

Thyroid and male reproduction

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ABSTRACT

Male reproduction is governed by the classical hypothalamo-hypophyseal testicular axis: Hypothalamic gonadotropin releasing hormone (GnRH), pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH) and the gonadal steroid, principally, testosterone. Thyroid hormones have been shown to exert a modulatory influence on this axis and consequently the sexual and spermatogenic function of man. This review will examine the modulatory influence of thyroid hormones on male reproduction.

Key words: Hyperthyroidism, hypothyroidism, Leydig cells, Sertoli cells, sperm function, Tri-iodothyronine

EXPRESSION OF THYROID HORMONE RECEPTORS ON TESTIS, REPRODUCTIVE TRACT AND ACCESSORY SEX GLANDS

Thyroid hormones exert their biological effects by binding to a specific nuclear thyroid hormone receptor (TR) which belongs to a family of ligand-dependent transcription factors. The receptor is encoded by two genes, c-erb A α and c-erb A β . Alternative splicing of the c-erb A α gene encodes three separate protein receptors α_1 , α_2 and α_3 whereas; the c-erbA β gene encodes β_1 and β_2 receptors.^[3]

Thyroid hormone receptors are widely distributed in the different compartments of the testis. In human fetal and adult Sertoli cells only the TR α_1 and TR α_2 isoforms are expressed; TR α_2 expression being higher at all stages and the TR α_2 /TR α_1 ratio increases progressively from fetal to adult life. The TR β isoform is absent in human Sertoli cells both in the fetal and adult stage.^[4] In rats, the TR α_1 is the predominant isoform expressed both in immature proliferating Sertoli cells and mature adult Sertoli cells.

mRNAs of TR α_2 , TR α_3 and TR β_1 are detected in Sertoli cells during development, but, their corresponding proteins are absent.^[5]

Rat mesenchymal stem cells, immature and adult Leydig cells express the TR α isoform, their expression being maximal in the postnatal age and decreases to almost negligible levels in adulthood. T₃ binds specifically to nuclei of goat Leydig cells and consequently stimulates androgen production from these cells.^[6,7]

The presence of thyroid hormone receptors on germ cells suggests a probable role of thyroid hormones in sustaining different population of germ cells. Thyroid hormone receptors are identified on different stages of developing rat germ cells such as gonocyte, spermatogonia, preleptotene, leptotene, pachytene, zygotene, round and elongating spermatids. Both TR α and TR β_1 are expressed during different stages of germ cell development. TR β_1 first appears in intermediate type spermatogonia while TR α first appear in type B spermatogonia.^[5]

The epithelial cells from the different segments of rat epididymis, caput, corpus and cauda, express thyroid hormone receptors. However, unlike classical TRs, the TRs in the epithelial cells of the epididymis are predominantly located in the cytoplasm. Both the TR α_1 and TR β_1 isoforms are expressed in all three segments of the epididymis. Both the protein as well as mRNA levels of TR isoforms increase significantly in hypothyroid rats.^[8] TR β_1 are also identified

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DOI:
10.4103/2230-8210.126523

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on the nuclear membrane of PZ HPV-10 cell line derived from prostatic tissue.^[9]

TESTICULAR ACTIONS OF THYROID HORMONES

Sertoli cells

Sertoli cells provide support and sustain the developing germ cells. Each Sertoli cell supports a limited number of developing germ cells; the ratio of Sertoli cells to germ cells are 1: 11 in humans and 1:50 in adult rat testis respectively.^[10,11] Therefore, the number of Sertoli cells is one of the indicator of daily sperm production (DSP) of the testis. The development and maturation of Sertoli cells has two different stages; the proliferative stage and the terminally differentiated mature stage. In primates including human, Sertoli cell proliferation occurs over two distinct periods. The first expansion phase is similar to that in rodents. Additionally, there is a second proliferative stage in the peripubertal life before proliferation finally stops. The maturation of immature Sertoli cells to the mature stage is characterized by certain morphological changes which include the nucleus enlarging and becoming tripartite and the nucleolus becoming more prominent, loss of proliferative ability and formation of inter-Sertoli cell junctions.^[12,13]

In Sertoli cells, expression of certain genes and proteins are associated with its maturational status. Anti-Mullerian hormones (AMH), aromatase, neural cell adhesion molecule (NCAM) are expressed exclusively by immature Sertoli cells whereas; p27^{Kip1}, p21^{Cip1}, androgen receptor (AR) are characteristic markers of mature Sertoli cells.^[12]

T₃ suppresses the expression of immature Sertoli cells markers, including AMH, aromatase and NCAM. Hypothyroidism induced in neonatal rats, delayed the fall in AMH mRNA levels and T₃ treatment decreased AMH mRNA in Sertoli cells.^[14] In mouse Sertoli cell line, TM4, T₃ decreased transcription of aromatase gene.^[15] T₃ down-regulates NCAM production in Sertoli cell-germ cell co-cultures.^[16]

T₃ increases the levels of cell cycle inhibitory proteins p27^{Kip1} and p21^{Cip1} in Sprague-Dawley rats. These inhibitory proteins are negative regulators of cyclin-dependent kinases Cdk2 and Cdk4 required for G1 to S transition in the cell cycle.^[17] Additionally, AR mRNA levels in 5- and 20-day-old cultured Sertoli cells were significantly increased by T₃.^[18]

In contrast, T₃ maintains inter-Sertoli cell junctions by regulating the levels of gap junction protein, Connexin. T₃ increased the levels of Connexin 43 (Cx43; the most

abundant gap junction protein) in Sertoli cell cultures.^[19] Two specific inhibitors of Cx43, AGA and oleamide, significantly lowered the T₃ induced Cx43 levels in cultured Sertoli cells.^[19]

Leydig cells

The stem cells for both fetal and adult Leydig cells populations are the mesenchymal cells in the testis interstitium, which are spindle-shaped and non-steroidogenic. While some of the mesenchymal cells differentiate into fetal Leydig cells others retain their undifferentiated characteristics and serve as precursor cells for the adult Leydig cells in the postnatal testis.^[20]

Thyroid hormones regulate Leydig cell development and steroidogenesis. Hardy *et al.*, (1993)^[21] have shown that propylthiouracil (PTU) treated hypothyroid rats showed a significant increase in Leydig cell number when compared to euthyroid controls. However the isolated Leydig cells from these hypothyroid rats had a 50% lower hCG binding sites and exhibited lower steroid producing potential.^[21] They later showed that the increase in adult Leydig cell population was mainly due to proliferation of immature Leydig cells.^[7] In their elegant studies, Ariyaratne *et al.*, (2000)^[22] have shown that thyroid hormone is essential for differentiation of mesenchymal stem cells into Leydig cells in adult rats. Ethane dimethanesulfonate (EDS), a specific destroyer of Leydig cells, completely eliminated Leydig cell population in adult Sprague- Dawley rats by 2 days of treatment. The Leydig cells reappeared by 14 and 21 days in euthyroid and T₃ treated rats respectively. The number of new Leydig cells was double in the T₃ treated rats in comparison to euthyroid controls. In PTU treated hypothyroid rats, Leydig cells did not appear at all. In the rats made hypothyroid by propyl thiouracil (PTU) treatment or thyroidectomy, Leydig cells did not appear at all. Thus, they have concluded that T₃ facilitates the differentiation of mesenchymal stem cells into Leydig cells.^[22]

Thyroid hormones also influence Leydig cell steroidogenesis. Leydig cells synthesize steroid hormones from cholesterol. Leydig cells take up lipoprotein-cholesterol ester from the circulation *via* specific lipoprotein receptors. A small fraction of cholesterol is also *de-novo* synthesized from acetyl CoA in the Leydig cells. Cholesterol derived from either source is esterified by cholesterol acyl transferase and stored in Leydig cells cytoplasmic lipid droplets. Upon LH stimulation, cholesteryl ester is hydrolysed and is released from the lipid droplets. Steroidogenic acute regulatory protein (StAR), a *de novo* synthesized labile protein, catalyzes the translocation of cholesterol from outer to the inner mitochondrial membrane. Steroidogenic factor 1 (SF-1), a 52 KDa orphan nuclear receptor transcription

factor, regulates the transcription of StAR gene. The StAR gene promoter has two conserved regions that govern basal and cAMP-regulated gene expression. SF-1 bind to the distal site on StAR promoter region with high affinity whereas; binding affinity between the proximal site and SF-1 is only moderate. Binding of SF-1 to either of the binding sites enhances basal and cAMP stimulated StAR gene transcription.^[23,24]

In the inner mitochondrial membrane, cholesterol is converted to pregnenolone catalyzed by cytochrome P450 side-chain cleavage enzyme (cyt P450_{sc} enzyme) using nicotinamide adenine dinucleotide phosphate-oxidase NADPH as a cofactor. Pregnenolone then diffuses out to cytoplasmic endoplasmic reticulum where remaining steps of testosterone biosynthesis are carried out. The conversion of pregnenolone to testosterone occurs *via* two distinct pathways, $\Delta 4$ and $\Delta 5$ pathway as shown in Figure 1.

Jana and Bhattacharya (1994)^[7] have shown that T₃ stimulates testosterone production by goat Leydig cells in a dose dependent manner. They have later shown that T₃ induces *de novo* synthesis of a 52 KDa soluble protein, which augments the androgen production in the Leydig cells.^[25] Similarly, T₃ increased testosterone production by the rat and mouse Leydig cells *in vitro*,^[26,27] and its precursor, progesterone, by a Leydig tumor cell derived line, MLTC-1 by about 300%.^[27] T₃ treatment for 8h increased StAR mRNA in MLTC-1 and mouse Leydig cells.^[27] However, chronic stimulation of MLTC-1 cells with T₃ beyond 8h and uptill 30h decreased StAR mRNA and protein levels and also the steroid production. T₃ does not alter StAR mRNA stability but decreases its transcription. The reduction in progesterone production upon T₃ exposure (30h) was partially restored by 22R hydroxycholesterol (lipid soluble side-chain oxygenated sterol which can directly diffuse

from the outer mitochondrial to the inner mitochondrial membrane without the need for StAR) or pregnenolone. These results confirm that the site of steroidogenic inhibition by T₃ is the StAR protein. T₃ treatment for 30h shows a significant increase in P450_{sc} enzyme responsible for catalyzing the conversion of cholesterol to pregnenolone, but also a decrease in pregnenolone metabolizing enzyme 3 β -HSD mRNA. Thus, a chronic exposure of the cells to T₃ inhibits StAR mRNA, StAR protein and pregnenolone to progesterone converting enzyme 3 β -HSD.^[28]

T₃ also increases hCG binding to the MLTC-1 cells; the binding peaks at 16h and thereafter the binding decreases in a time dependent manner. In hypothyroid mice, there was a significant increase in LHR-mRNA and hCG binding in the Leydig cells. In contrast, in hyperthyroid mice, LHR-mRNA and hCG binding decreases. Manna *et al.*, (2001)^[28] showed that action of T₃ is mediated *via* a 173-bp fragment on the Luteinizing hormone receptor LHR gene promoter region. Mutation studies showed that the SF-1 binding region on the mouse StAR promoter region is involved in T₃ response.^[28]

SPERMS AND FERTILITY

Sperm count

Hypothyroidism induced in rats by PTU treatment during the critical period of the first postnatal week resulted in a significant increase in testis weight, DSP and efficiency of sperm production.^[29] The rise in sperm production could be attributed to different causes such as (1) a rise in gonadotropins, LH and FSH, which are important for spermatogenesis and germ cell survival (2) increase in Sertoli cell number and (3) decrease in germ cell apoptosis. Serum gonadotropins are reduced in PTU treated hypothyroid rats eliminating the fact that the rise in sperm production was due to increase in LH and FSH levels.^[30] The hypothyroid rats have significantly higher number of Sertoli cells which in turn supports the recruitment and survival of greater number of germ cells.^[31] In rats large number of germ cells undergoes apoptosis during the third postnatal week; this period is characterized by a significant rise in pro-apoptotic proteins of both the intrinsic and extrinsic apoptotic pathways. Silva *et al.*, (2011)^[32] studied whether the apoptosis of germ cells is due to an intrinsic property of germ cell or whether it is dependent on Sertoli cell. In rats made hypothyroid by PTU treatment there was a delay in differentiation of immature Sertoli cells to the mature state as was evident by delayed expression of clusterin, a marker of differentiated Sertoli cell. In these hypothyroid rats the delay in Sertoli cell differentiation resulted in (1) delay in differentiation of spermatocyte to the more matured germ cell stage and (2) delay in germ cell apoptosis; maximum

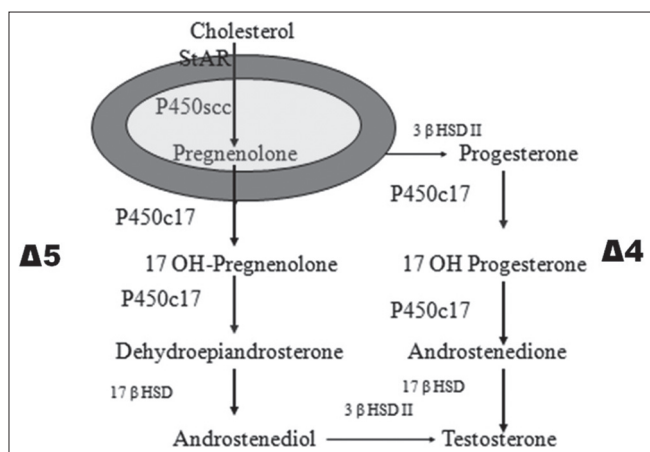


Figure 1: Schematic representation of Leydig cell steroidogenesis

apoptosis seen on day 45 as compared to day 25. Thus, even though thyroid hormones does not act directly on germ cell apoptosis but it delays Sertoli cell maturation which results in delayed germ cell apoptosis.^[32]

Sperm morphology

Morphological abnormalities in the development or proper shaping of sperm head may result in deformed heads and greatly reduces its potential to fertilize a mature oocyte. Morphological deformities in the tail region result due to defects in different parts of the tail. Both hyperthyroid and hypothyroid men had lower proportion of morphologically normal sperm.^[33,34] Thyroid hormones exert their effect on cell cytoskeleton. Zamoner *et al.*, (2008)^[35] reported that in Sertoli cells of hypothyroid rats, the phosphorylation and the immunoreactivity of cytoskeleton-associated vimentin protein was increased without any change in its expression. This results in loss of Sertoli cell cytoskeleton integrity. The high proportion of morphologically abnormal sperm observed in altered thyroid state could be due to effect of thyroid hormones on sperm cytoskeleton.

Motility

Hypothyroid and hyperthyroid patients showed a decrease in progressive forward motility of the sperm.^[36-38] Thyroid hormones increases basal metabolic rate and stimulate oxygen consumption in metabolically active cells. Thyroid hormones stimulate cellular oxygen consumption by promoting the action of Na^+/K^+ ATPases,^[39] increasing mitochondrial number and mitochondrial gene expression.^[3] The role of T_3 on ATP generation in males is speculated. However, specific studies on the modulatory effect of T_3 on sperm ATPases and energy production needs to be studied.

Fertility

Gestational exposure of rats to methimazole (MMI) resulted in a reduction in testicular interstitial fluid (TFI) levels of testosterone and estradiol. The hypothyroid rats showed reduced pregnancy induction capacity when mated with normal females and the litter size was also substantially reduced. Additionally, the ratio of male to female pups was also reduced significantly.^[40]

THYROID HORMONES AND REPRODUCTIVE TRACT AND SEX-ACCESSORY GLANDS

Thyroid hormones influence both epididymal structure and its secretory activity. Gestational exposure of rats to MMI resulted in reduced epididymal weight but sperm content in cauda and corpus epididymis remained unchanged. The caudal sperm forward motility decreased significantly. The epididymal secretions such as sialic acid, glycerophosphotylcholine and carnitine were also reduced

in these hypothyroid rats. Gestational MMI exposure also decreased 5 α -reductase and AR levels.^[40]

Thyroid hormones regulate the contractile activity of vas deferens in response to prostaglandin E_2 (PGE_2). Removal of thyroid glands in albino rats completely inhibited the contractile activity of vas deferens in response to PGE_2 . But treatment of T_4 further increased the contractibility of vas deferens in response to PGE_2 .^[41]

In PTU induced hypothyroid rats there was a significant fall in their seminal vesicle and prostate gland weight.^[42] Gestational MMI exposure to rats decreased AR mRNA levels in dorsolateral prostate lobe but surprisingly increased AR mRNA levels in ventral lobe in pups.^[43] Maran *et al.* (1998)^[44] reported a stimulatory effect of thyroid hormones on rat prostatic glycosidase activities such as β -glucosidase, β -galactosidase, β -N-acetylglucosaminidase and β -N-acetylgalactosaminidase while opposite effects were reported in thyroidectomized rats.^[44] Thyroid hormones regulate glycoprotein metabolism differently in the different prostatic lobes. T_3 decreased hexosamines and sialic acid concentrations in ventral prostatic tissues of 30 and 60 day old thyroidectomized rats. Fucose concentrations in the ventral prostatic tissues increased at 30 days but decreased at 60 days in these hypothyroid rats.^[44] In the dorsolateral prostatic lobe, hypothyroidism enhanced the concentration of hexosamines but resulted in a decrease in fucose, sialic acid and fructose levels irrespective of the duration of hypothyroidism. In the anterior prostate, hypothyroidism decreased fucose, fructose and hexosamines levels and increased sialic acid concentration in 60-day old rats.^[44] Thyroid hormones influence the risk of prostate cancer through their function in cell differentiation, growth and metabolism. Hypothyroid men showed a decreased risk of prostate cancer when compared to euthyroid men, although no associations between hyperthyroidism and risk of prostate cancer could be established.^[45]

THYROID HORMONES AND OXIDATIVE STRESS

Reactive oxygen species (ROS) are highly chemically reactive reduced forms of oxygen and their products with other molecules. All ROS including superoxide radical, hydroxyl radical, hydrogen peroxide contain one or more unpaired electrons. Mitochondria are the primary biological source of ROS. Under physiological conditions ROS can oxidize a number of biological molecules such as unsaturated fatty acids, sulphhydryl proteins and nucleic acids.^[46] To counteract the effects of ROS, cells produce many anti-oxidant molecules such as superoxide dismutase (SOD), glutathione

peroxidase (GSHPx), catalase (CAT), tocopherols, carotenoids. Oxidative stress results when ROS levels are much higher than anti-oxidant levels in the cells.^[46]

In the semen, sperm and some leukocytes contaminating the seminal plasma are the principal source of ROS. The sperm plasma membrane has a high amount of polyunsaturated fatty acids such as docosahexaenoic acid, which are rapidly oxidized by ROS thereby decreasing the flexibility and motility of the sperm tail.^[47] ROS also decreases ATP generated by sperm mitochondrial sheath which provides energy for sperm motility.^[48] Teratozoospermic sperm in most cases retain excess residual body which is normally lost during sperm maturation. These residual bodies has large amount of glucose-6-phosphate dehydrogenase which generates NADPH. NADPH produces ROS catalyzed by the NADPH oxidase enzyme present in sperm plasma membrane.^[49]

Thyroid hormones are important in maintaining the balance between ROS and anti-oxidant molecules in many tissues including testis. Zamoner *et al.*, (2008)^[35] showed that the oxygen consumption of testis in the new-born hypothyroid rats was significantly lower than the euthyroid controls but there was no difference in their lipid peroxide levels. The anti-oxidant SOD activity was significantly lowered in hypothyroid group in comparison to controls. These observations suggest that although ROS levels do not increase but reduced anti-oxidant activity could lead to oxidative stress in a hypothyroid state.^[35] In PTU treated rats hydrogen peroxide and

protein carbonyl content level in the crude homogenate of testis increased but the endogenous lipid peroxide levels remain unaltered. There was a fall in SOD and catalase activity in hypothyroid rats but on addition of T₃, only the catalase activity was enhanced without any change in SOD activity.^[42]

REPRODUCTIVE DYSFUNCTIONS IN THYROID DISORDERS

Thyroid disorders are mainly categorized into two groups; hypothyroidism and hyperthyroidism. As explained earlier thyroid hormones are important regulator of male reproductive function so any alterations in their serum levels have profound effects on male reproduction.

HYPOTHYROIDISM

Reproductive endocrine profile

Several studies have shown a fall in circulating testosterone levels in hypothyroid patients^[50,51] whereas; Velazquez and Arata (1997)^[52] found no change in free testosterone levels in hypothyroid men. Jaya Kumar (1990)^[50] found a rise in serum LH and FSH levels, but Velazquez and Arata (1997)^[52] found a rise in only FSH levels without any change in LH levels. Donnelly and White, (2000)^[51] found no change in serum LH and FSH levels in hypothyroid men. Thyroid hormones also alter pituitary response to GnRH. Administration of GnRH to hypothyroid patients resulted in an attenuated LH response.^[53]

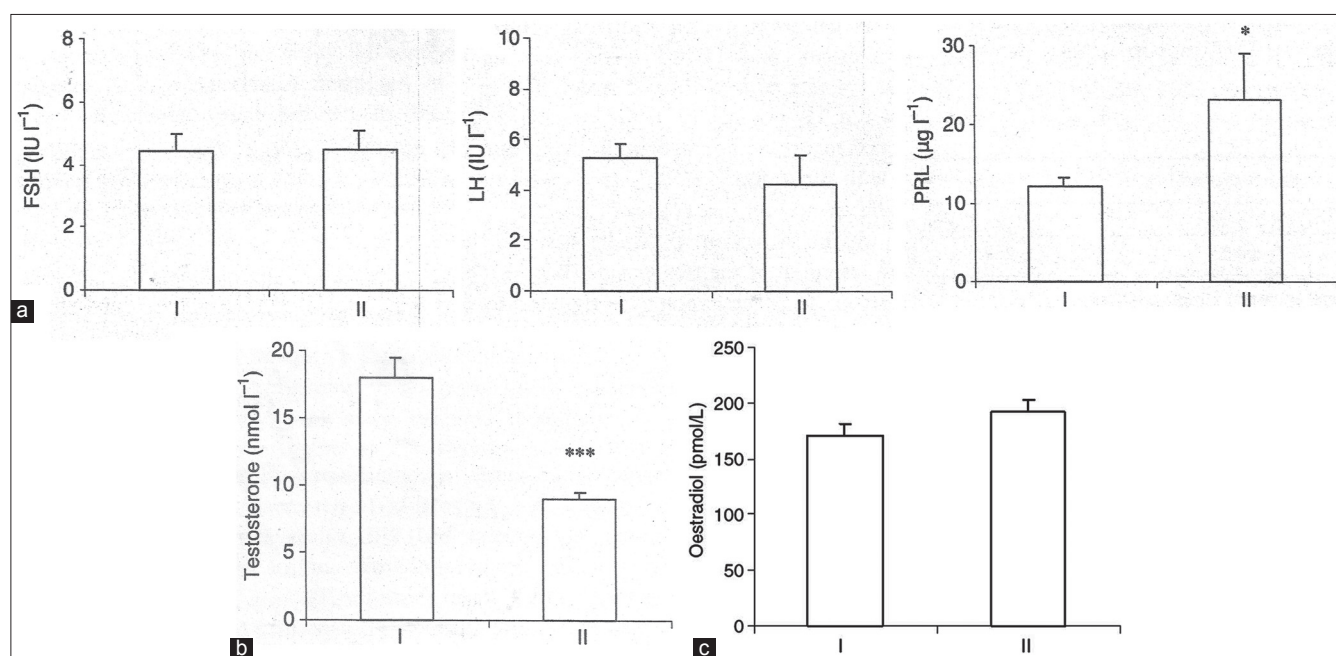


Figure 2: (a-c) Serum hormone levels in hypothyroid men. Bar I and II represent mean \pm SEM of normal ($n=22$) and hypothyroid patients ($n=12$) (adapted from Kumar *et al*, 2007, Andrologia)

In hypothyroid males we found a significant decrease in gonadal steroids – progesterone and total testosterone. Bioavailable testosterone (Bio T) or physiologically available testosterone, calculated by Morris's formula,^[54] was also reduced in hypothyroid men [Figure 2]. Morris *et al.*, (2004)^[54] measured serum total T and sex hormone binding globulin (SHBG) by ELISA, Bio T by Tremblay and Dube's method (1974),^[55] calculated percent free T, and free T by Nanjee's formula (1985)^[56] and Vermeulen's computer program (1999)^[57] respectively. On the basis of above calculations, they developed and validated an equation for the calculation of Bio T. They concluded that total T is the best predictor of Bio T. The fall in testosterone levels could be due to 1) low cholesterol uptake by Leydig cells as evident from high serum cholesterol levels 2) lower conversion of progesterone to testosterone as suggested by low testosterone/progesterone ratio 3) higher conversion of estradiol to testosterone as suggested by high estradiol to testosterone ratio and 4) hyperprolactinemia. High prolactin suppresses 17 α -hydroxylase enzyme in rat testicular cells which catalyzes the conversion of progesterone to 17 α -hydroxy progesterone.^[58] Though serum testosterone and Bio T levels were low in hypothyroid men we reported a normal levels of gonadotropins. This raised a question about the role of testosterone on the feedback inhibition of pituitary gonadotropin release. In our studies serum levels of estradiol were not different between euthyroid and hypothyroid men, suggesting the primary role of estradiol in the feedback regulation of pituitary gonadotropin secretion.^[59,60] Our suggestions about estradiol being the prime regulator of negative feedback on pituitary levels of gonadotropins instead of testosterone are corroborated by the findings of Rochira *et al.*, Their study showed that basal and GnRH stimulated LH and FSH secretion was higher than normal in aromatase-deficient men with normal testosterone levels. However, estrogen administration to these aromatase deficient men resulted in a decrease in pulse amplitude and frequency of LH and pulse amplitude of FSH establishing the role of estrogens on gonadotropin secretion in adult male with aromatase deficiency.^[61,62]

Cretinism

A study on Chinese cretins revealed some interesting facts about their endocrine profile and sexual function. Clinically these patients showed symptoms of both myxoedematous and neurological cretinism. The majority of cretins (65%) had normal TSH levels; however, 35% was hypothyroid with very high TSH levels (mean value 180mIU/L). The hypothyroid cretins showed a significant rise in serum LH, FSH and prolactin levels. 13% of euthyroid cretins and 39% of hypothyroid cretins showed testicular volume lower than 10ml suggesting hypogonadism.^[63]

Role of TSH vs thyroid hormones

Hypothyroid men had high TSH levels and low thyroid hormone levels confounding the exact mechanism of inhibition of testicular steroidogenesis - whether it is low levels of T₃ or raised TSH. To address this issue we studied subclinically hypothyroid men with TSH levels lower than 10mIU/L. These patients showed an endocrine profile similar to frankly hypothyroid males. They showed a significant fall in levels of serum testosterone and progesterone, rise in serum prolactin levels but no significant change in pituitary gonadotropin and estradiol levels. As only TSH is raised in subclinical hypothyroid patients without any change in thyroid hormones this could suggest a possible role of TSH in regulating gonadal steroidogenesis in males. However, till date there is no direct evidence suggesting that TSH regulates gonadal steroidogenesis in males. An earlier report had shown that TSH increased cAMP in human cryopreserved testicular slices. But the TSH doses used were very high (100-20,000 microIU/ml) and the preparation could have been contaminated by other pituitary hormones.^[64]

To confirm the role of TSH further we recruited normal men with TSH values $1.75 \pm 0.82 \mu\text{IU/ml}$ and men with low TSH, mean values $0.13 \pm 0.12 \mu\text{IU/ml}$ (Reference range of TSH 0.35–4.94 $\mu\text{IU/ml}$) and normal T₃ and T₄. These patients did not show a change in serum LH, FSH, testosterone, BioT, progesterone, estradiol and SHBG levels. However, lack of effect of low TSH does not rule out the lack of action of raised levels of TSH on gonadal functions (unpublished data from our laboratory).

Sperm function and semen

In hypothyroidism, men with normal sperm morphology were significantly lower than controls. But on treatment with levothyroxine there was a significant improvement on morphology. Sperm motility was also reduced in hypothyroid men but the reduction was not significantly lower than controls. Subclinical hypothyroidism does not affect semen and sperm parameters in adult males.^[65]

Sexual function

Hypothyroid males also show altered sexual behavior. In adult hypothyroid males impaired sexual behavior including hypoactive sexual desire (HSD), erectile dysfunction (ED) and ejaculatory disorders are prevalent. In these hypothyroid patients the sexual behavior improved with restoration of euthyroid status.^[2] The exact cause of sexual dysfunction in hypothyroidism is not clear. It could be attributed to persistent mild hyperprolactinemia as reported in the hypothyroid patients or it could be due to a rise in estrogen to testosterone ratio as reported by us and other or due to a CNS effect.

Hyperthyroidism

In our studies we found serum levels of testosterone and estradiol were raised in hyperthyroid men but their progesterone levels were similar to euthyroid controls.^[66] A similar rise in serum testosterone and estradiol levels was also reported by others.^[37,38]

Testosterone to progesterone ratio in the hyperthyroid group was significantly higher, suggesting a higher conversion rate of progesterone to testosterone. However, normal levels of progesterone in spite of increased conversion to testosterone imply an increased synthesis of progesterone. Fall in serum total cholesterol and LDL-C levels in hyperthyroid men suggest an increased utilization of serum cholesterol which may further be utilized for increased progesterone synthesis.^[66]

The increase in serum estradiol levels indicates an increase in peripheral aromatization of testosterone to estradiol. Further, hyperthyroidism being a state of hyperdynamic circulation is expected to increase the tissue blood flow and thereby an increased availability of testosterone to the peripheral tissues for aromatization.^[67]

Hudson and Edwards, (1992)^[37] have reported an increase in dialyzable free estradiol and without any change in dialyzable free testosterone. They calculated a decrease in free testosterone to free estradiol ratio suggesting an increased aromatization of testosterone. However, we^[66] reported an increase in calculated bioT and an unaltered total estradiol to testosterone ratio in hyperthyroid patients. The doubling of estradiol levels *per se* too suggested a rise in aromatization.

Kumar *et al.*, (2012)^[66] also reported an increase in serum SHBG in hyperthyroid men. SHBG decreases the metabolic clearance rate of testosterone which could partly attribute to increase in serum total testosterone levels observed in hyperthyroid men. In spite of raised SHBG levels, there is sufficient amount of circulating unbound testosterone available as evident by a rise in calculated bioT.

During the pre-ovulatory stage, the positive feedback of estradiol is characterized by 3-12-fold rise in LH levels in cycling females.^[59,60] Barbarino *et al.*, (1983)^[68] demonstrated that maintenance of serum estradiol concentration similar to that present in women at mid-cycle, for a period of 96-122h, lead to a surge of LH in both intact and castrated men. However, the magnitude of LH surge was not as huge as found in females. In our studies, we too report a similar positive feedback effect of raised estradiol levels on LH secretion; suggesting a key role of estrogen in regulating serum gonadotropin levels. The rise in LH was not as high as seen in cycling females which could result due to high testosterone levels [Figure 3].^[66] Boucekkine and

Semrouni (1990)^[69] examined the effect of estradiol on basal and GnRH – stimulated gonadotropin secretion in patients with Klinefelter's syndrome. Injections of estradiol to these patients for five days, induced an initial decline in the serum levels of FSH and LH, followed by a 6.6-fold rise in estradiol levels on day three and 1.7- fold increase in LH levels on day four of the injection. However, FSH levels remained suppressed till day seven. Their results also demonstrated the establishment of a positive feedback of estradiol on LH secretion in patients with Klinefelter's syndrome.

SPERM FUNCTION AND SEMEN

In hyperthyroid men there was a significant decrease in semen volume, sperm count, sperm motility and number of morphologically normal sperm.^[33,34]

BREASTS

Hyperthyroidism is often associated with gynecomastia.^[70] Karnath (2008)^[70] suggested a rise in serum SHBG and a subsequent fall in unbound testosterone as a probable cause for development of gynecomastia in hyperthyroid men. As we had already mentioned Hudson and Edwards, (1992)^[37] reported a fall in free testosterone to free estradiol ratio and suggested this hormonal imbalance could lead to gynecomastia. However, we^[66] calculated a raised bioT levels without any change in estradiol to testosterone ratio in hyperthyroid patients. Therefore, the ratio of estradiol to testosterone might not be as important as the rise of estradiol and its action through its specific receptors in the development of gynecomastia.

SEXUAL FUNCTIONS

In hyperthyroid men also, as was in hypothyroid patient impairment in sexual behavior was reported. There is a

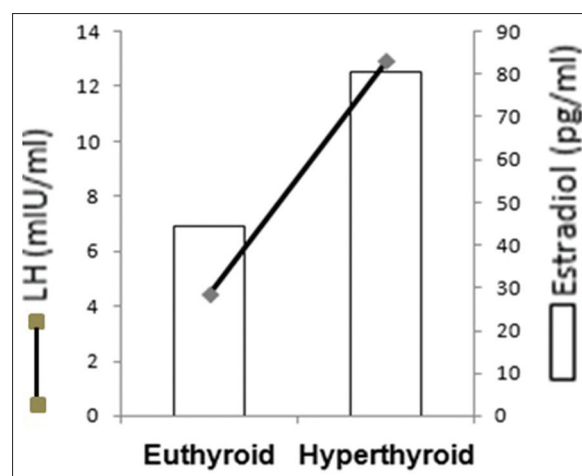


Figure 3: Serum LH and estradiol levels in hyperthyroid ($n=12$) and euthyroid ($n=17$) males (adapted from Kumar *et al.*, 2011, *Andrologia*)

higher prevalence of HSD, ED and ejaculatory disorders in them. A return to euthyroid state reversed the abnormality in sexual functions.^[2]

CONCLUSION

In conclusion, normal putative thyroid activity seems a requisite for male reproductive functions. Thyroid disorders distinctly affect the reproductive health of the male. However, the knowledge about the interaction between the two classical endocrine axes, hypothalamo-hypophyseal-testicular axis and hypothalamo-pituitary-thyroid axis, is still rudimentary and needs further investigation.

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Cite this article as: Kumar A, Shekhar S, Dhole B. Thyroid and male reproduction. *Indian J Endocr Metab* 2014;18:23-31.

Source of Support: Nil, **Conflict of Interest:** None declared.