51,706-713.

Burgus R., Dunn T.F., Desiderio D., Ward D.N., Vale W. and Guillemin R. (1970). Charactersization of ovine hypothalamic hypophysiotropic TSH-releasing factor. Nature 226, 321-325.

Buzzard J.J., Morrison J.R., O'Bryan M.K., Song Q. and Wreford NG (2000). Developmental expression pf thytoid hormone receptors in the rat testis. Biol. Reprod. 62, 664-669.

Byscove A.G. (1986). Differentiation of mammalian embryonic gonad. Physiol. Rev. 66,77-112.

Canale D., Agostini M., Giorgilli G., Caglieresi C., Scartabelli G., Nordini V., Jannini E.A., Martino E., Pinchera A. and Macchia E. (2001). Thyroid hormone receptors in neonatal, prepubertal and adult rat testis. J. Androl. 22, 284-288.

Chemes H.E. (1996). Leydig cell development in humans. In: The Leydig Cell. Payne A.H., Hardy M.P. and Russell L.D. (eds). Cache River Press. Vienna, IL. pp 175-202.

Chandrasekhar Y., D'Occhio M.J. and Setchell B.P. (1986). Reproductive hormone secretion and spermatogenic function in thyrodectomized rams receiving graded doses of exogenous thyroxine. J. Endocrinol. 111, 245-253.

Cheek A.O., Kow K., Chen J. and McLachlan J.A. (1999). Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid biding globulin. Environ. Health Perspect. 107, 273-278.

Chin W.W. (1991). Nuclear thyroid hormone receptors. In: Nuclear hormone receptors. Parker M.G. (ed). Academic Press. New York. pp 79-102.

Christensen A.K. (1996). The history of studies on Leydig cells: the first century. In: The Leydig Cell. Payne A.H., Hardy M.P. and Russell L.D. (eds). Cache River Press. Vienna, IL. pp 1-30.

Cizza G., Brady L.S., Calogero A.E., Bagdy G., Lynn A.B., Kling M.A., Blackman M.R.. Chrousos G.P. and Gold P.W. (1992). Central hypothyroidism is associated with advanced age in male Fischer 344/N rats: *in vivo* and *in vitro* studies. Endocrinology 131, 2672-2680.

Cizza G., Brady L.S., Pascak K., Blackman M.R., Gold P.W. and Chrousos G.P. (1995). Stress-induced inhibition of the hypothalamic-pituitary-thyroid axis is attenuated in the aged Fischer 344/N male rat. Neuroendocrinology 62, 506-513.

Collins W.T. (1980). Fine structural lesions and hormonal alterations in thyroid glands of perinatal rats exposed in utero and by the milk to polychlorinated biphenyls. Am. J. Pathol. 99, 125-142.

Cooke P.S., Zhao Y.D. and Hansen L.G. (1996). Neonatal polycholrinated biphenyl treatment increases adult testis size and sperm production in the rat. Toxicol. Appl. Pharmacol. 136, 112-117.

Cristovao F.C., Bisi H., Mendonca B.B., Bianco A.C. and Bloise W. (2002). Severe and mild neonatal hypothyroidism mediate opposite effects on Leydig cells of rats. Thyroid 12, 13-18.

D'Armiento M. and Jannini E.A. (1992). Thyroid hormone action. In: Thyroid disease: Basic science, pathology, clinical and laboratory diagnoses. Troncone L., Shapiro B., Satta M.A. and Monaco F. (eds). CRC Press. Boca Raton, FL. pp 145-154

Desaulniers D., Leingartner K., Wade M., Fintelman E., Yagminas A. and Foster W.G. (1999). Effects of acute exposure to PCBs 126 and 153 on anterior pituitary and thyroid hormones and FSH isoforms in adult Sprague Dawley male rats. Toxicol. Sci. 47, 158-169.

Evans R.M. (1988). The steroid and thyroid hormone receptor super-family. Science 240, 889-895.

Fawcett D.W., Neaves W.B. and Flores M.N. (1973). Comparative observations on intertubular lymphatics an the organization of the interstitial tissue of the mammalian testis. Biol. Reprod. 9, 500-532.

Fang P., Gu J., Kim U.J., Carnell N.E. and Wilber J.F. (1993). Identification, localization and developmental studies of rat prepro thyrotropin-releasing hormone mRNA in the testis. Neuropeptides 24, 63-69.

Foldes J., Banos C., Feher T., Bodrogi L., Szalay F., Borvendeg J., Csillag J. and Tako J. (1982). Functional relationships of the hypothalamic-pituitary-testicular system in Grave's disease. Act. Med. Acad. Sci. Hung. 39, 109-116.

Gudernatsch J.F. (1912). Feeding experiments on tadpoles. 1. The influence of specific organs given as food on growth and differentiation. A contribution to the knowledge of organs with internal secretion. Wilhelm Roux Arch. Entwicklungsmech. Organismen 35, 457-483.

Haider S.G., Lau D., Schowchau G. and Hilscher B. (1995). Morphological studies on the origin of adult-type Leydig cells in the rat testis. Int. J. Anat. Embryol. 100, 535-541.

Hardy M.P., Sharma R.S., Arambepola N.K., Sottas C.M., Russell L.D. Bunick D., Hess R.A. and Cooke P.S. (1996). Increased proliferation of Leydig cells induced by neonatal hypothyroidism in the rat. J. Androl. 17, 231-237.

Hoffman W.H., Kovacs K.T., Gala R.R., Keel B.A., Jarrell T.S., Ellegood J.O. and Burec C.L. (1991). Macroorchidism and testicular fibrosis associated with autoimmune thyroiditis. J. Endocrinol. Invest. 14, 609-616.

Hollander N. and Hollander V.P. (1958). The microdetermination of testosterone in human spermatic vein blood. J. Clin. Endocrinol. 18, 966-971.

Ichihara I., Kawamura H. and Pelliniemi L.J. (1993). Ultrastructure and morphometry of testicular Leydig cells and the interstitial components correlated with testosterone in aging rats. Cell Tissue Res. 271,241-255.

Jana N.R., Halder S. and Bhattacharya S. (1996). Thyroid hormone induces a 52 kDa soluble protein in goat testis Leydig cell which stimulates androgen release. Biochim. Biophys. Acta 1292, 209-214.

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

Jannini E.A., Dolci S., Ulisse S. and Nicodem V.M. (1994). Developmental regulation of the thyroid hormone receptor α1 mRNA expression in the rat testis. Mole. Endocrinol. 8, 89-96.

Jannini E.A., Ulisse S. and D'Armiento M. (1995). Thyroid hormone and male gonadal function. Edocr. Rev. 16, 443-459.

Jannini E.A., Crescenzi A., Rucci N., Screponi E., Carosa E., de Matteis A., Macchia E., d'Amati G. and D'Armiento M. (2000). Ontogenic pattern of thyroid hormone receptor expression in the human testis. J. Clin. Endocrinol. Metab. 85, 3453-3457.

Josso N. (1986). Anti-Mullerian hormone: new perspective for sexist molecule. Endocr. Rev. 4, 421-433.

Kato Y., Haraguchi K., Shibahara T., Masuda Y. and Kimura R. (1998). Reduction of thyroid hormone levels by methylsulfonyl metabolites of polychlorinated biphenyl congeners in rats. Arch. Toxicol. 72, 541-544.

Kato Y., Haraguchi K., Shibahara T., Yumoto S., Masuda Y., Kimura R. (1999). Reduction of thyroid hormone levels by methylsulfonyl metabolites of of tetra- and pentachlorinated biphenyls in male Sprague Dawley rats. Toxicol. Sci. 48, 51-54.

Kim I., Ariyaratne H.B.S. and Mendis-Handagama S.L.M.C. (2000).

Effects of continuous and intermittent exposure of lactating mothers to Aroclor 1242 on testicular steroidogenic function in the adult offspring. Tissue Cell 33, 169-177.

Kim I., Ariyaratne H.B.S. and Mendis-Handagama S.L.M.C. (2002). Changes in the testis interstitium of Brown Norway rats with aging and effects of luteinizing and thyroid hormones on the aged testes in enhancing the steroidogenic potential. Biol. Reprod. 66,1359-1366.

Kirschner M.A. and Coffman G.D. (1968). Measurement of plasma testosterone and Δ^4-androstenedione using electron capture gas-liquid chromatography. J. Clin. Invest. 47, 38-47.

Kumar P.N., Aruldhas M.M. and Juneja S.C. (1996). Influence of hyperthyroidism induced at puberty on the epididymal lipids, number and motility of spermatozoa in rats. Reprod. Fertil. Dev. 8, 373-378.

Langer P., Tajtakova M., Fodor G., Kocan A., Bohov P., Michalek J. and Kreze A. (1998). Increased thyroid volume and prevalence of thyroid disorders in an area heavily polluted by polychlorinated biphenyls. Eur. J. Endocrinol. 139, 402-409.

Lazar M.A. (1993). Thyroid hormone receptors: multiple forms, multiple possibilities. Endocr. Rev. 14, 184-193.

Lee M.M. and Donohoe P.K. (1993). Mullerian inhibiting substance: a gonadal hormone with multiple functions. Endocr. Rev. 14, 152-164.

Leydig F. (1850). Zur Anatomie der mannlichen Geschlechtsorgane und Analdrusen der Saugetgiere. Z. Wiss. Zool. 2, 1-57.

Li Z., Zhang Y., Liu X. and Xu R. (2002). Correlation of expression of preprothyrotropin-releasing hormone and receptor with rat testis development. Chin. Med. J. (Engl). 115, 12-16.

Liu X.P., Li Z., Zhang Y.Q. and Xu R.J. (2001). Expression of thyrotropin-releasing hormone receptor mRNA in the rat testis development. Shi Yan Sheng Wu Xue Bao 34,109-114

Lording D.W. and de Kretser D.M. (1972). Comparative ultrastructural and histochemical studies of the interstitial cells of the rat testis during fetal and postnatal development. J. Reprod. Fert. 29, 261-269.

Lue M., Faure R., Ruel J. and Dussault J.H. (1988). A monoclonal antibody to the rat nuclear triiodothyronine receptor: production and characterization. Endocrinology 123,180-186.

Macchia E., Nakai A., Janiga A., Sakurai A., Fisfalen M.E., Gardner P., Soltani K. and DeGroot L. (1990). Characterization of site-specific poly-clonal antibodies to c-erbA peptides recognizing human thyroid hormone receptors α1, α2 and ß and native 3,5,3'-triiodothyronine receptor and study of tissue distribution of the antigen. Endocrinology 126, 3232-3239.

Mancini R.E., Vilar O., Lavieri J.C., Andrada J.A. and Heinrich J.J. (1963). Development of Leydig cells in the normal human testis. A cytological, cytochemical and quantitative study. Am. J. Anat. 112, 203-214.

Manna P.R., Tena-Sempere M. and Huhtaniemi I.T. (1999). Molecular mechanisms of thyroid hormone stimulated steroidogenesis in mouse Leydig tumor cells. J. Biol. Chem. 274, 5909-5918.

Manna P.R., Roy P., Clark B.J., Stocco D.M. and Huhtaniemi I.T. (2001a). Interaction of thyroid hormone and steroidogenic acute regulatory (StAR) protein in the regulation of murine Leydig cell steroidogenesis. J. Steroid Biochem. Mol. Biol. 76,167-177.

Manna P.R., Kero J., Tena-Sempere M., Pakarinan P., Stocco D.M. and Huhtaniemi I.T. (2001b). Assessment of mechanisms of thyroid hormone action in mouse Leydig cells: Regulation of the steroidogenic acute regulatory protein, steroidogenesis and luteinizing hormone receptor function. Endocrinology 14, 319-331.

Maran R.R., Arunakaran J., Jayaraj D.A., Ravichandran K., Ravisankar B. and Aruldhas M.M. (2000a). Transient neonatal hypothyroidism alters plasma and testicular sex steroid concentration in puberal rats. Endocr. Res. 26, 411-429.

Maran R.R., Arunakaran J., Aruldhas M.M. (2000b). T3 directly stimulates basal and modulates LH induced testosterone and oestradiol production by rat Leydig cells in-vitro. Endocr. J. 4, 417-428.

Maran R.R., Ravichandran K., Arunakaran, J. and Aruldhas M.M. (2001). Impact of neonatal hypothyroidism on Leydig cell number, plasma and testicular interstitial fluid sex steroids concentration. Endocr. Res. 27, 119-141.

Mendis-Handagama S.L.M.C. and Ariyaratne H.B.S. (2001). Differentiation of the adult Leydig cell population in the postnatal testis. Biol. Reprod. 65, 660-671.

Mendis-Handagama S.L.M.C. and Gelber S.J.(1995). Signs of aging are apparent in the testis interstitium of Sprague Dawley rats at 6 months of age. Tissue Cell 27, 689-699.

Mendis-Handagama S.L.M.C. and Sharma O.P. (1994). Effects of neonatal administration of the reversible goitrogen propylthiouracil on the testis interstitium in adult rats. J. Reprod. Fert. 100, 85-92.

Mendis-Handagama S.L.M.C., Risbridger G.P. and de Kreter D.M. (1987). Morphometric analysis of the components of the neonatal and adult rat testis interstitium. Int. J. Androl. 10, 525-534.

Mendis-Handagama S.L.M.C., Ariyaratne H.B.S., Teunissen van Manen K.R., Haupt R.L. (1998). Differentiation of adult Leydig cells in the neonatal rat testis is arrested by hypothyroidism. Biol. Reprod. 59, 351-357.

Mori H., Hiromoto N., Nakahara M. and Shiraishi, T. (1982). Stereological analysis of Leydig cell ultrastructure in aged humans. J. Clin. Endocrinol. Metab. 55, 634-641.

Murray M.B., Zilz N.D., McCreary N.L., MacDonald M.J. and Towle H.C. (1988). Isolation and characterization of rat cDNA clones for two distinct thyroid hormone receptors. J. Biol. Chem. 263,12770-12777.

Nagayama J., Okamura K., Iida T., Hirakawa H., Matsueds T., Tsuji H., Hasegawa M., Sato K., Ma H.Y., Yanagawa,T., Igarashi H., Fukushige J. and Watanabe T. (1998). Postnatal exposure to chlorinated dioxins and related chemicals on thyroid hormone status in Japanese breast-fed infants. Chemosphere 37, 1789-1793.

Ness D.K., Schantz S.L., Moshtaghian J. and Hansen L.G. (1993). Effects of perinatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. Toxicol. Lett. 68, 311-323.

Niemi M. and Kormano M. (1964). Cell renewal in the interstitial tissue of postnatal prepubertal rat testis. Endocrinology 74, 996-998.

Norman A.W. and Litwick G.L. (1997). Thyroid Hormones. In: Hormones. Norman A.W. and Litwick G.L (eds). Academic Press. San Diego. pp 169-191.

Oppenheimer J.H., Schwartz H.L., Surks M.I. (1974). Tissue differences in the concentration of triiodothyronine nuclear binding sites in the rat: liver, kidney, pituitary, heart, brain, spleen and testis. Endocrinology 95, 897-903.

Oppenheimer J.H. (1991). Thyroid hormone action at the molecular level. In: Werner and Ingbar's The Thyroid. ed 6. Braverman L.E. and Utiger R.D. (eds). Lippincott. Philadelphia. pp 204-224.

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

Palmero S., Benahmed M., Morera A.M., Trucchi, P., Fugassa E. (1992). Identification of nuclear tri-iodothyronine receptors in Sertoli

cells from immature piglet testes. J. Mol. Endocrinol. 9, 55-59.

Racine C., Rey R., Forrest M., Louis F., Ferre A., Huhtaniemi I., Josso N. and Clementi N. (1998). Receptors for anti-Mullerian hormone on Leydig cells are responsible for its effects on steroidogenesis and cell differentiation. Proc. Natl. Acad. Sci. USA 95, 594-599.

Rao J.N., Debeljuk L., Bartke A., Gao Y.P., Wilber J.F. and Fang P. (1997). The detection of thyrotropin-releasing hormone (TRH) and TRH receptor gene expression in Siberian hamster testis. Peptides 18, 1217-1222.

Rojdmark S., Berg A. and Kallner G. (1988). Hypothalamic-pituitary-testicular axis in patients with hyperthyroidism. Hom. Res. 29, 185-190.

Roosen-Runge E.C. and Anderson A.D. (1959). The development of the interstitial cells in the testis of albino rat. Acta Anat. 37,125-137.

Russell L.D., de Franca L.R., Hess R. and Cooke P. (1995). Characteristics of mitotic cells in developing and adult rat testes with observations on cell lineage. Tissue Cell 27,105-128.

Saeed A. and Hansen L.G. (1985). Morphometric changes in the prepubertal female rat thyroid gland following acute exposure to 2,2',4,4'-tetrachlorobiphenyl and Aroclor 1242. J. Toxicol. Environ. Health. 51, 503-513.

Santos A., Freake H.C., Rosenburg M.E., Schwartz H.L. and Oppenheimer J.H. (1988). Triiodothyronine nuclear binding capacity in rat tissues correlates with a 6.0 kilobase (kb) and not a 2.6 kb messenger ribonucleic acid hybridization signal generated by a human c-erbA probe. Mol. Endocrinol. 2, 992-998.

Sasaki S., Nakamura H., Tagami T., Osamura Y. and Imura H. (1991). Demonstration of nuclear 3,5,3'-triiodothyronine receptor proteins in gonadotrophs and corticotrophs in rat anterior pituitary by double immunohistochemical staining. Endocrinology 129, 511-516.

Satoh T., Fang P., Kim U.J. and Wilber J.F. (1994). Identification of thyrotropin-releasing hormone receptor in the rat testis. Neuropeptides 27, 195-202.

Schally A.V., Redding T.W., Boweres C.Y. and Barrett J.F. (1969). Isolation of properties of porcine thyrotropin-releasing hormone. J. Biol. Chem. 244, 4077-4088.

Stocco D.M. (1996). Acute regulation of Leydig cell steroidogenesis. In: The Leydig cell. Payne A.H., Hardy M.P. and Russell L.D. (eds). Cache River Press. Vienna, IL. pp 241-258.

Strait K.A., Cshwartz H.L., Perez-Castillo A. and Oppenheimer J.H. (1990). Relationship of c-erb A mRNA content to tissue triiodothyronine nuclear binding capacity and function in developing and adult rats. J. Biol. Chem. 265,10514-10521.

Tagami T., Nakamura H., Sasaki S., Mori T., Yoshioka, H., Yoshida H. and Imura H. (1990). Immunohistochemical localization of nuclear 3,5,3'-triiodothyronine receptor proteins in rat tissues studied with anti-serum against c-erbA/T3 receptor. Endocrinology 127, 1727-1734.

Tahmaz L., Gokalp A., Kibar Y., Kocak I., Yalcin O. and Ozercan Y. (2000). Effect of hypothyroidism on the testes in mature rats and treatment with levothyroxine and zinc. Andrologia 32, 85-89.

Teerds K.J., de Rooij D.G., de Jong F.H. and van Haaster L.H. (1998). Development of the adult Leydig cell population in the rat testis is affected by neonatal thyroid hormone levels. Biol. Reprod. 59, 344-350.

Valenty S., Guido R., Fazzuoli L., Barreca A., Giusti M. and Giordano G. (1997).Decreased steroidogenesis and cAMP production in-vitro by Leydig cells isolated from rats made hypothyroid during adulthood. Int. J. Androl. 20, 279-286.

Vermulen A. (1976). Leydig cell function in old age. In: Hypothalamus, pituitary and aging. Everitte A.V. and Burgess J.A. (eds). Charles C. Thomas. Springfield, IL. pp 458-463.

Wilber J.F. and Xu A.H. (1998). The thyrotropin releasing hormone gene: cloning, characterization and transcriptional regulation in the central nervous system, heart and testis. Thyroid 8, 897-901.

Wolstad S. and Jensen B.M. (1999). Thyroid hormones in grey seal pups (*Halichoerus grypus*). Comp. Biochem. Physiol. A. Mol. Integr-Physiol 122, 157-162.

Wortsman J., Rosner W. and Dufau M.L. (1987). Abnormal testicular function in men with primary hypothyroidism. Am. J. Med. 82, 207-212.

Zhang S.H., Zhang Y.Q. and Vacca-Galloway L.L. (1995). Identification of thyrotropin-releasing hormone receptor mRNA in the Leydig cells of the mouse testis. Neuropeptides 29, 309-313.

Zhou B., Satts L.M. and Hutson J.M. (1993). Germ cell development in neonatal mouse testes in utero requires Mullerian inhibiting substance. J. Urol. 150, 613-616.

Zraly Z., Bendova J., Svecova D., Faldikova L., Veznik Z. and Zajicova A. (1997). Effects of oral intake of nitrates on reproductive function of bulls. Vet. Med. (Praha) 42, 345-354.

Accepted April 5, 2004